# Prospective Study on Usual Dietary Phytoestrogen Intake and Cardiovascular Disease Risk in Western Women

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*Circulation* 2005;111;465-471
DOI: 10.1161/01.CIR.0000153814.87631.B0
Circulation is published by the American Heart Association. 7272 Greenville Avenue, Dallas, TX 72514
 Print ISSN: 0009-7322. Online ISSN: 1524-4539

# Prospective Study on Usual Dietary Phytoestrogen Intake and Cardiovascular Disease Risk in Western Women

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***Background***—Phytoestrogens have been suggested to lower cardiovascular disease risk, but existing research focused on non-Western high intake levels and on risk factors. We investigated whether habitual low phytoestrogen intake is associated with manifest cardiovascular disease risk.

***Methods and Results***—Between 1993 and 1997, 16 165 women 49 to 70 years old and free from cardiovascular disease were enrolled in the Dutch Prospect-EPIC cohort (European Prospective study Into Cancer and nutrition) and followed up for a median period of 75 months. At enrollment, women filled in questionnaires on chronic disease risk factors and nutrition. Intake of phytoestrogens was estimated using the food frequency questionnaire covering regular dietary intake of 178 food items in the year before enrollment. Cox regression analysis was used to estimate hazard ratios of cardiovascular disease for quartiles of phytoestrogen intake adjusted for age at intake, body mass index, smoking, physical activity, hypertension, hypercholesterolemia, use of hormone replacement therapy, menopausal status, and intake of total energy, total fiber, vegetables, fruit, and alcohol. In total, 372 women experienced a coronary event (CHD) (International Classification of Diseases, Ninth Revision, Clinical Modification [ICD-9], 410 to 414, 427.5) and 147 women a cerebrovascular event (CVD) (ICD-9, 430 to 438) during follow-up. Overall, neither isoflavones nor lignans were associated with decreased cardiovascular disease risk. When stratifying for ever versus never smokers, CHD risk decreased with increasing lignan intake for ever smokers.

***Conclusions***—Our results do not support the presence of a protective effect of higher intake of phytoestrogens in low doses on cardiovascular disease risk, although a small risk reduction with higher lignan intake cannot be excluded for smokers. **(*Circulation*. 2005;111:465-471.)**

**Key Words:** epidemiology ■ nutrition ■ women ■ coronary disease ■ cerebrovascular disorders

Phytoestrogens are plant-derived substances that are structurally comparable to 17$\beta$-estradiol and that may have estrogenic effects. The 2 main groups of phytoestrogens are isoflavones and lignans. The major isoflavones are genistein, daidzein, formononetin, and biochanin A. Colonic bacteria also produce the active metabolites enterolactone and enterodiol from the dietary lignans matairesinol and secoisolariciresinol. Genistein and daidzein bind to estrogen receptor-$\beta$ with high affinity and to estrogen receptor-$\alpha$ with lower affinity, but they have a high transactivational potency for both receptors,[1–3] whereas lignans show only minimal binding affinity.[4]

**See p 385**

In Western populations, beans and peas (45%), tea and coffee (25%), nuts (10%), and grains, rice, and cereals (5%) are the main sources of isoflavone intake. Fruits (25%), vegetables (20%), berries (15%), grains, rice, and cereals (10%), tea and coffee (10%), and nuts (10%) are the main sources of lignan intake.[5]

In some[6] but not all[7] metabolic studies, soy containing naturally occurring isoflavones have been found to exert lipid-lowering effects in humans.[6] Other favorable cardiovascular effects of soy or isoflavone supplementation have also been described, such as effects on vasodilatation and arterial compliance.[8,9]

Previous studies have concentrated on isoflavones, particularly those from soy. Moreover, they focused on intervention with doses comparable to intake levels in Asia, whereas the relevance of intake found in Western countries remains to be elucidated. In addition, the vast majority of studies reported effects on cardiovascular risk factors, notably lipid levels, or intermediate end points, whereas studies with clinical end points are still lacking.

The importance of lignans is not yet resolved, although they might be more important phytoestrogen sources than isoflavones in Western populations. Statistically significant risk reductions for myocardial infarction and overall cardiovascular mortality have been found in men with relatively

Received July 1, 2004; revision received October 28, 2004; accepted November 2, 2004.

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*Circulation* **is available at http://www.circulationaha.org** **DOI: 10.1161/01.CIR.0000153814.87631.B0**

high serum enterolactone levels.[10,11] In women, the incidence of cardiovascular events increases after the dramatic decline in endogenous estrogen levels after menopause. It could be hypothesized that in premenopausal women, estrogen receptors could be occupied with circulating estradiol to a larger extent than in postmenopausal women. As a consequence, postmenopausal women may potentially benefit more from higher phytoestrogen exposure.

To explore the relationship between dietary phytoestrogen intake and the incidence of cardiovascular disease, we studied a cohort of 16 165 women 49 to 70 years old at study entrance, who were followed up for a median period of 75 months (range, 0.3 to 102 months).

## Methods

### Population

Between 1993 and 1997, we recruited 17 357 women 49 to 70 years old among breast cancer screening participants in the PROSPECT-EPIC cohort, which is 1 of 2 Dutch contributions to the European Prospective Investigation into Cancer and Nutrition (EPIC).[12,13] We excluded 355 women who did not consent to linkage with vital status registries, 117 women with missing questionnaires, and 92 women who reported an energy intake of <500 kcal/d or >6000 kcal/d. Furthermore, 628 women reported a history of CHD (International Classification of Diseases, Ninth Revision, Clinical Modification [ICD-9], 410 to 414, 427.5) or cerebrovascular disease (ICD-9, 430 to 438) before the baseline measurements, and were therefore excluded from the analysis, leaving 16 165 women available for analysis.

### Baseline Measurements

At baseline, we collected information on demographic characteristics, presence of chronic diseases, and risk factors for chronic diseases, such as blood pressure, reproductive history, family history, smoking habits, alcohol intake, and physical activity. Height and weight were measured, and body mass index (BMI) was calculated as weight divided by height squared ($kg/m^2$). Smokers were categorized as current, past, or never smokers. Oral contraceptive (OC) use and postmenopausal hormone replacement therapy (HRT) use were defined as ever versus never. Hypertension, hypercholesterolemia, and diabetes mellitus were defined as present when women reported that these disorders had been diagnosed by a physician. For assessment of physical activity, we used a questionnaire previously validated in an elderly population.[14] For 1220 women, we could not calculate a total physical activity score because 1 or more questions on this questionnaire were missing. These missing total scores were imputed by means of linear regression modeling using SPSS version 10.0 (SPSS Inc). Such modeling predicts the value of a missing variable using all available data on the individual questions of the questionnaire and reduces bias because missing data may not occur at random.[15]

Levels of estradiol and testosterone were measured by use of the following commercially available double-antibody radioimmunoassay kits (Diagnostic System Laboratories Inc): estradiol, DSL-39100; testosterone, DSL-4100.

### Food Frequency Questionnaire

The validated food frequency questionnaire (FFQ) estimates the usual frequency of consumption of 79 main food items over the preceding 12 months.[16,17] Moreover, the FFQ comprises questions regarding nutritional habits, preparation methods, and additions. Color photographs of 28 dishes were used to estimate habitual portion sizes. Food consumption data were converted into macronutrients and micronutrients by use of an updated version of the computerized Dutch food composition table 1996. Overall, the questionnaire enables estimation of the average daily consumption of 178 food items. All nutrients were adjusted for total energy intake using the regression residual method.[18]

### Identifying Food Sources of Phytoestrogens

To locate published laboratory analysis data for the phytoestrogen contents of food items, we conducted a search of the medical (Medline) and agricultural (Agricola) scientific literature and contacted several experts in the field of phytoestrogens. We also searched the literature with the terms phytoestrogens, plant estrogens, isoflavones, lignans, enterolactone, and enterodiol.

### Scoring Phytoestrogen Intake

A detailed description of the scoring of phytoestrogen intake has been published previously.[19] Briefly, we calculated and assigned, for each food item in the FFQ, values for the isoflavones daidzein, genistein, formononetin, and biochanin A and for the lignans matairesinol and secoisolariciresinol. Each food item was then scored in 1 of 7 categories according to its phytoestrogen content. We multiplied the score of each food item by its daily consumption (in grams) and then summed across foods to get a total individual intake score for each phytoestrogen.

### Morbidity and Mortality Follow-Up

Data on morbidity were obtained from the Dutch Center for Health Care Information, which holds a standardized computerized register of hospital discharge diagnoses. Admission files are filed continuously from all general and university hospitals in the Netherlands since 1990. Whenever a patient is discharged from a hospital, data on sex, date of birth, dates of admission and discharge, 1 mandatory principal diagnosis, and up to 9 optional additional diagnoses are recorded. All diagnoses are coded according to the ICD-9. Follow-up was complete until January 1, 2002. The database was linked to the cohort on the basis of birth date, sex, postal code, and general practitioner with a validated probabilistic method.[20]

Information on vital status was obtained through linkage with the municipal administration registries. Causes of death were obtained from the women's general practitioners.

For our analysis, coronary events (CHD) (ICD-9, 410 to 414, 427.5) and cerebrovascular events (CVD) (ICD-9, 430 to 438), whichever came first, were the end points of interest. In total, 519 women were newly diagnosed with cardiovascular disease during follow-up, 372 with ischemic heart disease and 147 with cerebrovascular disease.

### Data Analyses

Because the distributions of phytoestrogens were skewed, medians and interquartile ranges are presented.

Mortality because of noncardiovascular causes, loss to follow-up because of moves outside the Netherlands, and withdrawn alive were considered censoring events. Cox regression analysis[21] was used to quantify the effect of phytoestrogen intake on total cardiovascular disease risk, coronary heart disease, and cerebrovascular disease, respectively. Phytoestrogen intake was analyzed in 4 quartiles, with the lowest quartile as the reference category, and separate analyses were performed for isoflavones and lignans. To study whether established cardiovascular risk factors or nutrition variables caused confounding, we entered them first individually and next simultaneously into the Cox model with phytoestrogen intake to see whether the crude hazard ratio (HR) of phytoestrogen intake changed substantially. The factors we considered were age at intake (continuously), BMI (continuously), smoking (current, past, never), physical activity (continuous Voorrips score), diabetes mellitus (yes/no), hypertension (yes/no), hypercholesterolemia (yes/no), OC use (ever/never), HRT use (ever/never), energy intake (continuously per 100 kcal), animal protein intake (continuously), monounsaturated fat intake (continuously), fiber intake (continuously), alcohol intake (0 to 4.9/5.0 to 14.9/15.0 to 29/≥30), fruit intake (continuously), and vegetable intake (continuously). The effects of phytoestrogen intake were investigated in the following predetermined subgroups of BMI (≤25 versus >25), menopausal status (postmenopausal versus pre-

**TABLE 1. Baseline Characteristics of the Study Population of 16 132 Women**

| | Mean | SD | N | % |
|---|---|---|---|---|
| Age at enrollment, y | 57.1 | 6.0 | | |
| Systolic blood pressure, mm Hg | 133.1 | 20.0 | | |
| Diastolic blood pressure, mm Hg | 79.3 | 10.4 | | |
| Body mass index, kg/m$^2$ | 26.0 | 4.1 | | |
| Total Voorrips score | 6.9 | 5.0 | | |
| Estradiol,* pg/mL | 15.5 | 27.5 | | |
| Total testosterone,* ng/mL | 0.29 | 0.15 | | |
| Hypertension | | | 3082 | 19.1 |
| Hypercholesteremia | | | 788 | 4.9 |
| Diabetes mellitus | | | 434 | 2.7 |
| Smoking | | | | |
| Current | | | 3548 | 21.9 |
| Past | | | 5567 | 34.4 |
| Never | | | 7046 | 43.6 |
| Menopausal status | | | | |
| Premenopausal | | | 1674 | 10.4 |
| Postmenopausal | | | 12 144 | 75.1 |
| Perimenopausal or unknown | | | 2347 | 14.5 |
| Ever use OC† | | | 10 431 | 64.5 |
| Ever use HRT‡ | | | 4169 | 25.8 |
| Dietary intake | | | | |
| Total energy intake, kcal/d | 1798 | 436 | | |
| Carbohydrate intake, g/d§ | 195 | 28 | | |
| Animal protein intake, g/d§ | 46.3 | 11.0 | | |
| Total fat intake, g/d§ | 68.9 | 10.4 | | |
| Monounsaturated fat intake, g/d§ | 25.5 | 4.6 | | |
| Total fiber intake, g/d§ | 22.2 | 4.3 | | |
| Total alcohol intake, g/d§ | 9.1 | 13.0 | | |
| Total fruit intake, g/d§ | 232 | 137 | | |
| Total vegetable intake, g/d§ | 143 | 53 | | |

*Assessed in random sample of n=1829 participants.
†OC indicates oral contraceptives.
‡HRT indicates hormonal replacement therapy.
§All nutrient intakes are energy-adjusted.

menopausal and perimenopausal), HRT use (never versus ever), age at intake (≤56 versus >56), smoking (current versus past versus never), and hypercholesterolemia (present/absent). To test the significance of the subgroup effects, interaction terms of phytoestrogen intake with the above-mentioned variables were added to the Cox model, respectively.

## Results

Baseline characteristics of the study population are shown in Table 1. Higher phytoestrogen consumption was related to a healthier lifestyle, indicated by lower BMI, lower smoking rate, higher physical activity score, lower fat intake, and lower prevalence of hypertension, hypercholesterolemia, and diabetes mellitus (data not shown).

The regular intake of individual phytoestrogens and phytoestrogen classes is shown in Table 2. Overall, intake was low, and intake of lignans was almost 3 times as high as isoflavone intake.

**TABLE 2. Usual Intake of Phytoestrogens (mg/d) in the Year Before Enrollment Among Middle-Aged and Elderly Women in Utrecht and Vicinity, Netherlands**

| Phytoestrogen | Median, mg/d | P25 | P75 |
|---|---|---|---|
| Isoflavones | | | |
| Daidzein | 0.134 | 0.094 | 0.191 |
| Genistein | 0.142 | 0.091 | 0.225 |
| Formononetin | 0.079 | 0.056 | 0.112 |
| Biochanin A | 0.001 | 0.001 | 0.002 |
| Lignans | | | |
| Matairesinol | 0.074 | 0.043 | 0.106 |
| Secoisolariciresinol | 0.992 | 0.681 | 1.288 |
| Phytoestrogen classes | | | |
| Total isoflavones* | 0.369 | 0.257 | 0.541 |
| Total dietary lignans† | 1.081 | 0.738 | 1.392 |
| Total phytoestrogens | 2.202 | 1.717 | 2.768 |

*Sum of daidzein, genistein, formononetin, and biochanin A.
†Sum of matairesinol and secoisolariciresinol

The number of cardiovascular events and total follow-up is presented per quartile of intake in Table 3.

Tables 4, 5, and 6 show the hazard ratios (HRs) and their 95% CIs for the higher quartiles of isoflavone and dietary lignan intake compared with the first quartile, adjusted for various classic cardiovascular risk factors in a multivariate model. When fully adjusted, there was no association between isoflavone intake or lignan intake and cardiovascular disease risk.

The findings were the same across different categories of BMI, menopausal status, HRT use, age at intake, and hypercholesterolemia. For smokers (past or current), the risk of coronary heart disease was lower with increasing lignan intake (HR of the fourth versus the first quartile of lignan intake, 0.63; 95% CI, 0.41 to 0.98), and for never smokers the risk was not influenced by lignan intake ($P$ for interaction, 0.01).

## Discussion

In this population of middle-aged and elderly women, we did not find an inverse association between phytoestrogen intake and cardiovascular disease risk after adjustment for potential confounders.

To the best of our knowledge, the present study is the first to analyze daily food intake of phytoestrogens in relation to manifest cardiovascular disease risk in women. However, to interpret the results, some issues need to be addressed. Although our FFQ was not specifically designed to capture intake of phytoestrogens, it included detailed data on almost all habitually consumed bread, cereals, and vegetable and fruit items, which are the food groups most likely to contain phytoestrogens in Western diets. The relative validity for the estimate of vegetable intake, the most important source of isoflavones in this population, was moderate (Spearman rank correlation coefficient [$\rho$] of 0.4 comparing the FFQ with 12- to 24-hour recalls), but for bread and cereals, other important contributors, it was rather high ($\rho$=0.8 and 0.7, respectively.[17] Soy foods and flaxseed, the richest sources of isofla-

**TABLE 3. Data on Follow-Up Time and Number of Cardiovascular Events per Quartile of Total Phytoestrogen Intake**

| | First Quartile | Second Quartile | Third Quartile | Fourth Quartile |
|---|---|---|---|---|
| Isoflavones | | | | |
| Subjects | 4040 | 4041 | 4041 | 4040 |
| Total follow-up time, mo | 304 169 | 302 196 | 302 352 | 298 206 |
| Average follow-up time, mo | 75.3 | 74.8 | 74.8 | 73.8 |
| CHD cases | 95 | 99 | 103 | 74 |
| CVD cases | 42 | 34 | 38 | 33 |
| Total cardiovascular cases | 137 | 133 | 141 | 107 |
| Lignans | | | | |
| Subjects | 4041 | 4041 | 4042 | 4041 |
| Total follow-up time, mo | 303 298 | 301 021 | 301 594 | 301 199 |
| Average follow-up time, mo | 75.1 | 74.5 | 74.6 | 74.5 |
| CHD cases | 125 | 70 | 82 | 95 |
| CVD cases | 47 | 40 | 31 | 29 |
| Total cardiovascular cases | 172 | 110 | 113 | 124 |

vones and lignans, are uncommon foods in Western populations; however, the FFQ did include a question on soy. Conversely, the FFQ included detailed data on almost all habitually consumed vegetable and fruit items, which are the food groups most likely to contain phytoestrogens in Western diets. The industrial use of soy meal was not accounted for and could have caused the presence of phytoestrogens in certain food items (ie, donuts and white bread), although the processing of soy meal possibly reduces the amounts of phytoestrogens in these products. However, error in exposure measurement produced by missing data on some of the food items consumed in the Western diet cannot be entirely excluded.

By using an FFQ, we were able to quantify the average exposure to dietary phytoestrogens in the year preceding study enrollment. This is particularly important for a study of dietary phytoestrogen intake in Western populations, because the foods contributing to a high intake of phytoestrogen are most likely to be consumed weekly or monthly, not on a daily basis. Furthermore, our FFQ covers a 1-year period of food intake, thereby reducing biases caused by seasonal variation in intake. The fact that our FFQ reflects habitual and long-term intakes makes it superior to biochemical indicators, such as urinary excretion, which are often used to measure phytoestrogen exposure, because such biomarkers reflect only a short-term intake (24 hours),[22] which may be less relevant to risk-reducing effects.

We used Dutch food intake data combined with phytoestrogen content data from elsewhere, mostly the United

**TABLE 4. Hazard Ratios for Coronary Cardiovascular Disease Risk by Quartiles of Isoflavone Intake and Lignan Intake**

| | Hazard Ratio (95% CI) | | | |
|---|---|---|---|---|
| Variable in the Model | First Quartile | Second Quartile | Third Quartile | Fourth Quartile |
| Isoflavones | | | | |
| Unadjusted | 1 | 1.06 (0.80–1.41) | 1.12 (0.84–1.48) | 0.81 (0.60–1.10) |
| Basic model* | 1 | 1.17 (0.88–1.55) | 1.22 (0.92–1.62) | 0.98 (0.72–1.33) |
| Fully adjusted model† | 1 | 1.14 (0.85–1.53) | 1.15 (0.86–1.55) | 0.94 (0.68–1.30) |
| Lignans | | | | |
| Unadjusted | 1 | 0.57 (0.43–0.77) | 0.66 (0.50–0.88) | 0.78 (0.60–1.02) |
| Basic model* | 1 | 0.65 (0.48–0.87) | 0.74 (0.56–0.98) | 0.90 (0.69–1.18) |
| Fully adjusted model† | 1 | 0.69 (0.51–0.94) | 0.75 (0.55–1.01) | 0.92 (0.65–1.29) |

*Adjusted for age at intake (continuously), BMI (continuously), smoking (current, past, never), physical activity (continuous Voorrips score), diabetes mellitus (yes/no), hypertension (yes/no), hypercholesterolemia (yes/no), OC use (ever/never), HRT use (ever/never), and menopausal status (pre/peri/post).

†Adjusted for age at intake (continuously), BMI (continuously), smoking (current, past, never), physical activity (continuous Voorrips score), diabetes mellitus (yes/no), hypertension (yes/no), hypercholesterolemia (yes/no), OC use (ever/never), HRT use (ever/never), energy intake (continuously per 100 kcal), animal protein intake (continuously), monounsaturated fat intake (continuously), fiber intake (continuously), alcohol intake (0–4.9/5.0–14.9/15.0–29/≥30), fruit intake (continuously), and vegetable intake (continuously).

**TABLE 5. Hazard Ratios for Cerebrovascular Disease Risk by Quartiles of Isoflavone Intake and Lignan Intake**

| | Hazard Ratio (95% CI) | | | |
|---|---|---|---|---|
| Variable in the Model | First Quartile | Second Quartile | Third Quartile | Fourth Quartile |
| Isoflavones | | | | |
| Unadjusted | 1 | 0.81 (0.52–1.28) | 0.91 (0.59–1.41) | 0.80 (0.51–1.26) |
| Basic model* | 1 | 0.91 (0.58–1.43) | 1.02 (0.66–1.58) | 1.00 (0.63–1.59) |
| Fully adjusted model† | 1 | 0.96 (0.60–1.52) | 1.09 (0.68–1.73) | 1.05 (0.64–1.70) |
| Lignans | | | | |
| Unadjusted | 1 | 0.86 (0.56–1.31) | 0.66 (0.42–1.04) | 0.62 (0.39–0.99) |
| Basic model* | 1 | 1.02 (0.67–1.56) | 0.78 (0.49–1.23) | 0.76 (0.47–1.21) |
| Fully adjusted model† | 1 | 1.02 (0.66–1.59) | 0.80 (0.49–1.31) | 0.80 (0.45–1.42) |

*Adjusted for age at intake (continuously), BMI (continuously), smoking (current, past, never), physical activity (continuous Voorrips score), diabetes mellitus (yes/no), hypertension (yes/no), hypercholesterolemia (yes/no), OC use (ever/never), HRT use (ever/never), and menopausal status (pre/peri/post).

†Adjusted for age at intake (continuously), BMI (continuously), smoking (current, past, never), physical activity (continuous Voorrips score), diabetes mellitus (yes/no), hypertension (yes/no), hypercholesterolemia (yes/no), OC use (ever/never), HRT use (ever/never), energy intake (continuously per 100 kcal), animal protein intake (continuously), monounsaturated fat intake (continuously), fiber intake (continuously), alcohol intake (0–4.9/5.0–14.9/15.0–29/≥30), fruit intake (continuously), and vegetable intake (continuously).

Kingdom, United States, and Finland. Differences in phytoestrogen content of food items between types, brands, or different countries are unknown, because most measurements were performed in a few countries using only a few types or brands. However, by using categories instead of exact amounts of phytoestrogen content, these differences should not influence our results, provided that they are within a 10-fold range of the data we used for our classification. Moreover, we divided the cohort into quartile categories of intake and did not use continuous data on phytoestrogen intake. This fact further reduces the influence of error on the measured data but may also reduce the power to detect an association.

The lower number of clinical events in the fourth quartile of isoflavones suggests benefit at the highest (albeit still very low) intakes. Because isoflavones are measured with some degree of error, as are many of the covariates, we cannot exclude the possibility that adjustment for many potential

**TABLE 6. Hazard Ratios of Isoflavone Intake or Lignan Intake and Total Cardiovascular Disease Risk**

| | Hazard Ratio (95% CI) | | | |
|---|---|---|---|---|
| Variable in the Model | First Quartile | Second Quartile | Third Quartile | Fourth Quartile |
| Isoflavones | | | | |
| Unadjusted | 1 | 0.98 (0.77–1.25) | 1.05 (0.83–1.33) | 0.81 (0.63–1.04) |
| Basic model* | 1 | 1.09 (0.85–1.38) | 1.16 (0.91–1.47) | 0.98 (0.76–1.27) |
| Fully adjusted model† | 1 | 1.08 (0.85–1.39) | 1.13 (0.88–1.45) | 0.97 (0.74–1.27) |
| Lignans | | | | |
| Unadjusted | 1 | 0.65 (0.51–0.83) | 0.66 (0.52–0.84) | 0.74 (0.58–0.93) |
| Basic model* | 1 | 0.75 (0.59–0.95) | 0.75 (0.59–0.96) | 0.86 (0.68–1.09) |
| Fully adjusted model† | 1 | 0.78 (0.61–1.01) | 0.76 (0.59–0.99) | 0.89 (0.66–1.19) |

*Adjusted for age at intake (continuously), BMI (continuously), smoking (current, past, never), physical activity (continuous Voorrips score), diabetes mellitus (yes/no), hypertension (yes/no), hypercholesterolemia (yes/no), OC use (ever/never), HRT use (ever/never), and menopausal status (pre/peri/post).

†Adjusted for age at intake (continuously), BMI (continuously), smoking (current, past, never), physical activity (continuous Voorrips score), diabetes mellitus (yes/no), hypertension (yes/no), hypercholesterolemia (yes/no), OC use (ever/never), HRT use (ever/never), energy intake (continuously per 100 kcal), animal protein intake (continuously), monounsaturated fat intake (continuously), fiber intake (continuously), alcohol intake (0–4.9/5.0–14.9/15.0–29/≥30), fruit intake (continuously), and vegetable intake (continuously).

confounders may have masked a modest association between isoflavones or lignans and cardiovascular risk.

Until now, only a few research groups have quantified the dietary intake of phytoestrogens, notably to study the association between dietary intake of phytoestrogens and prostate cancer[23] and breast and thyroid cancer.[24,25] Although both groups used different food questionnaires and food composition databases than ours, the median dietary intake of different phytoestrogens reported in their results was similar to the intake reported in our study.[23,26]

Although we were not able to demonstrate protective effects of increased phytoestrogen intake on cardiovascular disease risk, there are several plausible mechanisms to expect such effects. The majority of intervention studies with soy protein, containing isoflavones, have shown favorable effects on lipid profile, as summarized in a meta-analysis published in 1995.[6] However, intake in most intervention studies is at levels comparable to isoflavone intake in Asian societies, 50 to 100 mg/d.[27] At lower intake levels, as typically found in Western societies, we have shown previously that greater phytoestrogen intake in women of comparable age in the Framingham Study was associated with a favorable metabolic cardiovascular profile.[28] Although a different FFQ was used for this study, intake of phytoestrogens was similar in Framingham and in PROSPECT-EPIC.[19] In a sample of the PROSPECT-EPIC population, we have shown that increased phytoestrogen intake is associated with decreased stiffness of the aorta.[29] This suggests that at low levels, phytoestrogens are able to exert effects on risk factors and intermediate measures of vascular disease. Our study is the first prospective study of the association between low levels of habitual intake of phytoestrogens and cardiovascular disease risk. It is possible that beneficial effects on metabolic risk factors and vascular stiffness do not translate into beneficial effects on clinical outcomes in the presence of other, more powerful risk factors for cardiovascular disease. This leaves the possibility for higher doses of phytoestrogens to exert effects. This deserves further research, in particular in prospective studies, and also in populations with higher levels of intake and randomized trials with clinically manifest end points.

One study has been published with data on lignans in a Western diet in relation to acute myocardial infarction risk in men[10] and cardiovascular mortality.[11] In this study, the risk of acute myocardial infarction was significantly lower in the fourth versus the first quartile, with an odds ratio (OR) of 0.35 (95% CI, 0.14 to 0.88). However, no adjustments were made for important confounders, such as physical activity and alcohol and fiber intake. In the second part of that study, which linked enterolactone levels to lower coronary heart disease–related mortality with an OR of 0.44 (95% CI, 0.20 to 0.96) and to lower total cardiovascular mortality (OR, 0.55; 95% CI, 0.29 to 1.01), the investigators did adjust for fiber and alcohol intake but not for physical activity. Other methodological issues limit the interpretation of these findings.

The cardiovascular protective mechanism of lignans may be through lowering blood lipid levels, because recent randomized placebo-controlled trials with flaxseed supplementation, a rich source of lignan, have shown beneficial effects.[30,31]

It could be hypothesized that in premenopausal women, estrogen receptors are occupied by endogenous estrogens and are not available for dietary isoflavones. The same could be argued for women with higher BMI, who convert testosterone to estradiol in peripheral fatty tissue, and for women who use HRT. Consequently, more pronounced effects should be expected in postmenopausal women. However, when we restricted our population to either postmenopausal women, women with BMI $\leq$25 kg/m$^2$, or women who have never used HRT, we did not find an inverse association between isoflavone or lignan intake and cardiovascular disease risk. We did observe an inverse association between high lignan intake and coronary heart disease in past or current smokers. Although a chance finding cannot be ruled out, it could also be hypothesized that because lignans are powerful antioxidants,[4,31,4,29] they protect women from the oxidative damage caused by smoking.

In conclusion, the results of this large prospective study among Dutch women do not support the presence of overall protective effects of dietary intake of isoflavones or lignans at habitually low levels on the occurrence of manifest cardiovascular disease risk. A small risk reduction with higher lignan intake cannot be excluded for smokers. Beneficial effects of higher levels of intake, as common in Asia, cannot be excluded either.

## Acknowledgments

This study was supported by grant 2100.0027 from Zorg Onderzoek Nederland and the Europe Against Cancer Program of the European Commission (SANCO). The skillful help of J.J.M.M. Drijvers, dietitian, and J.H. den Breeijen, MSc, with calculation of phytoestrogen intake is gratefully acknowledged.

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