Isoflavone Soy Protein Supplementation and Atherosclerosis Progression in Healthy Postmenopausal Women

A Randomized Controlled Trial

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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 progression.

Methods—In a double-blind, placebo-controlled trial, 350 postmenopausal women 45 to 92 years of age without diabetes and cardiovascular disease 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% CI) carotid artery intima-media thickness progression rate was 4.77 (3.39–6.16) μm/year in the ISP group and 5.68 (4.30–7.06) μm/year in the placebo group. Although carotid artery intima-media thickness 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 carotid artery intima-media thickness progression rate than placebo participants 2.16 (1.10 to 5.43) versus 6.79 (3.56–10.01) μm/year (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.

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

Clinical Trial Registration—URL: http://www.clinicaltrials.gov. Unique identifier: NCT00118846.

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Key Words: atherosclerosis ■ cardiovascular disease ■ intima-media thickness ■ isoflavones ■ menopause ■ soy ■ women

More than 40 million American women are currently postmenopausal and as the US population ages, the number of women entering menopause will steadily increase by >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-like activity.4,5 Evidence from epidemiological6–8 and nonhuman primate studies9–11 indi-
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indicates that isoflavone-rich soy protein (ISP) has antiatherogenic activity, evidence supported by a large body of data that demonstrates mechanistic and biological 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 people (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 to 50 g/day of soy protein20 and approximately 20 to 200 mg/day of isoflavones,20 whereas Americans ingest <5 g/day of soy protein21 and <1 mg/day 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/day) 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 effect of ISP supplementation on subclinical atherosclerosis progression is reported herein.

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 a 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 ≥160 mm Hg and/or diastolic blood pressure ≥110 mm Hg, 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 or soy, nut, or related food allergies. Participants were recruited from the general population of 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 aglycone equivalents of naturally occurring isoflavones and its glycodies (154 mg total isoflavone conjugates plus aglycons): genistein 52 mg aglycone equivalents (88 mg total), daidzein 36 mg aglycone equivalents (61 mg total), and glycitein 3 mg aglycone 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, ≥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 (http://stroke.ahajournals.org).

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, nonstudy 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. Dual-energy x-ray absorptiometry bone scans, mammograms, pelvic examinations with Papanicolaou 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 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 μm/year (based on unopposed estrogen randomized controlled trial data24) 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 Centers for Disease Control and Prevention Standardization Program; low-density lipoprotein cholesterol 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 postrandomization visits were used to determine equol producer status: equol never >20 nmol/L (nonproducer), 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 analytic testing in all production lots before release to assure that the products met the target levels for macro- and micronutrients.31

Statistical Analysis

Prerandomization sociodemographic and clinical characteristics (including CIMT) and percentage product compliance were compared between treatment groups with 2-sample t tests (Wilcoxon rank sum) for continuous variables and ×2 tests for categorical variables. On-treatment 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 before 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×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 (nonproducer, 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 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, NC); 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. Three hundred twenty-five 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 (interquartile 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). 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 Table I). Over the trial, average changes in fasting glucose, blood pressure, and weight did not significantly differ between treatment groups (Table 4). Compared with placebo, ISP participants on average had increased high-density lipoprotein cholesterol levels ($P=0.03$).

Clinical Events

There were no deaths and 1 CVD event (stroke in an ISP participant) during the trial. Five participants in the placebo group reported cancer (colon, breast, uterus, and squamous cell of the skin [3 events affecting 2 participants]); 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 versus placebo participants that accounted for the categorical differences were cold/influenza symptoms (26.9% versus 13.1%), pharyngitis/pharyngodynia (5.1% versus 1.7%), and bronchitis/respiratory infection (7.4% versus 4.0%) for the respiratory system and urinary tract infection.

### Table 1. Baseline Characteristics*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Placebo (n=163)</th>
<th>ISP (n=162)</th>
<th>No CIMT Follow-Up (n=25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIMT, mm</td>
<td>0.811 (0.098)</td>
<td>0.810 (0.105)</td>
<td>0.813 (0.082)</td>
</tr>
<tr>
<td>Age, y</td>
<td>60.9 (6.9)</td>
<td>60.8 (7.2)</td>
<td>62.3 (7.8)</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White (non-Hispanic)</td>
<td>110 (67%)</td>
<td>97 (60%)</td>
<td>16 (64%)</td>
</tr>
<tr>
<td>Black (non-Hispanic)</td>
<td>10 (6%)</td>
<td>12 (7%)</td>
<td>3 (12%)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>21 (13%)</td>
<td>31 (19%)</td>
<td>4 (16%)</td>
</tr>
<tr>
<td>Asian</td>
<td>22 (14%)</td>
<td>21 (13%)</td>
<td>2 (8%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>. . .</td>
<td>1 (&lt;1%)</td>
<td>. . .</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ High school</td>
<td>5 (3%)</td>
<td>13 (8%)</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>&gt; High school</td>
<td>158 (97%)</td>
<td>149 (92%)</td>
<td>24 (96%)</td>
</tr>
<tr>
<td>Smoking history</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>5 (3%)</td>
<td>2 (1%)</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>Former</td>
<td>59 (36%)</td>
<td>68 (42%)</td>
<td>8 (32%)</td>
</tr>
<tr>
<td>Never smoked</td>
<td>99 (61%)</td>
<td>92 (57%)</td>
<td>16 (64%)</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>25.8 (23.2, 30.0)</td>
<td>26.0 (22.8, 29.6)</td>
<td>25.0 (23.0, 27.6)</td>
</tr>
<tr>
<td>Past use of hormone therapy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>111 (68%)</td>
<td>118 (73%)</td>
<td>16 (64%)</td>
</tr>
<tr>
<td>No</td>
<td>52 (32%)</td>
<td>44 (27%)</td>
<td>9 (36%)</td>
</tr>
<tr>
<td>Age at menopause, y†</td>
<td>50.0 (48.0, 52.0)</td>
<td>50.0 (47.0, 52.0)</td>
<td>48.5 (46.0, 50.0)</td>
</tr>
<tr>
<td>Time since menopause, y</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5</td>
<td>35 (22%)</td>
<td>33 (20%)</td>
<td>5 (20%)</td>
</tr>
<tr>
<td>5–10</td>
<td>43 (26%)</td>
<td>46 (28%)</td>
<td>6 (24%)</td>
</tr>
<tr>
<td>&gt;10</td>
<td>65 (40%)</td>
<td>69 (43%)</td>
<td>11 (44%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>20 (12%)</td>
<td>14 (9%)</td>
<td>3 (12%)</td>
</tr>
<tr>
<td>Type of menopause</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Natural</td>
<td>144 (88%)</td>
<td>150 (93%)</td>
<td>20 (80%)</td>
</tr>
<tr>
<td>Surgical</td>
<td>18 (11%)</td>
<td>12 (7%)</td>
<td>5 (20%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>1 (&lt;1%)</td>
<td>. . .</td>
<td>. . .</td>
</tr>
</tbody>
</table>

*Mean (SD) or median (25th, 75th percentile) for continuous variables and no. (%).
†Age at menopause could not be determined in 37 subjects.
(10.9% versus 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 pain or tenderness (5.1% versus 1.7%) and vaginal bleeding/spotting (6.9% versus 2.3%) were more evident in the ISP versus placebo participants; endometrial hyperplasia (0% versus 0.006%) and endometrial biopsies (3.4% versus 2.9%) 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 hormone 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 but prevents atherosclerosis development.15,36 Recently, the timing hypothesis has been shown to be operative with the selective estrogen receptor modulator raloxifene whereby women <60 years of age who received selective estrogen...
receptor modulator therapy had a statistically significant reduction in CVD, whereas women older than 60 years of age had no CVD benefit relative to placebo. Our subgroup analysis suggests that ISP may be the third class of estrogen receptor-binding molecules to behave in accordance with the timing hypothesis.

Equol, a product of intestinal bacterial metabolism of daidzein, is superior to all other isoflavones in its antioxidant activity. 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 nonproducers. Because the clinical effectiveness of ISP may be a function of the ability to biotransform daidzein to the more potent metabolite equol, we examined the effect of ISP on atherosclerosis progression in equol producers versus equol nonproducers. We found no difference in atherosclerosis progression comparing placebo with treatment groups defined by equol production.

Although shown to require the estrogen receptor to be antiatherogenic, soy isoflavones may also have non-estrogen receptor-mediated antiatherogenic effects through traditional CVD risk factors, including alteration of lipids and blood pressure. The ISP effects on these 2 major CVD traditional CVD risk factors, including alteration of lipids and antiatherogenic, soy isoflavones may also have non-

Table 4. Absolute Change in Blood Pressure, Weight and Plasma Lipids, Glucose, and Isoflavone Levels

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>ISP</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low-density lipoprotein cholesterol, mg/dl</td>
<td>1.3 (0.2–2.5)</td>
<td>2.7 (1.4–3.9)</td>
<td>0.03</td>
</tr>
<tr>
<td>Total triglycerides, mg/dL</td>
<td>8.0 (3.5–19.4)</td>
<td>1.6 (6.3–9.5)</td>
<td>0.09</td>
</tr>
<tr>
<td>Plasma genistein, nmol/L</td>
<td>-7.5 (-76.7–61.7)</td>
<td>467.1 (366.4–567.8)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Plasma daidzein, nmol/L</td>
<td>11.8 (36.3–59.8)</td>
<td>337.9 (273.5–402.3)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Plasma glycitein, nmol/L</td>
<td>2.9 (-0.04–5.9)</td>
<td>10.3 (7.3–13.3)</td>
<td>0.0002</td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>-2.7 (-4.4 to -0.9)</td>
<td>-0.8 (-2.7–1.0)</td>
<td>0.12</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>-1.7 (-3.7–0.3)</td>
<td>-2.3 (-4.3 to -0.3)</td>
<td>0.51</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>-2.0 (-3.5 to -0.6)</td>
<td>-2.5 (-4.0 to -1.0)</td>
<td>0.53</td>
</tr>
<tr>
<td>Weight, lbs</td>
<td>0.3 (-0.9–1.6)</td>
<td>0.4 (-0.8–1.5)</td>
<td>0.93</td>
</tr>
</tbody>
</table>

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

ISP indicates isoflavone soy protein treatment; CI, confidence interval.

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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. In the context of nonhuman 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 in which ISP was used as a supplement to rather than a replacement of a Western diet. Longer or sole exposure to ISP like 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 (approximately 90%) with confirmed high plasma concentrations. 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 on 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 nonsoy food products (e.g., flavones, coumestans, and lignans), or to the more recently developed equol products. Duration of therapy may be a limitation because the cardioprotective effect of ISP observed in epidemiological studies is in populations of individuals who are exposed over a lifetime 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 because 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. Although these latter findings are consistent with those predicted from epidemiological studies, our findings are limited by the number of subgroup analyses performed.

Conclusions
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 is feasible. Mechanistically, the effect of ISP is consistent with postmenopausal hormone therapy and partly accounted for by a rise in high-density lipoprotein cholesterol. These results warrant further investigation.

Appendix

WISH Research Group Members
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Disclosures
None.

References
Soy Protein and Progression of Atherosclerosis


Isoflavone Soy Protein Supplementation and Atherosclerosis Progression in Healthy Postmenopausal Women: A Randomized Controlled Trial
Howard N. Hodis, Wendy J. Mack, Naoko Kono, Stanley P. Azen, Donna Shoupe, Juliana Hwang-Levine, Diana Petitti, Lora Whitfield-Maxwell, Mingzhu Yan, Adrian A. Franke, Robert H. Selzer and for the Women's Isoflavone Soy Health Research Group

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Randomization Procedures

Trial eligibility was assessed at screening visit and confirmed at the data coordinating center. Assignment to ISP or placebo in a 1:1 ratio used stratified blocked randomization (block size of 4) within 2 strata of carotid artery intima-media thickness (<0.75 mm or ≥0.75 mm), as assessed by high-resolution B-mode ultrasonography. Randomization lists from a computerized random number generator (SAS statistical software) were prepared prior to trial initiation by the trial statistician. For each stratum, the randomization list included the product identification number and treatment code (active or placebo). Blinded study product was prepared based on the randomization list. Upon determination of trial eligibility for a given participant, clinic staff pulled the next study product in sequence from the appropriate stratum and recorded the product identification number. The statistician monitored the fidelity of the randomization process. Participants, investigators, staff, imaging specialists and data monitors were masked to treatment assignment.

Ultrasound Imaging and Carotid Artery Intima-Media Thickness Measurements

Ultrasound imaging of far wall CIMT was conducted using standardized procedures and technology specifically developed for longitudinal measurements (Patents 2005, 2006, 2011) (24-27). In brief, the jugular vein and carotid artery were imaged longitudinally with the former stacked above the latter. All images contained internal anatomical landmarks for reproducing probe angulation. The baseline image for each individual was used as an online guide for follow-up examinations on a split-screen system designed for repeat image acquisition for longitudinal studies. For each individual, depth of field, gain, monitor intensity setting, and all other instrumentation settings used at baseline examination were maintained for all follow-up examinations. All examinations were recorded with the electrocardiogram signal. These techniques have resulted in significant reductions in measurement variability (26,27). Far wall CIMT was measured using automated computerized edge detection software (26,27). CIMT was determined as the average of 70 to 100 measurements between the intima-lumen and media-adventitia interfaces along a 1 cm length just distal to the carotid artery bulb at the same point of the cardiac cycle. This method standardizes the location and the distance over which CIMT is measured, ensuring comparability within and across participants (26,27). This CIMT method is correlated with the change in coronary artery disease assessed by quantitative coronary
angiography (28) and is predictive of clinical coronary events (29). The coefficient of variation of the 350 repeated baseline CIMT measurements was <1%.

**Plasma Isoflavone and Equol Level Measurements**
Plasma isoflavone and equol levels were measured by high-pressure liquid chromatography with isotope dilution electrospray ionization (negative mode) tandem mass spectrometry (31). Samples from each individual were run in one batch to limit variability. Between-day coefficients of variation ranged 4-18% for all analytes, while intra-day variation was half or less of that (31).
Table 1. Absolute Change in Blood Pressure, Weight and Plasma Lipids, Glucose and Isoflavone Levels*

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Placebo</th>
<th>ISP</th>
<th>p-value†</th>
<th>Change</th>
<th>Placebo</th>
<th>ISP</th>
<th>p-value‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cholesterol – mg/dl§</td>
<td>221.2</td>
<td>218.3</td>
<td>0.38</td>
<td>-0.1</td>
<td>-1.4</td>
<td>0.55</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(216.3-226.1)</td>
<td>(213.8-222.7)</td>
<td></td>
<td>(-4.6-4.3)</td>
<td>(-5.4-2.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL-Cholesterol – mg/dl§</td>
<td>62.7</td>
<td>61.8</td>
<td>0.64</td>
<td>1.3</td>
<td>2.7</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(60.0-65.4)</td>
<td>(59.4-64.2)</td>
<td></td>
<td>(0.2-2.5)</td>
<td>(1.4-3.9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL-Cholesterol – mg/dl§</td>
<td>136.1</td>
<td>133.7</td>
<td>0.43</td>
<td>-2.3</td>
<td>-4.0</td>
<td>0.41</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(131.4-140.9)</td>
<td>(129.7-137.6)</td>
<td></td>
<td>(-6.7-2.0)</td>
<td>(-7.8-0.2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Triglycerides – mg/dl§</td>
<td>99.7</td>
<td>100.3</td>
<td>0.91</td>
<td>8.0</td>
<td>1.6</td>
<td>0.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(92.4-107.6)</td>
<td>(93.0-108.2)</td>
<td></td>
<td>(-3.5-19.4)</td>
<td>(-6.3-9.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma genistein – nmol/L</td>
<td></td>
<td></td>
<td>8.4</td>
<td>12.7</td>
<td>0.33</td>
<td>-7.5</td>
<td>467.1</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td></td>
<td>(4.0, 39.9)</td>
<td>(4.5, 55.0)</td>
<td></td>
<td>(-76.7-61.7)</td>
<td>(366.4-567.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma daidzein – nmol/L</td>
<td></td>
<td></td>
<td>11.8</td>
<td>15.5</td>
<td>0.90</td>
<td>11.8</td>
<td>337.9</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td></td>
<td>(4.5, 52.3)</td>
<td>(3.6, 58.1)</td>
<td></td>
<td>(-36.3-59.8)</td>
<td>(273.5-402.3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma glycitein – nmol/L</td>
<td></td>
<td></td>
<td>2.3</td>
<td>1.9</td>
<td>0.74</td>
<td>2.9</td>
<td>10.3</td>
<td>0.0002</td>
</tr>
<tr>
<td></td>
<td>(0.5, 6.4)</td>
<td>(0.5, 7.4)</td>
<td></td>
<td>(-0.04-5.9)</td>
<td>(7.3-13.3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose – mg/dl#</td>
<td>96.2</td>
<td>94.9</td>
<td>0.24</td>
<td>-2.7</td>
<td>-0.8</td>
<td>0.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(94.5-98.0)</td>
<td>(93.5-96.4)</td>
<td></td>
<td>(-4.4-0.9)</td>
<td>(-2.7-1.0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic Blood Pressure – mmHg**</td>
<td>118.2</td>
<td>117.9</td>
<td>0.83</td>
<td>-1.7</td>
<td>-2.3</td>
<td>0.51</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(116.0-120.5)</td>
<td>(115.8-120.0)</td>
<td></td>
<td>(-3.7-0.3)</td>
<td>(-4.3-0.3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diastolic Blood Pressure – mmHg**</td>
<td>74.9</td>
<td>75.1</td>
<td>0.85</td>
<td>-2.0</td>
<td>-2.5</td>
<td>0.53</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(73.6-76.3)</td>
<td>(73.8-76.5)</td>
<td></td>
<td>(-3.5-0.6)</td>
<td>(-4.0-1.0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight – lbs**</td>
<td>152.3</td>
<td>152.6</td>
<td>0.93</td>
<td>0.3</td>
<td>0.4</td>
<td>0.93</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(147.3-157.3)</td>
<td>(147.9-157.4)</td>
<td></td>
<td>(-0.9-1.6)</td>
<td>(-0.8-1.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
* Numbers in table are mean (95% confidence interval) except for baseline plasma isoflavones which are median (25\textsuperscript{th}, 75\textsuperscript{th} percentile).

† Treatment groups compared by 2-sample t-test; baseline plasma isoflavones compared by Wilcoxon 2-sample test.

‡ 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.

§ Sample size: Placebo = 153; ISP = 158.

|| Sample size: Placebo = 148; ISP = 155.

# Sample size: Placebo = 141; ISP = 152.

** Sample size: Placebo = 161; ISP = 159.

Median (25\textsuperscript{th}, 75\textsuperscript{th} 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.