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Isoflavone Soy Protein Supplementation and Atherosclerosis Progression in Healthy Postmenopausal Women: A Randomized Controlled Trial

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Abstract

Background and Purpose—Although epidemiological and experimental studies suggest that dietary intake of soy may be cardioprotective, use of isoflavone soy protein (ISP) supplementation as a primary preventive therapy remains unexplored. We determined whether ISP reduces subclinical atherosclerosis assessed as carotid artery intima-media thickness (CIMT) progression.

Methods—In a double-blind, placebo-controlled trial, 350 postmenopausal women 45–92 years of age without diabetes and cardiovascular disease (CVD) were randomized to 2 evenly divided daily doses of 25 g soy protein containing 91 mg aglycon isoflavone equivalents or placebo for 2.7-years.

Results—Overall, mean (95% confidence interval) CIMT progression rate was 4.77(3.39–6.16) $\mu\text{m}/\text{year}$ in the ISP group and 5.68(4.30–7.06) $\mu\text{m}/\text{year}$ in the placebo group. Although CIMT progression was reduced on average by 16% in the ISP group relative to the placebo group, this treatment effect was not statistically significant ($p=0.36$). Among the subgroup of women who were randomized within 5 years of menopause, ISP participants had on average a 68% lower CIMT progression rate than placebo participants 2.16(–1.10–5.43) vs. 6.79(3.56–10.01) $\mu\text{m}/\text{year}$.

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DISCLOSURES

None

$p=0.05$). ISP supplementation had a null effect on women who were >5 years beyond menopause when randomized. There were no major adverse events from ISP supplementation.

Conclusion—ISP supplementation did not significantly reduce subclinical atherosclerosis progression in postmenopausal women. Subgroup analysis suggest that ISP supplementation may reduce subclinical atherosclerosis in healthy young (median age, 53 years) women at low-risk for CVD who were <5 years postmenopausal. These first trial results of their kind warrant further investigation.

Keywords

Atherosclerosis; Cardiovascular disease; Intima-media thickness; Isoflavones; Menopause; Soy; Women

More than 40 million American women are currently postmenopausal and as the United States population ages, the number of women entering menopause will steadily increase by more than 1 million American women per year (1). Fear of estrogen-based hormone therapy has resulted in an escalating use of nutraceuticals, specifically soy protein products and soy-rich diets containing isoflavones as a postmenopausal therapeutic option (2,3).

Soy isoflavones are estrogen-like compounds that are structurally similar to 17β -estradiol and possess selective estrogen receptor modulator (SERM)-like activity (4,5). Evidence from epidemiological (6–8) and non-human primate studies (9–11) indicates that isoflavone-rich soy protein (ISP) has anti-atherogenic activity, evidence supported by a large body of data that demonstrates mechanistic and biologic plausibility (12–15).

As such, ISP may provide a safe and effective strategy for extending premenopausal cardioprotection afforded by endogenous estrogen into menopause without the increased risk of breast and uterine cancer and thromboembolic events associated with traditional hormone therapy, and without the side effects limiting sex steroid hormone use (16–18). In particular, a population-based 12.5-year prospective study in 40,462 Japanese (27,435 women) has shown that a high soy and isoflavone dietary intake is associated with reduced risk of cerebral and myocardial infarctions and cardiovascular disease (CVD) mortality among postmenopausal women (8). Asian populations consume approximately 30–50 g/d of soy protein (19) and approximately 20–200 mg/d of isoflavones (20) whereas Americans ingest <5 g/d of soy protein (21) and <1 mg/d of isoflavones (22). As such, increased dietary intake of ISP in the American diet has been recommended (23).

However, whether ISP can be used for the prevention of CVD is unknown. We hypothesized that ISP supplementation designed to provide the principle isoflavones similar in composition and exposure (150 mg/d) to a soy-based Asian diet will reduce atherosclerosis in postmenopausal women.

The Women's Isoflavone Soy Health (WISH) trial is a randomized controlled trial designed to determine the impact of ISP supplementation on health outcomes (atherosclerosis, osteoporosis, cognition and breast density) in a healthy population of postmenopausal women without pre-existing CVD. The aim of this study was to determine the effect of ISP supplementation on subclinical atherosclerosis progression.

MATERIALS AND METHODS

Study Population and Design

WISH was a randomized, double-blind, placebo-controlled trial conducted from April 12, 2004 to March 19, 2009. Participants were postmenopausal women without vaginal bleeding

>1 year and serum estradiol <20 pg/ml. Exclusion criteria were clinical signs, symptoms or personal history of CVD, diabetes mellitus or fasting serum glucose >6.99 mmol/L (126 mg/dl), fasting triglycerides >5.64 mmol/L (500 mg/dL), systolic blood pressure \geq 160 mmHg and/or diastolic blood pressure \geq 110 mmHg, untreated thyroid disease, serum creatinine >2 mg/dL, life-threatening illness with prognosis <5 years, alcohol intake >5 drinks/day or substance abuse, taking menopausal hormone therapy and soy, nut or related food allergies. Participants were recruited from the general population from the Greater Los Angeles area predominantly through media advertisement. The University of Southern California Institutional Review Board approved the study protocol; all participants provided written informed consent.

Participants were randomly assigned in a 1:1 ratio to daily 25 g soy protein containing 91 mg aglycon equivalents of naturally occurring isoflavones and its glycosides (154 mg total isoflavone conjugates plus aglycons): genistein 52 mg aglycon equivalents (88 mg total), daidzein 36 mg aglycon equivalents (61 mg total) and glycitein 3 mg aglycon equivalents (5 mg total) or daily total milk protein matched placebo (0 isoflavones) within 2 strata of carotid artery intima-media thickness (CIMT) (<0.75 mm, \geq 0.75 mm). The placebo and active treatments were taken in 2 evenly divided doses daily delivered in either beverage powder-food packs or food bars to provide variety and to maintain compliance. ISP and placebo products were prepared without charge by the Solae Company (St. Louis, MO) and were identical in taste and appearance. Within each stratum, blocked randomization was implemented with a masked block size. Participants, investigators, staff, imaging specialists and data monitors were masked to treatment assignment. The randomization procedure is more fully described in the Supplemental Material.

Clinic visits occurred every month for the first 6 months and then every other month for the remainder of the trial. At every clinic visit, data regarding dietary intake, product compliance, non-study medications and nutritional products, clinical adverse events as well as vital signs were ascertained. Every 6 months, laboratory determinations were performed (including ultrasound determinations of CIMT, lipids and isoflavones) and lifestyle and medical questionnaires administered. DXA bone scans, mammograms, pelvic examinations with Pap smears, transvaginal ultrasounds (and endometrial biopsies when indicated) and chemistry panels and complete blood counts were performed at baseline and annually. Cognitive assessments were completed at baseline and at the final follow-up visit (2.5-years).

The initial 2.5-year treatment period was increased to 3 years (an optional additional 6 month study visit) by the External Data and Safety Monitoring Board to increase the chance of detecting treatment group differences on the primary endpoint. Interim analyses of the primary trial endpoint were not performed.

The primary trial endpoint was the rate of change in the right distal common carotid artery intima-media thickness (CIMT). Sample size based on CIMT progression required 150 participants/arm (including an anticipated 10% annual dropout rate) to detect a difference in the rate of CIMT progression of 12.4 μ m/year (based on unopposed estrogen randomized controlled trial data (24)) at 0.05-significance (2-sided) with 90% power. A total of 350 participants were recruited.

Assessment of Atherosclerosis Progression

High-resolution B-mode ultrasound images of the right common carotid artery were obtained with a 7.5-MHz linear array transducer attached to an ATL Apogee ultrasound system (Bothell, WA). Ultrasound imaging and measurement of far wall CIMT were completed as previously described masked to treatment group and to participant

characteristics (Patents 2005, 2006, 2011) (24–29). Ultrasound imaging and CIMT measurement are more fully described in the Supplemental Material.

Laboratory Measurements

Participants fasted 8 hours before sample collections. Plasma lipids were measured using an enzymatic method under the CDC Standardization Program; LDL-C was calculated (30). Plasma isoflavone and equol levels were measured by high-pressure liquid chromatography (see Supplemental Material for further details) (31). Among ISP participants, plasma equol (a daidzein metabolite) levels at post-randomization visits were used to determine equol-producer status: equol never >20 nmol/L (non-producer), equol >20 nmol/L at some visits (intermittent producer) or equol >20 nmol/L at all visits (consistent producer). Protein and isoflavone (soy products) content was determined by analytical testing in all production lots before release to assure that the products met the target levels for macro- and micro-nutrients (31).

Statistical Analysis

Pre-randomization socio-demographic and clinical characteristics (including CIMT) and percentage product compliance were compared between treatment groups with 2-sample t-tests (or Wilcoxon rank sum) for continuous variables and chi-square tests for categorical variables. On-trial changes from baseline in plasma lipids, isoflavone levels, glucose, blood pressure and weight were compared using generalized estimating equations (dependent variables with repeated measurements) using an exchangeable correlation structure; independent variables were treatment groups and the CIMT randomization strata. Triglycerides were log transformed prior to analysis.

An intention-to-treat analysis was performed for all participants who had carotid ultrasonography at baseline and at least 1 follow-up visit. A linear mixed effects model was used to compare treatment groups on average CIMT change rates. CIMT was regressed on follow-up time (in years), with adjustment for the randomization stratification factor (baseline CIMT). The regression coefficient associated with trial follow-up time estimated the average CIMT annual rate of change. A treatment \times follow-up time interaction term evaluated whether the treatment groups differed in average CIMT progression rates. Post-hoc subgroup analyses examined treatment group differences on CIMT progression rates by participant age (<55, 56–60 or >60 years), time-since-menopause (<5, 5–10 or >10 years), equol production status (non-producer, intermittent producer or consistent producer) and ethnicity.

In ancillary analyses, mixed effects models were used to evaluate the association of baseline and on-trial plasma isoflavone and equol levels (all modeled as continuous variables) with the CIMT progression rate. Interaction terms of isoflavone levels with follow-up time evaluated in the overall sample whether isoflavone levels modified CIMT progression.

Treatment group comparisons on adverse events among all randomized participants used the Fisher's exact test. Major adverse events included deaths, cardiovascular events, cerebrovascular events, arterial revascularization procedures and cancers.

Statistical analyses used SAS 9.2 software (SAS, Inc., Cary, North Carolina); statistical testing was conducted at a 2-tailed 0.05 significance level.

This was an investigator-initiated and -conducted trial. The authors were solely responsible for the design, conduct, data collection, data management, statistical analysis and data interpretation. The funding source played no role in these functions. The funding source had no role in deciding whether or where the study would be submitted for publication.

RESULTS

Baseline Characteristics

Of the 1063 individuals screened (Figure 1), 350 were randomized (175 placebo, 175 ISP). Of the randomized participants, 280(136 placebo, 144 ISP) completed the initially planned 2.5-year trial period; of these, 165(81 placebo, 84 ISP) participated in the trial extension. 325 participants (163 placebo, 162 ISP) contributed to the primary end point analysis.

Treatment groups did not significantly differ at baseline for demographic, clinical and atherosclerosis characteristics (Table 1). The average age was 60.9 years and 36% were from an ethnic minority.

CIMT Progression Rates

The 325 participants with CIMT data had a median(range) of 2.8(0.5–3.6) years of follow-up in the placebo group and 2.7(0.5–3.6) years of follow-up in the ISP group ($p=0.83$). Participants contributed an average of 5.8(range, 2–8) CIMT measures in the placebo group and 6.1(range, 2–8) measures in the ISP group ($p=0.07$). Although the progression rate of CIMT was reduced on average by 16% in the ISP group relative to the placebo group (Figure 2), this treatment effect was not statistically significant overall ($p=0.36$) (Table 2). Treatment groups did not significantly differ on CIMT progression among post-hoc analysis of age, ethnic and equol-production subgroups (Tables 2 and 3). Among women who had experienced menopause within the past 5 years (median age = 53 years, range = 45–62 years), ISP participants had on average a 68% lower CIMT progression rate than placebo participants ($p=0.05$) (Table 2). Asian participants receiving ISP had on average a 64% lower CIMT progression rate than those Asian participants receiving placebo ($p=0.08$). In mixed effects models in the entire sample of 325 participants, baseline and on-trial plasma isoflavone or equol levels were not significantly associated with CIMT progression rate.

Compliance

Median (inter-quartile range) compliance assessed over the 3-year treatment period by package and bar count was 86.5% (47.5%–96.0%) among the placebo-treated participants and 91.0% (75.0%–98.0%) among the ISP-treated participants ($p=0.008$). For subjects who were lost to follow-up, compliance was censored at last contact. For the participants who discontinued study product, zero adherence was assigned from the time they discontinued the study product until the last follow-up visit. Compliance was confirmed by plasma and urine isoflavone measurements (Table 4) (31). No participant reported on-trial use of soy/ isoflavone supplements.

Metabolic and Blood Pressure Variables

Treatment groups did not differ on average baseline levels of fasting plasma lipids, isoflavone levels or glucose, blood pressure or body weight (Supplemental Material Table 1). Over the trial, average changes in fasting glucose, blood pressure and weight did not significantly differ between treatment groups (Table 4). Compared to placebo, ISP participants on average increased HDL-C levels ($p=0.03$).

Clinical Events

There were no deaths and 1 CVD event (stroke in an ISP participant) during the trial. Five cancers were reported in placebo participants (colon, breast, uterus and 2 squamous cell of the skin), none were reported in ISP participants, $p=0.06$. Overall categorical differences ($p<0.05$) in adverse events occurred for the respiratory and urinary systems. The adverse events in the ISP vs. placebo participants that accounted for the categorical differences were

cold/influenza symptoms (32.6% vs. 16.6%), pharyngitis/pharyngodynia (6.3% vs. 2.3%) and bronchitis/respiratory infection (9.1% vs. 4.0%) for the respiratory system and urinary tract infection (12.0% vs. 2.9%) for the urinary system. There were no other differences by treatment group across any other category including the gastrointestinal and gynecological systems although breast tenderness (5.7% vs. 1.1%) and vaginal bleeding/spotting (10.3% vs. 2.9%) were more evident in the ISP vs. placebo participants; endometrial hyperplasia (0.003% vs. 0%) and endometrial biopsies (2.9% vs. 3.4%) were not different between treatment groups.

DISCUSSION

In the overall cohort of healthy postmenopausal women, ISP supplementation did not significantly reduce the progression of subclinical atherosclerosis relative to placebo. In subgroup analysis, ISP supplementation significantly reduced progression of subclinical atherosclerosis in women who were within 5 years of menopause with a null effect on the progression of subclinical atherosclerosis in the women who were >5 years beyond menopause when randomized.

The age range for this trial was developed to address the effect of ISP supplementation over the postmenopausal age range for potential generalization to all postmenopausal women. Although the trial was not specifically designed to test the effects of ISP supplementation in younger women, the trial results indicate that a beneficial treatment effect may be limited to women who initiate ISP supplementation when close to menopause (within <5 years). This suggests a potential benefit over a narrower age range. The approximate 46% reduction in progression of CIMT in the ISP versus placebo group in the women <55 years of age is consistent with this possibility. It is possible that the broad age range of our population may have diluted a possible treatment effect of ISP supplementation in younger postmenopausal women.

Findings from WISH are consistent with data that support the timing hypothesis that posits that CVD is reduced in young postmenopausal women who initiate hormone therapy in close proximity to menopause versus a null CVD effect in women who initiate therapy when distant from menopause (24,32,33). Accumulating data from human and animal studies indicate that postmenopausal hormone therapy has little effect in reversing atherosclerosis once it is established, whereas it significantly reduces the extent of atherosclerosis if initiated at an early stage (24,32). Animal studies have demonstrated the same phenomenon whereby soy isoflavone administration does not reverse established atherosclerosis (34) but prevents atherosclerosis development (35,36). Recently, the timing hypothesis has been shown to be operative with the SERM raloxifene whereby women <60 years of age who received SERM therapy had a statistically significant reduction in CVD whereas women older than 60 years of age had no CVD benefit relative to placebo (37). Our subgroup analysis suggests that ISP may be the third class of estrogen receptor-binding molecules (4,5) to behave in accordance with the timing hypothesis (33).

Equol, a product of intestinal bacterial metabolism of daidzein is superior to all other isoflavones in its antioxidant activity (38,39). However, equol is not produced by all individuals and it has been suggested that the maximal clinical responses to ISP are observed in equol-producers versus equol non-producers (38). Since the clinical effectiveness of ISP may be a function of the ability to biotransform daidzein to the more potent estrogenic isoflavone equol, we examined the effect of ISP on atherosclerosis progression in equol producers versus equol non-producers. We found no difference in atherosclerosis progression comparing placebo to treatment groups defined by equol production.

Although shown to require the estrogen receptor (ER) to be anti-atherogenic (40–43), soy isoflavones may also have non-ER-mediated anti-atherogenic effects through traditional CVD risk factors including alteration of lipids and blood pressure (12,13,44). The ISP effects on these 2 major CVD risk factors are inconsistent across the literature (45). We found no effect of ISP on blood pressure but did detect a 2-fold greater increase in HDL-C in the ISP-treated group relative to the placebo-treated group. We previously reported that 17 β -estradiol therapy significantly reduced CIMT progression with approximately 30% of the variability of this effect due to lipid alteration including the HDL-C raising effect of estrogen therapy (24).

Epidemiological, cross-cultural and migrant studies indicate that the Western diet plays an important role in the incidence of coronary heart disease as well as breast, ovary, colon and prostate cancer (7,45). Among Pacific Rim (Asian) countries where the intake of ISP is very high, the incidence of these chronic diseases is much lower than in Western countries (6–8). In the context of non-human primate studies in which dietary intake was completely replaced with ISP and epidemiological studies of Asian populations, the effects of ISP may not have been fully expressed in WISH where ISP was used as a supplement to rather than a replacement of a Western diet. Longer or sole exposure to ISP as in Asian populations may be required for the full expression of the effects of ISP on atherosclerosis progression. Although we did not find a difference in treatment effect across ethnic groups, the treatment group difference in CIMT progression was of borderline significance in the small subgroup of Asian women. Additionally, within the ISP-treatment group, the rate of CIMT progression in Asians was less than half the CIMT rate as the other ethnic groups. Taken together, the cumulated data suggest that a potential gene-ISP interaction may exist for the expression of a beneficial effect of ISP.

WISH was the largest and longest randomized controlled trial of ISP supplementation examining CVD in general and progression of atherosclerosis specifically; isoflavone dosage was high, tolerable and without serious adverse events. As such, participant withdrawal was low and compliance with study products was excellent (about 90%) with confirmed high plasma concentrations (31). However, sample size and duration of therapy may not have been sufficient to detect a smaller change in atherosclerosis progression than that originally hypothesized and used to guide our sample size estimates. As the first large-scale randomized controlled trial examining the effects of ISP on atherosclerosis progression, certain limitations result from the disadvantage of not having antecedent trials upon which to guide design. Practicality mandated use of only 1 readily available form of high-dose ISP such that the results may not be necessarily generalizable to isolated individual isoflavones, other classes of phytoestrogens more abundant in non-soy food products (e.g., flavones, coumestans and lignans) or to the more recently developed equol products. Duration of therapy may be a limitation since the cardioprotective effect of ISP observed in epidemiological studies is in populations of individuals who are exposed over a life-time to high levels of soy beginning in-utero and to a large dietary ingestion of soy from birth. Another limitation of the trial results from the disadvantage of experience in administration of ISP in different populations of women since our results suggest that certain populations of women such as Asians and women in close proximity to menopause (younger women in a narrower age range) may have a more effective response to ISP supplementation. While these latter findings are consistent with those predicted from epidemiological studies our findings are limited by the number of subgroup analyses performed.

SUMMARY

In conclusion, ISP supplementation over a 2.7-year period did not significantly reduce the progression of subclinical atherosclerosis in postmenopausal women. Subgroup analyses suggest a possible benefit of ISP supplementation among women who were randomized within 5 years of menopause; this hypothesis will require further study. ISP supplementation appears to be safe and long-term high compliance feasible. Mechanistically, the effect of ISP is consistent with postmenopausal hormone therapy and partly accounted for by a rise in HDL-C. These results warrant further investigation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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APPENDIX

WISH Research Group Members

Study Chairman: Howard N. Hodis MD*; Clinical Center Staff: Martha Charlson RD, Irma Flores MA, Martha Huerta, Thelma LaBree MA, Sonia Lavender MA, Violetta McElreath RN, Janie Teran, Liny Zurbrugg RN, Philip Zurbrugg; Ultrasound Image Acquisition and Processing Laboratory: Robert H. Selzer MS* (Director), Mei Feng MD, Yanjie Li MD, Lora Whitfield-Maxwell RN, Ming Yan MD, PhD; Data Coordinating Center: Wendy J. Mack PhD* (Director), Stanley P. Azen PhD*, Farzana Choudhury MS, Carlos Carballo, Chun Ju-Chien, Laurie Dustin MS, Adrian Herbert, Michael Hutchinson, Naoko Kono MPH, George Martinez, Nitya Mathew, Olga Morales, Connie Wu MS, Mingzhu Xiang, MS; Core Lipid/Lipoprotein Laboratory: Juliana Hwang-Levine PharmD* (Director), Gail Izumi CLS, Arletta Ramirez CLS, Luci Rodriguez; Gynecology: Donna Shoupe MD*; Neurocognition: Victor W. Henderson MD*, Carol A. McCleary, PhD, Jan A. St. John MPH; Bone Density and Metabolic Laboratory: Robert Rude MD* (Director), Livia Y. Wei; Mammography Breast Density Laboratory: Anna H. Wu PhD* (Director), Chiu-chen Tseng MS, Giske Ursin PhD; Isoflavone Laboratory: Adrian A. Franke PhD* (Director), Sandra M. Hebshi, Ian Pagano; Data Safety Monitoring Board: Meir Stampfer MD (Chairman), Ronald M. Krauss MD, J. Christopher Gallagher MD; Josh Berman MD, PHD, Catherine Stoney PhD and Shan S. Wong PhD (NCCAM ex-officios); Lisa Begg, DrPH, RN and Rebecca B. Costello PhD (ODS ex-officios).

*Primary trial investigators

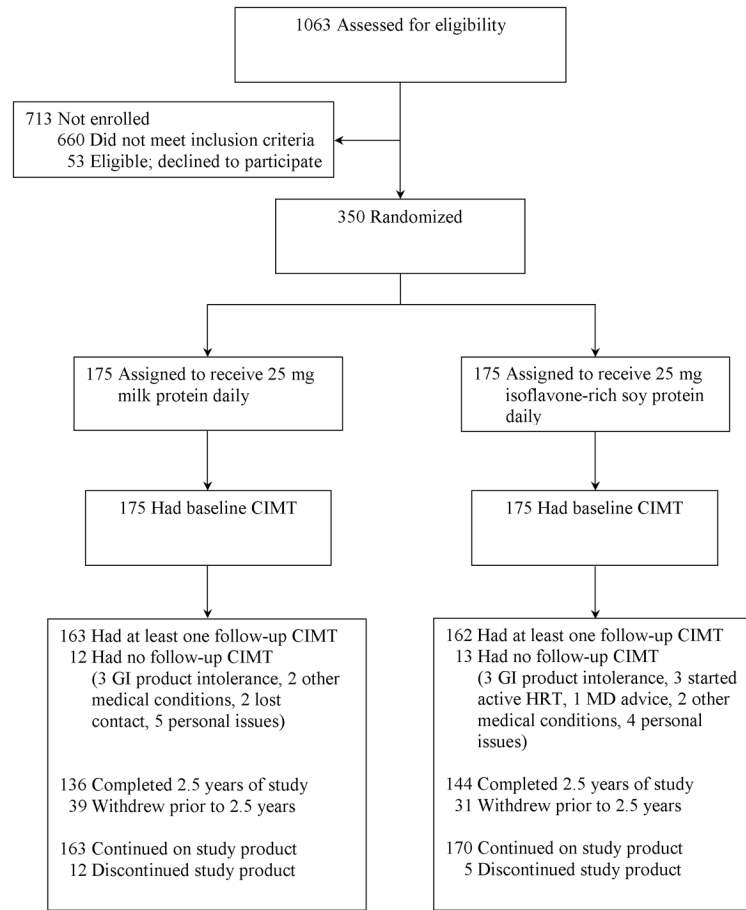
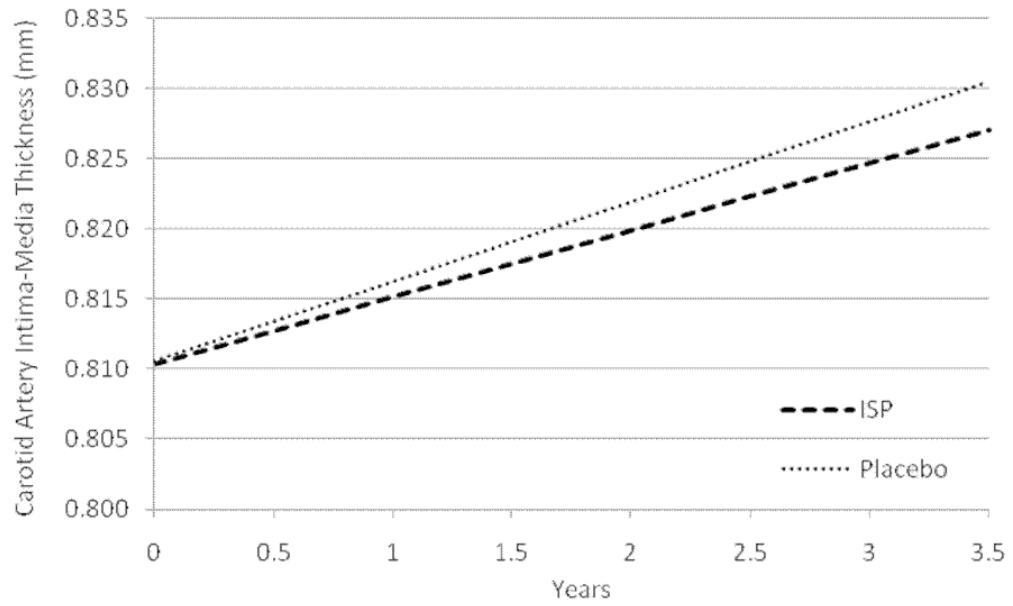


Figure 1. Women’s Isoflavone Soy Health (WISH) trial flow for carotid artery intima-media thickness outcome.



Number at follow-up	0	0.5	1	1.5	2	2.5	3	3.5
ISP	162	150	151	148	143	152	76	11
Placebo	163	141	141	134	134	154	69	6

Figure 2. Carotid artery intima-media thickness progression rates estimated by linear mixed models (by treatment group) with numbers of subjects evaluated at each follow-up ultrasound scanning time; $p=0.35$ for difference between treatment groups. ISP = isoflavone soy protein treatment group.

Table 1

Baseline Characteristics*

Variable	Placebo n = 163	ISP n = 162	No CIMT Follow-up n = 25
CIMT – mm	0.811(0.098)	0.810 (0.105)	0.813 (0.082)
Age – yrs	60.9 (6.9)	60.8 (7.2)	62.3 (7.8)
Ethnicity			
White (non-Hispanic)	110 (67%)	97 (60%)	16 (64%)
Black (non-Hispanic)	10 (6%)	12 (7%)	3 (12%)
Hispanic	21 (13%)	31 (19%)	4 (16%)
Asian	22 (14%)	21 (13%)	2 (8%)
Unknown	-	1 (<1%)	-
Education			
≤ High school	5 (3%)	13 (8%)	1 (4%)
> High school	158 (97%)	149 (92%)	24 (96%)
Smoking history			
Current	5 (3%)	2 (1%)	1 (4%)
Former	59 (36%)	68 (42%)	8 (32%)
Never smoked	99 (61%)	92 (57%)	16 (64%)
Body mass index – kg/m ²	25.8 (23.2, 30.0)	26.0 (22.8, 29.6)	25.0 (23.0, 27.6)
Past use of hormone therapy			
Yes	111 (68%)	118 (73%)	16 (64%)
No	52 (32%)	44 (27%)	9 (36%)
Age at menopause – yrs [†]	50.0 (48.0, 52.0)	50.0 (47.0, 52.0)	48.5 (46.0, 50.0)
Time-since-menopause – yrs			
<5	35 (22%)	33 (20%)	5 (20%)
5–10	43 (26%)	46 (28%)	6 (24%)
>10	65 (40%)	69 (43%)	11 (44%)
Unknown	20 (12%)	14 (9%)	3 (12%)
Type of menopause			
Natural	144 (88%)	150 (93%)	20 (80%)
Surgical	18 (11%)	12 (7%)	5 (20%)
Unknown	1 (<1%)	-	-

* Mean (SD) or median (25th, 75th percentile) for continuous variables and n (%).

[†] Age at menopause could not be determined in 37 subjects.

ISP = isoflavone soy protein treatment group.

CIMT = carotid artery intima-media thickness.

Table 2

Carotid Artery Intima-Media Thickness Progression Rates*

Subject group	N [†]	Placebo	ISP	Differences in rates	p-value [‡]
All Subjects	325 (163/162)	5.68 (4.30–7.06)	4.77 (3.39–6.16)	-0.91 (-2.86–1.05)	0.36
Age at randomization – yrs					
≤55	82 (44/38)	5.41 (2.70–8.11)	2.92 (0.05–5.79)	-2.49 (-6.44–1.45)	0.21
56–60	81 (40/41)	5.76 (3.21–8.32)	4.19 (1.64–6.74)	-1.57 (-5.18–2.04)	0.39
>60	162 (79/83)	5.78 (3.74–7.82)	5.89 (3.90–7.88)	0.11 (-2.74–2.96)	0.94
Time-since-menopause – yrs					
<5	68 (35/33)	6.79 (3.56–10.00)	2.16 (-1.10–5.43)	-4.62 (-9.21–-0.04)	0.05
5–10	89 (43/46)	6.46 (4.10–8.83)	5.36 (3.08–7.64)	-1.10 (-4.39–2.19)	0.51
>10	134 (65/69)	5.24 (2.98–7.50)	4.78 (2.58–6.98)	-0.46 (-3.62–2.70)	0.77
Ethnicity [§]					
White (non-Hispanic)	207 (110/97)	5.89 (4.16–7.62)	5.19 (3.35–7.03)	-0.70 (-3.22–1.83)	0.59
Black (non-Hispanic)	22 (10/12)	6.69 (1.25–12.13)	6.27 (1.31–11.22)	-0.43 (-7.79–6.93)	0.91
Hispanic	52 (21/31)	3.42 (-0.29–7.12)	4.63 (1.55–7.70)	1.21 (-3.60–6.02)	0.62
Asian	43 (22/21)	6.37 (2.95–9.79)	2.07 (-1.40–5.55)	-4.30 (-9.18–0.58)	0.08

* Mean (95% confidence interval) change rate in $\mu\text{m}/\text{year}$.

[†]Total sample size (placebo treatment group sample size/isoflavone soy protein treatment group sample size).

[‡]P-value for treatment group differences in carotid artery intima-media thickness rate analyzed by linear mixed effects models adjusting for carotid artery intima-media thickness randomization strata.

[§]Ethnicity unknown for 1 participant and excluded from the model.

ISP = isoflavone soy protein treatment group.

Table 3

Carotid Artery Intima-Media Thickness Progression Rate by Isoflavone Soy Protein Treatment Group Equol Producer Status versus Placebo Treatment

Study group	N*	CIMT [†]	p-value [‡]	p-value [§]
Placebo-treatment	163	5.68 (4.29–7.07)	Reference	0.84
Isoflavone soy protein-treatment				
Non-equol producer	76	5.09 (3.07–7.12)	0.64	
Intermittent equol producer	35	4.52 (1.55–7.50)	0.49	
Consistent equol producer	39	4.56 (1.74–7.39)	0.49	

* Sample size; isoflavone soy protein treatment group sample size does not total 162 subjects since 12 participants did not have sufficient follow-up equol measures to be classified.

[†] Mean (95% confidence interval) carotid artery intima-media thickness change rate in $\mu\text{m}/\text{year}$.

[‡] p-value for difference from placebo.

[§] Overall p-value across isoflavone soy protein treatment equol producer categories compared to placebo treatment adjusted for CIMT randomization strata.

Table 4

Absolute Change in Blood Pressure, Weight and Plasma Lipids, Glucose and Isoflavone Levels

	Placebo	ISP	p-value*
Total Cholesterol – mg/dl	–0.1 (–4.6–4.3)	–1.4 (–5.4–2.5)	0.55
HDL-Cholesterol – mg/dl	1.3 (0.2–2.5)	2.7 (1.4–3.9)	0.03
LDL-Cholesterol – mg/dl	–2.3 (–6.7–2.0)	–4.0 (–7.8– –0.2)	0.41
Total Triglycerides – mg/dl	8.0 (–3.5–19.4)	1.6 (–6.3–9.5)	0.09
Plasma genistein – nmol/L	–7.5 (–76.7–61.7)	467.1 (366.4–567.8)	<.0001
Plasma daidzein – nmol/L	11.8 (–36.3–59.8)	337.9 (273.5–402.3)	<.0001
Plasma glycitein – nmol/L	2.9 (–0.04–5.9)	10.3 (7.3–13.3)	0.0002
Glucose – mg/dl	–2.7 (–4.4– –0.9)	–0.8 (–2.7–1.0)	0.12
Systolic Blood Pressure – mmHg	–1.7 (–3.7–0.3)	–2.3 (–4.3– –0.3)	0.51
Diastolic Blood Pressure – mmHg	–2.0 (–3.5– –0.6)	–2.5 (–4.0– –1.0)	0.53
Weight – lbs	0.3 (–0.9–1.6)	0.4 (–0.8–1.5)	0.93

* Mean (95% confidence interval) change from baseline, adjusted for randomization stratum.

Treatment groups compared using generalized estimating equations with identity link function and exchangeable correlation structure.

Median (25th, 75th percentile) for change from baseline variables with non-normal distributions:

Total triglycerides: Placebo = 2.5 (–14.5, 21.0); ISP = 1.0 (–19.5, 19.0).

Plasma genistein: Placebo = 0.6 (–6.1, 21.2); ISP = 273.9 (70.3, 679.3).

Plasma daidzein: Placebo = 2.1 (–6.4, 28.3); ISP = 202.9 (49.9, 484.9).

Plasma glycitein: Placebo = 0.0 (–0.6, 2.1); ISP = 4.2 (0, 14.3).

ISP = isoflavone soy protein treatment group.