SUPPLEMENTAL MATERIAL

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*

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

* Numbers in table are mean (95% confidence interval) except for baseline plasma isoflavones which are median (25^{th} , 75^{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^{th} , 75^{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.