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Arterioscler. Thromb. Vasc. Biol. 2002;22;1316-1322; originally published online Jun 20, 2002;
DOI: 10.1161/01.ATV.0000027176.83618.1A
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association. 7272 Greenville Avenue, Dallas, TX 72514
 Print ISSN: 1079-5642. Online ISSN: 1524-4636

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Objective—Phytoestrogens have been postulated to protect against cardiovascular diseases, but few studies have focused on the effect of Western dietary phytoestrogen intake.

Methods and Results—Four hundred three women with natural menopause either between 1987 and 1989 or between 1969 and 1979 were selected from the baseline data of the PROSPECT study (n=17 395). Isoflavone and lignan intake was calculated from a food-frequency questionnaire. Aortic stiffness was noninvasively assessed by pulse-wave velocity measurement of the aorta. Linear regression analysis was used. After adjustment for age, body mass index, smoking, physical activity, mean arterial pressure, follow-up time, energy intake, dietary fiber intake, glucose, and high density lipoprotein cholesterol, increasing dietary isoflavone intake was associated with decreased aortic stiffness: −0.51 m/s (95% CI −1.00 to −0.03, fourth versus first quartile, *P* for trend=0.07). Increasing dietary intake of lignans was also associated with decreased aortic pulse-wave velocity: −0.42 m/s (95% CI −0.93 to 0.11, fourth versus first quartile, *P* for trend=0.06). Results were most pronounced in older women: for isoflavones, −0.94 m/s (95% CI −1.65 to −0.22, *P* for trend=0.02), and for lignans, −0.80 m/s (95% CI −1.85 to −0.05), fourth versus first quartile.

Conclusions—The results of our study support the view that phytoestrogens have a protective effect on the risk of atherosclerosis and arterial degeneration through an effect on arterial walls, especially among older women. **(*Arterioscler Thromb Vasc Biol*. 2002;22:1316-1322.)**

Key Words: phytoestrogens ■ dietary intake ■ aortic stiffness ■ postmenopausal women

Phytoestrogens are naturally occurring plant compounds with a chemical structure comparable to that of estradiol.[1] Phytoestrogens can be divided into 3 major categories: isoflavones, lignans (precursors and naturally occurring), and coumestans.[2] Because phytoestrogens are capable of binding to the estrogen receptor, they may have the potential to mimic estrogenic effects.[3,4]

See p 1245

Phytoestrogens have been suggested to lower the risk of coronary heart disease (CHD). In nonhuman primates, isoflavones in soy protein have been found to improve cardiovascular disease risk factors,[5] in particular, serum lipids, and to inhibit the progression of coronary artery atherosclerotic lesions.[6–8] A meta-analysis of the effects of soy protein supplement action on serum lipids in humans found decreases in serum concentrations of 9.3% for total cholesterol, 12.9% for LDL cholesterol, and 10.5% for triglycerides.[9] Phytoestrogens have been reported to have antioxidative effects on LDL cholesterol.[10,11] Another possibility of phytoestrogen action is their possible effect on arterial walls through an effect on vascular reactivity. Animal studies found an improvement of vascular reactivity after supplementation of soy-containing isoflavones compared with isoflavone-devoid soy.[12,13] In Australian postmenopausal and perimenopausal women, systemic arterial compliance (or arterial stiffness) has been found to improve by >22% after supplementation of high amounts of tablets containing soy isoflavones[14] or isoflavones from red clover.[15]

Most investigators of the possible effects of phytoestrogens on tissues studied phytoestrogen supplements that contain amounts of phytoestrogens that are normally only reached in Asian populations (20 to 80 mg/d.)[16] Very little is known about the effects on tissues of the amount of phytoestrogens that Western populations consume in their usual diet (<1 mg/d).[17] In a very recent study among postmenopausal women, women with a usual dietary intake of genistein (one of the isoflavones) of ≥1.0 mg/d had a significantly lower body mass index (BMI), waist circumference, and fasting insulin level than did those with no daily genistein consumption.[18] Furthermore, in adjusted analyses, genistein, daidzein (both isoflavones), and total isoflavone intake were each

Received April 10, 2002; revision accepted June 11, 2002.

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Arterioscler Thromb Vasc Biol. **is available at http://www.atvbaha.org** **DOI: 10.1161/01.ATV.0000027176.83618.1A**

positively associated with HDL cholesterol and negatively related to post–glucose-challenge insulin. Earlier work from our group has shown a high usual dietary intake of phytoestrogens to be associated with a favorable metabolic cardiovascular risk profile.[19] All of these previous findings suggest the protective role of dietary soy intake against cardiovascular disease (risk factors) in postmenopausal women.

Because atherosclerosis and arterial stiffness are likely to predate the development of symptomatic CHD, pulse-wave velocity (PWV) measurements might be useful to detect cardiovascular risk at an early stage, before clinical manifestations have occurred. Phytoestrogens may have a positive effect on the postmenopausal risk of atherosclerosis and arterial degeneration, possibly through an effect on vascular walls. Therefore, we are interested in the relationship between the usual dietary intake of phytoestrogens and stiffness of the aorta, measured by PWV, in postmenopausal women.

Methods

Subjects

For the present study, participants were recruited from the PROSPECT study, 1 of the 2 Dutch cohorts participating in the European Prospective Investigation Into Cancer and Nutrition (EPIC).[20] In PROSPECT, a total of 17 365 healthy breast-cancer–screening participants, aged 49 to 70 years, living in Utrecht and surroundings were enrolled between 1993 and 1997. The purpose of the PROSPECT-EPIC study is to assess the relationship between nutrition and cancer and other chronic diseases. For the present study, 2 groups of women who had experienced a natural menopause either between 1987 and 1989 or between 1969 and 1979 were selected from the baseline data of PROSPECT. In addition, women were required to have an intact uterus and at least 1 intact ovary and should not have used sex steroids after the reported date of last menstruation. These criteria were used because the primary objective of the present study was to elucidate the role of endogenous estrogens on markers of frailty and to determine whether this role was different for women with a longer menopausal time span (20 to 30 years) compared with women with a shorter menopausal time span (8 to 12 years). Of 1802 eligible women (1149 with shorter menopausal and 653 with longer menopausal time spans), 902 women (451 with shorter menopausal and 451 with longer menopausal time spans) were invited by a personal letter from the principal investigator of PROSPECT, and 553 (61%) answered positively. The aim of the present study was to enroll 200 women in each group (2 groups with different intervals of time since menopause: women with a shorter menopausal time span and women with a longer menopausal time span). Eventually, 403 participants (207 with shorter menopausal and 196 with longer menopausal time spans) were included in the study. Women were considered sufficiently healthy to participate when they were physically and mentally able to visit the study center independently. Each participant underwent all tests and assessments during 2 visits to the study center. The present study was approved by the Institutional Review Board of the University Medical Center Utrecht, and written informed consent was obtained from all participants. Data collection took place between September 1999 and March 2000.

FFQ and Scoring Phytoestrogen Intake

At the baseline visit of the PROSPECT study, a validated food-frequency questionnaire (FFQ) was administered, designed to estimate regular intake of 178 food items in the year before enrollment.[21,22] As previously described in detail,[17] by using information in the literature, we calculated and assigned for each food item in the FFQ the values for the isoflavones (daidzein, genistein, formononetin, biochanin A, and coumestrol) and for the lignans (matairesinol and secoisolariciresinol). Each phytoestrogen content of a food item was then scored in 7 categories (Table 1), according to the following procedure. All values found in the literature were converted to milligrams per 100 g food. Values expressed on a dry weight basis were concerted to wet weight basis either by using the moisture content for that particular food or by using adjustments for the method of preparation. When the specific phytoestrogen content was reported as "a trace" or "traceable," the value of 0.00001 mg/100 g was appointed. This value is based on sensitivity of the method used. When more values were reported from the same or different original sources in the literature, we used the highest value to score the phytoestrogen content of a food. If wet and dry weights were reported from different original sources in the literature, we used the reported wet weight value to score. If the questionnaire listed similar food items on the same line, we used the phytoestrogen data for the food most commonly eaten. If values for the most common food were unavailable, any value found on 1 of the other food items in line was used. If there was no information about the phytoestrogen content of a food item, a value was assigned by using data of a similar food item if available; if not, the value zero was imputed. By use of the Nutrition Data system of the University of Minnesota, the amount of phytoestrogen in breakfast cereals was estimated, with the fiber content of the cereal used as a proxy for the phytoestrogen content. The average phytoestrogen content of wheat, triticale, and rye was used to estimate the amount of phytoestrogen per gram fiber. Finally, we multiplied the score of each food item by the serving size of the food. This final amount of each food item was multiplied by the frequency of the consumption of that food item and then summed across foods to obtain the total individual dietary intake of each group of phytoestrogens.

TABLE 1. Scoring of Phytoestrogen Content of Food Item

Phytoestrogen Content (mg/100 g Wet Weight)*	Scoring Value (mg/100 g)
Non detectable, 0	0
$0<*<0.001$	0.0005
$0.001\leq*<0.01$	0.005
$0.01\leq*<0.1$	0.05
$0.1\leq*1$	0.5
$1\leq*<10$	5
≥10	50

Measurements

Information on health was obtained by taking the medical history, by registration of current medication, and by physical examination. A standardized history of estrogen use, alcohol consumption, and smoking was obtained from all women as part of the medical history. Anthropometric measures included height, weight, and waist and hip circumferences. From these measures, BMI (kg/m^2) and body fat distribution were calculated. The Voorrips questionnaire[23] was administered to assess physical activity. This questionnaire has been validated in an elderly population.[23] Blood pressure and heart rate were measured during the first visit by an oscillometric automated device (DINAMAP 8100, Critikon). Measurements were conducted before 11:00 AM after an overnight fast. After 5 minutes of rest, blood pressure was taken at the right brachial artery simultaneously with heart rate measurement, twice with the participant in supine position, with 1 minute between each measurement. Systolic and diastolic blood pressures were taken as the average of the 2 measurements. Mean arterial pressure was calculated as diastolic blood pressure+1/3(systolic blood pressure−diastolic blood pressure). Pulse pressure was defined as systolic blood pressure minus diastolic blood pressure. Fasting venous blood samples were obtained between 8:00 and 11:00 AM. Total cholesterol, HDL cholesterol, triglyceride, glucose, and albumin levels were reflectometrically measured by using commercial enzymatic kits with a Vitros

250 (dry chemistry, Johnson & Johnson). Glucose was measured by the glucose oxidase/peroxidase method. Total cholesterol was measured by adaptation of the cholesterol oxidase/peroxidase method (fixed time). Triglycerides were measured by the lipase/glycerol kinase/glycerol phosphate oxidase/peroxidase method (fixed time). HDL cholesterol was measured after precipitation with dextran sulfate/Mg^{2+}. The LDL cholesterol concentration was estimated by using the Friedewald formula.[24]

Aortic Stiffness

The Sphygmocor system (PWV system, PWV Medical) was used to noninvasively measure stiffness of the aorta.[25] After the subject had rested for 5 to 10 minutes in supine position, aortic PWV was measured by sequentially recordings of arterial pressure waveform at the carotid artery and the femoral artery by using a hand-held micromanometer-tipped probe on the skin at the site of maximum arterial pulsation. Gating the recordings at those 2 sites to the ECG allowed the PWV to be measured. Recordings were taken when a reproducible signal was obtained with high-amplitude excursion, ie, usually 10 consecutive beats to cover the complete respiratory cycle. The wave transit time was calculated by the system software, with the R wave of a simultaneously recorded ECG used as a reference frame. Distances from the carotid-sampling site to the suprasternal notch and suprasternal notch to the femoral artery were measured with a compass.[26] The aortic PWV (m/s) was automatically calculated as the distance between the suprasternal notch and the femoral artery minus the distance between the carotid sampling site and the suprasternal notch, divided by the time interval between systolic R wave and femoral systolic upstroke minus the time interval between systolic R wave and carotid systolic upstroke. Aortic PWV was determined as the mean of at least 3 consecutive beats recorded during 10 seconds of data acquisition. A higher PWV indicates increased aortic stiffness. All measurements were performed by the same observer. A reproducibility study was performed among 27 participants, who underwent a second PWV measurement within 2 weeks after the first examination. The intraclass correlation coefficient was 89.6%, indicating that 89.6% of the variance in the PWV measurements was due to patient differences, whereas 10.4% could be attributed to differences between visits.

Data Analysis

Dietary intake of total phytoestrogens, isoflavones, and lignans was categorized into quartiles. The relationship of dietary intake of isoflavones and lignans with aortic PWV was determined by linear regression analysis. Aortic PWV was included in the model as a continuous variable (m/s). Data on aortic PWV were not available for 18 women. Data on dietary intake of isoflavones and lignans were not available for 3 women. Residual analysis was performed to identify possible outliers (defined as $\geq$3 SD remote from the mean). Three outliers were detected, of which it was very likely that errors in measurement of PWV were the cause. All together, 379 women remained for analysis.

Because dietary intake of phytoestrogens was calculated from the baseline FFQ of PROSPECT and because aortic PWV was assessed in the present study several years later, all analyses are adjusted for follow-up time. Other confounders that were considered in the analysis included age (y), BMI (kg/m^2), smoking (never, past, current), physical activity (Voorrips score), mean arterial pressure (mm Hg), time since menopause (short, long), energy intake (kJ/d), fiber intake (g/d), fruit intake (g/d), vegetable intake (g/d), alcohol intake (g/d), glucose level (mmol/L), and HDL cholesterol level (mmol/L).

Results were considered statistically significant at a 2-sided $P<0.05$. Statistical analyses were performed by using the program SPSS, version 9.0.

Results

General Characteristics

General characteristics of the total study population by quartiles of total phytoestrogen intake are presented in Table 2. The mean age of the total study group was 66.2 years (SD 3.8). The mean aortic PWV of all participants was 9.14 (SD 2.00). The dietary intakes of isoflavones and lignans of the total study population and of each postmenopausal time-span group separately are presented in Table 3. The median dietary intakes of isoflavones and lignans were 0.14 (interquartile range 0.09 to 0.24) and 0.63 mg/d (interquartile range 0.33 to 0.89 mg/d), respectively.

Linear Regression Analysis

In Table 4, adjusted regression coefficients, CIs, and probability values by quartile categories of isoflavones and lignans for all participants are given. The final regression models included follow-up time (mo), age (y), smoking (never, past, current), BMI (kg/m^2), physical activity (Voorrips score), mean arterial pressure (mm Hg), energy intake (kJ/d), dietary fiber intake (g/d), glucose level (mmol/L), and HDL cholesterol level (mmol/L). Other potential confounders did not materially change the regression coefficients. A high dietary intake of isoflavones was statistically significantly associated with decreased aortic stiffness (fourth quartile -0.52 [95% CI -1.00 to -0.04] compared with the first quartile). An increased dietary intake of lignans was also associated with decreased aortic stiffness (PWV in second quartile, -0.47 m/s [95% CI -0.92 to -0.02], PWV in third quartile, -0.78 m/s [95% CI -1.25 to -0.31], and PWV in fourth quartile, -0.42 m/s [95% CI -0.93 to 0.10] compared with the first quartile).

Subgroup Analysis

For women with a longer postmenopausal time span, a higher dietary intake of almost all phytoestrogen subgroups was associated with a less stiff aorta (Table 5). A dietary intake of isoflavones of $>$0.24 mg/d significantly decreased aortic PWV by 0.94 m/s (95% CI -1.65 to -0.23). Likewise, an increased dietary intake of lignans was associated with a less stiff aorta: with -0.71 m/s (95% CI -1.32 to -0.09), -0.75 m/s (95% CI -1.42 to -0.08), and -0.81 m/s (95% CI -1.54 to -0.07) for the second, third, and fourth quartiles, respectively, compared with the first quartile (Figures 1 and 2).

Discussion

Overall, we found that a higher regular intake of phytoestrogens within Western countries is associated with lower aortic stiffness among postmenopausal women, regardless of the low range of intake in this population.

Before we interpret our results, some issues concerning the measurement of aortic stiffness and the FFQ need to be addressed. In the present study, arterial stiffness was determined by measuring PWV. Observational studies have consistently shown that unfavorable levels of established cardiovascular risk factors are related to increased aortic stiffness.[27,28] Increased arterial stiffness has been related to atherosclerosis,[29,30] and recently, it has been shown to strongly predict cardiovascular events.[31–36]

Another issue that needs to be addressed concerns the usage of the FFQ in the present study. With the FFQ that we have used, we were able to quantify the usual dietary intake of phytoestrogens in the previous year. This is especially

TABLE 2. General Characteristics of the Study Population, by Quartiles of Total Phytoestrogen Intake (Mean±SD)

Characteristic	Quartile 1 N=94	Quartile 2 N=95	Quartile 3 N=95	Quartile 4 N=95
Age, y	67.3±3.7	66.0±3.8	65.6±3.9	65.9±3.6
Age at menopause, y	48.8±4.4	50.0±3.9	50.1±4.8	50.0±4.8
BMI, kg/m^2	25.9±4.1	27.1±4.1	25.5±3.8	25.3±4.3
Waist-Hip ratio	$0.8\pm7.0\times10^{-2}$	$0.8\pm7.4\times10^{-2}$	$0.8\pm6.6\times10^{-2}$	$0.8\pm6.5\times10^{-2}$
Smoking, pack-years	18.5±20.5	12.5±16.9	11.8±17.0	14.1±18.2
Physical activity, Voorrips score	11.4±6.6	13.9±7.7	14.7±6.5	14.2±7.1
Follow-up time, months	47.6±9.5	46.0±9.0	45.6±8.4	45.6±8.8
Glucose, mmol/L	5.29±1.24	5.26±1.06	5.28±8.36	5.15±1.19
Triglycerides, mmol/L	1.49±0.45	1.45±0.79	1.43±0.67	1.43±0.73
Cholesterol, mmol/L	6.40±1.06	6.29±0.96	6.22±1.08	6.34±1.06
HDL cholesterol, mmol/L	1.50±0.36	1.57±0.44	1.53±0.44	1.54±0.38
LDL cholesterol, mmol/L	4.22±1.02	4.06±1.01	4.05±0.95	4.14±1.04
LDL-HDL ratio	2.97±0.96	2.83±1.18	2.86±1.14	2.93±1.28
Oestradiol, pmol/L	22.75±16.41	19.23±11.65	19.94±12.43	19.08±12.37
Oestron, pmol/L	52.05±51.26	45.71±26.81	43.25±31.89	46.05±29.46
Pulse wave velocity, m/s	9.77±2.15	9.16±1.99	8.97±1.89	8.68±1.81
Systolic BP, mm Hg	151.1±22.7	145.2±19.4	147.4±21.5	147.1±19.2
Diastolic BP, mm Hg	76.5±15.0	74.5±12.9	77.0±14.5	75.6±11.7
Heart rate, bpm	69.9±11.7	68.8±8.5	69.4±8.5	68.4±10.5
Mean arterial pressure, mm Hg	101.4±16.4	98.1±14.1	100.5±15.7	99.4±13.0
Energy intake, kJ/d	6714±1551	7102±1716	7451±1303	8093±1686
Protein intake, g/d	61.8±14.4	67.7±16.1	71.4±12.9	76.2±20.1
Fat intake, g/d	66.6±19.6	67.1±21.8	69.1±19.2	71.7±21.2
Carbohydrate intake, g/d	176.2±49.0	189.2±51.9	201.8±43.1	230.6±47.0
Dietary fiber intake, g/d	18.3±5.2	21.9±4.7	24.1±3.4	28.1±6.0
Alcohol intake, g/d	7.1±11.4	8.7±12.4	8.7±11.7	7.6±10.5

BP indicates blood pressure.

important for a study on dietary intake of phytoestrogens, because food items containing high amounts of phytoestrogens are more likely to be consumed weekly or monthly and not on a daily basis. Dietary assessment methods, such as 24-hour dietary recall and food record methods, represent a relatively short period of intake. The underlying principle of the FFQ is that a long-term dietary pattern can be the conceptually important exposure rather than intake of a few days.[37] By using information in the literature, values for isoflavones and lignans were assigned for each food item in the FFQ. We decided to score the highest value reported in the literature into 7 categories (Table 1) and to use the scores instead of the exact measurements of phytoestrogen content reported in the literature. By using this method, we avoided the suggestion of a degree of precision for which the reported data in the literature are too limited and too preliminary. In addition, this system will decrease the degree of misclassification of our determinant of interest, dietary intake of phytoestrogens, considerably.

A further concern is residual confounding due to unmeasured or unknown factors. Increased phytoestrogen intake could be a marker of healthy lifestyle. To address this, we adjusted all analysis for BMI, smoking, and physical activity as most important lifestyle indicators. However, we cannot entirely exclude residual confounding by other unmeasured lifestyle indicators.

Furthermore, confounding due to other nutrient intakes is potentially important. However, the availability of extensive FFQ data on other nutrients enabled adjustment in the multivariate analyses. We adjusted for dietary fiber intake because dietary phytoestrogens are present in foods that contain fiber as well and because dietary fiber intake is

TABLE 3. Dietary Isoflavone and Lignan Intake of the Study Population

Phytoestrogen Intake, mg/d	Median (Interquartile Range)	Mean±SD Total Study Group	Mean±SD Group Short Postmenopausal Time Span	Mean±SD Group Long Postmenopausal Time Span
Isoflavones	0.14 (0.09–0.24)	0.57±1.97	0.65±2.35	0.49±1.46
Lignans	0.63 (0.33–0.87)	0.62±0.32	0.67±0.31	0.56±0.31*

*$P<0.05$, significant difference in mean dietary intake between postmenopausal time span groups.

TABLE 4. Results of the Linear Regression Analysis* for the Total Study Group

Phytoestrogen Intake, mg/d	Regression Coefficient	95% Confidence Interval	*P* Trend
Isoflavones			
≤0.09	Reference		
≤0.14	−0.33	−0.80, 0.14	
≤0.24	−0.22	−0.71, 0.27	
>0.24	−0.51	−1.00, −0.03	0.07
Lignans			
≤0.33	Reference		
≤0.63	−0.48	−0.94, −0.03	
≤0.87	−0.76	−1.24, −0.29	
>0.87	−0.41	−0.93, 0.11	0.06

*Adjusted for follow-up time (mo), age (y), smoking (never, past, current), BMI (kg/m^2), physical activity (Voorrips score), mean arterial pressure (mm Hg), energy intake (kJ/day), dietary fiber intake (g/d), glucose level (mmol/L), and HDL cholesterol (mmol/L).

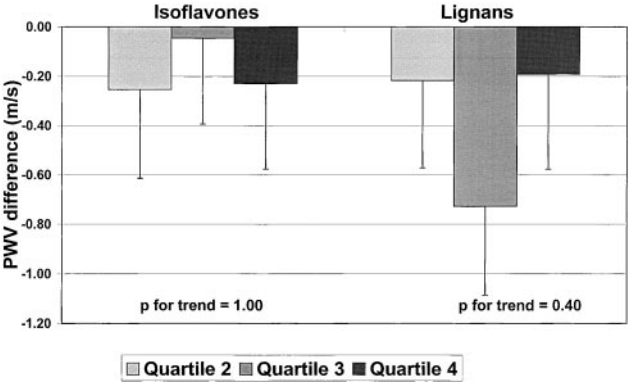

Figure 1. PWV difference (+SE) in m/s with first quartile for isoflavones and lignans in women with shorter menopausal time spans.

inversely associated with CHD.[38,39] A high dietary lignan intake was associated with a low aortic stiffness. A possible explanation could be that high lignan consumers eat more plant foods of lower energy, causing a lower BMI; therefore, they have a less stiff aorta. However, our results were adjusted for BMI and total energy intake. Additional adjustment for alcohol, fruit, and vegetable intake did not materially influence the results. However, even after proper adjustment for confounders, a causal interpretation of our findings is inherently restricted by the cross-sectional nature of the design.

Because phytoestrogens also exert an effect on lipid profiles,[9] it could be hypothesized that the effect on arterial walls that we found in the present study is reached through an effect on lipids.[40] However, in the present study, the lipid levels were not correlated with dietary intake of phytoestrogens, so a direct effect on arterial walls may be assumed.

We found that the associations between dietary intake of phytoestrogens and aortic stiffness were more pronounced in older women with longer postmenopausal time spans (20 to 30 years). Several explanations are posed. A first possible explanation for this finding is that women with shorter postmenopausal time spans still have higher plasma estradiol levels than women with longer postmenopausal time spans. Therefore, the effect of phytoestrogens is possibly overshadowed in the first group, and the latter group could have more estrogen receptors available. However, from further analysis in the present study, we found that estradiol and estrone levels do not differ significantly between the 2 postmenopausal time-span groups and are not related to age, time since menopause, or aortic stiffness. Additionally, no effect modification by endogenous estradiol was found (interaction between estradiol level and dietary intake of phytoestrogens was not significant; $P=0.68$). Endogenously produced estradiol through aromatization of estrone produced in peripheral fat tissue likewise did not modify our results, inasmuch as the interaction between BMI (kg/m^2) and dietary intake of phytoestrogens was not significant ($P=0.07$).

TABLE 5. Results of the Linear Regression Analysis* by Menopause Group

	Women With Short Postmenopausal Time Span (8–12 y), N=199				Women With Long Postmenopausal Time Span (20–30 y), N=180			
Phytoestrogen Intake, mg/d	N	Regression Coefficient	95% Confidence Interval	*P* Trend	N	Regression Coefficient	95% Confidence Interval	*P* Trend
Isoflavones								
≤0.09	47	Reference			47	Reference		
≤0.14	45	−0.25	−0.97, 0.46		50	−0.28	−0.93, 0.38	
≤0.24	53	−0.05	−0.73, 0.64		42	−0.34	−1.07, 0.40	
>0.24	54	−0.23	−0.92, 0.46	1.00	41	−0.94	−1.65, −0.22	0.02
Lignans								
≤0.33	36	Reference			58	Reference		
≤0.63	48	−0.22	−0.92, 0.48		47	−0.69	−1.31, −0.07	
≤0.87	56	−0.73	−1.43, −0.02		39	−0.74	−1.42, −0.06	
>0.87	59	−0.19	−0.95, 0.57	0.40	36	−0.80	−1.54, −0.05	0.03

*Adjusted for follow-up time (mo), age (y), smoking (never, past, current), BMI (kg/m^2), physical activity (Voorrips score), mean arterial pressure (mm Hg), energy intake (kJ/day), dietary fiber intake (g/d), glucose level (mmol/L), and HDL cholesterol (mmol/L).

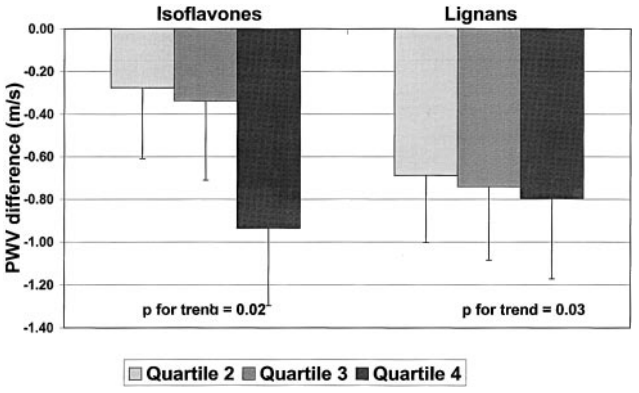

Figure 2. PWV difference (+SE) in m/s with first quartile for isoflavones and lignans in women with longer menopausal time spans.

Another possible explanation for the differences found in the effects of phytoestrogens between both postmenopausal time-span groups might be that the initial level of aortic PWV might have a powerful effect on changes in aortic PWV. A higher initial PWV may be lowered more strongly than a lower initial PWV. Because women with a longer postmenopausal time span are significantly older and have a significantly higher initial PWV, an effect of phytoestrogens on PWV is perhaps shown sooner than in women with a shorter postmenopausal time span, who are younger and have lower (and therefore better) initial aortic stiffness. In addition, information from Table 5 shows that, overall, a higher number of women with a longer postmenopausal time span have a lower intake and that a higher number of women with a shorter postmenopausal time span have a higher intake of dietary intake of phytoestrogens. All this may indicate that even when the dietary intake of phytoestrogens is low, an initial higher aortic stiffness can be decreased more strongly.

In conclusion, the present study found that phytoestrogens may have a protective effect on the risk of atherosclerosis and arterial degeneration through an effect on arterial walls, especially among older women.

References

1. Anderson JJB, Anthony M, Messina M, Garner SC. Effects of phytooestrogens on tissues. *Nutr Res Rev*. 1999;12:75–116.
2. Knight DC, Eden JA. Phytoestrogens: a short review. *Maturitas*. 1995; 22:167–175.
3. Miksicek RJ. Estrogenic flavonoids: structural requirements for biological activity. *Proc Soc Exp Biol Med*. 1995;208:44–50.
4. Kuiper GG, Lemmen JG, Carlsson B, Corton JC, Safe SH, van der Saag PT, van der BB, Gustafsson JA. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. *Endocrinology*. 1998;139: 4252–4263.
5. Anthony MS, Clarkson TB, Hughes CL Jr, Morgan TM, Burke GL. Soybean isoflavones improve cardiovascular risk factors without affecting the reproductive system of peripubertal rhesus monkeys. *J Nutr*. 1996;126:43–50.
6. Anthony MS, Clarkson TB, Bullock BC, Wagner JD. Soy protein versus soy phytoestrogens in the prevention of diet-induced coronary artery atherosclerosis of male cynomolgus monkeys. *Arterioscler Thromb Vasc Biol*. 1997;17:2524–2531.
7. Wagner JD, Cefalu WT, Anthony MS, Litwak KN, Zhang L, Clarkson TB. Dietary soy protein and estrogen replacement therapy improve cardiovascular risk factors and decrease aortic cholesteryl ester content in ovariectomized cynomolgus monkeys. *Metabolism*. 1997;46:698–705.
8. Clarkson TB, Anthony MS, Morgan TM. Inhibition of postmenopausal atherosclerosis progression: a comparison of the effects of conjugated equine estrogens and soy phytoestrogens. *J Clin Endocrinol Metab*. 2001;86:41–47.
9. Anderson JW, Johnstone BM, Cook Newell ME. Meta-analysis of the effects of soy protein intake on serum lipids. *N Engl J Med*. 1995;333: 276–282.
10. Tikkanen MJ, Wahala K, Ojala S, Vihma V, Adlercreutz H. Effect of soybean phytoestrogen intake on low density lipoprotein oxidation resistance. *Proc Natl Acad Sci U S A*. 1998;95:3106–3110.
11. Wiseman H, O'Reilly JD, Adlercreutz H, Mallet AI, Bowey EA, Rowland IR, Sanders TA. Isoflavone phytoestrogens consumed in soy decrease F(2)-isoprostane concentrations and increase resistance of low-density lipoprotein to oxidation in humans. *Am J Clin Nutr*. 2000;72:395–400.
12. Honore EK, Williams JK, Anthony MS, Clarkson TB. Soy isoflavones enhance coronary vascular reactivity in atherosclerotic female macaques. *Fertil Steril*. 1997;67:148–154.
13. Mishra SK, Abbot SE, Choudhury Z, Cheng M, Khatab N, Maycock NJ, Zavery A, Aaronson PI. Endothelium-dependent relaxation of rat aorta and main pulmonary artery by the phytoestrogens genistein and daidzein. *Cardiovasc Res*. 2000;46:539–546.
14. Nestel PJ, Yamashita T, Sasahara T, Pomeroy S, Dart A, Komesaroff P, Owen A, Abbey M. Soy isoflavones improve systemic arterial compliance but not plasma lipids in menopausal and perimenopausal women. *Arterioscler Thromb Vasc Biol*. 1997;17:3392–3398.
15. Nestel PJ, Pomeroy S, Kay S, Komesaroff P, Behrsing J, Cameron JD, West L. Isoflavones from red clover improve systemic arterial compliance but not plasma lipids in menopausal women. *J Clin Endocrinol Metab*. 1999;84:895–898.
16. Adlercreutz H, Honjo H, Higashi A, Fotsis T, Hamalainen E, Hasegawa T, Okada H. Urinary excretion of lignans and isoflavonoid phytoestrogens in Japanese men and women consuming a traditional Japanese diet. *Am J Clin Nutr*. 1991;54:1093–1100.
17. de Kleijn MJ, van der Schouw YT, Wilson PW, Adlercreutz H, Mazur W, Grobbee DE, Jacques PF. Intake of dietary phytoestrogens is low in postmenopausal women in the United States: the Framingham study. *J Nutr*. 2001;131:1826–1832.
18. Goodman-Gruen D, Kritz-Silverstein D. Usual dietary isoflavone intake is associated with cardiovascular disease risk factors in postmenopausal women. *J Nutr*. 2001;131:1202–1206.
19. de Kleijn MJ, van der Schouw YT, Wilson PW, Grobbee DE, Jacques PF. Dietary intake of phytoestrogens is associated with favorable metabolic cardiovascular risk profile in postmenopausal US women: the Framingham Study. *J Nutr*. 2002;132:276–282.
20. Riboli E. Nutrition and cancer: background and rationale of the European Prospective Investigation into Cancer and Nutrition (EPIC). *Ann Oncol*. 1992;3:783–791.
21. Ocke MC, Bueno-de-Mesquita HB, Goddijn HE, Jansen A, Pols MA, Van Staveren WA, Kromhout D. The Dutch EPIC food frequency questionnaire, I: description of the questionnaire, and relative validity and reproducibility for food groups. *Int J Epidemiol*. 1997;26(suppl 1):S37–S48.
22. Ocke MC, Bueno-de-Mesquita HB, Pols MA, Smit HA, Van Staveren WA, Kromhout D. The Dutch EPIC food frequency questionnaire, II: relative validity and reproducibility for nutrients. *Int J Epidemiol*. 1997; 26(suppl 1):S49–S58.
23. Voorrips LE, Ravelli AC, Dongelmans PC, Deurenberg P, Van Staveren WA. A physical activity questionnaire for the elderly. *Med Sci Sports Exerc*. 1991;23:974–979.
24. Ettinger B, Friedman GD, Bush T, Quesenberry CP Jr. Reduced mortality associated with long-term postmenopausal estrogen therapy. *Obstet Gynecol*. 1996;87:6–12.
25. Lehmann ED, Hopkins KD, Gosling RG. Assessment of arterial distensibility by automatic pulse wave velocity measurement. *Hypertension*. 1996;27:1188–1191.
26. O'Rourke MF, Gallagher DE. Pulse wave analysis. *J Hypertens*. 1996; 14(suppl):S147–S157.
27. Amar J, Ruidavets JB, Chamontin B, Drouet L, Ferrieres J. Arterial stiffness and cardiovascular risk factors in a population-based study. *J Hypertens*. 2001;19:381–387.
28. Mackey RH, Sutton-Tyrrell K, Vaitkevicius PV, Sakkinen PA, Lyles MF, Spurgeon HA, Lakatta EG, Kuller LH. Correlates of aortic stiffness in elderly individuals: a subgroup of the Cardiovascular Health Study. *Am J Hypertens*. 2002;15:16–23.

29. Farrar DJ, Bond MG, Sawyer JK, Green HD. Pulse wave velocity and morphological changes associated with early atherosclerosis progression in the aortas of cynomolgus monkeys. *Cardiovasc Res*. 1984;18:107–118.
30. Van Popele NM, Grobbee DE, Bots ML, Asmar R, Topouchian J, Reneman RS, Hoeks AP, van der Kuip DA, Hofman A, Witteman JC. Association between arterial stiffness and atherosclerosis: the Rotterdam Study. *Stroke*. 2001;32:454–460.
31. Blacher J, Guerin AP, Pannier B, Marchais SJ, Safar ME, London GM. Impact of aortic stiffness on survival in end-stage renal disease. *Circulation*. 1999;99:2434–2439.
32. Meaume S, Benetos A, Henry OF, Rudnichi A, Safar ME. Aortic pulse wave velocity predicts cardiovascular mortality in subjects >70 years of age. *Arterioscler Thromb Vasc Biol*. 2001;21:2046–2050.
33. Boutouyrie P, Tropeano AI, Asmar R, Gautier I, Benetos A, Lacolley P, Laurent S. Aortic stiffness is an independent predictor of primary coronary events in hypertensive patients: a longitudinal study. *Hypertension*. 2002;39:10–15.
34. Blacher J, Pannier B, Guerin AP, Marchais SJ, Safar ME, London GM. Carotid arterial stiffness as a predictor of cardiovascular and all-cause mortality in end-stage renal disease. *Hypertension*. 1998;32:570–574.
35. Guerin AP, Blacher J, Pannier B, Marchais SJ, Safar ME, London GM. Impact of aortic stiffness attenuation on survival of patients in end-stage renal failure. *Circulation*. 2001;103:987–992.
36. Laurent S, Boutouyrie P, Asmar R, Gautier I, Laloux B, Guize L, Ducimetiere P, Benetos A. Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. *Hypertension*. 2001;37:1236–1241.
37. Willett WC. *Nutritional Epidemiology*. New York, NY: Oxford University Press; 1998.
38. Wolk A, Manson JE, Stampfer MJ, Colditz GA, Hu FB, Speizer FE, Hennekens CH, Willett WC. Long-term intake of dietary fiber and decreased risk of coronary heart disease among women. *JAMA*. 1999; 281:1998–2004.
39. Todd S, Woodward M, Tunstall-Pedoe H, Bolton-Smith C. Dietary antioxidant vitamins and fiber in the etiology of cardiovascular disease and all-causes mortality: results from the Scottish Heart Health Study. *Am J Epidemiol*. 1999;150:1073–1080.
40. Consensus conference. Lowering blood cholesterol to prevent heart disease. *JAMA*. 1985;253:2080–2086.