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Association between Urinary Phytoestrogens and C-reactive Protein in the Continuous National Health and Nutrition Examination Survey

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ABSTRACT

Objective: A reduced risk of some cancers and cardiovascular disease associated with phytoestrogen intake may be mediated through its effect on serum C-reactive protein (CRP; an inflammation biomarker). Therefore, this study examined the associations between urinary phytoestrogens and serum CRP.

Methods: Urinary phytoestrogen and serum CRP data obtained from 6009 participants aged ≥ 40 years in the continuous National Health and Nutrition Examination Survey during 1999–2010 were analyzed.

Results: After adjustment for confounders, urinary concentrations of total and all individual phytoestrogens were inversely associated with serum concentrations of CRP (all $p < 0.004$). The largest reductions in serum CRP (mg/L) per interquartile range increase in urinary phytoestrogens (ng/mL) were observed for total phytoestrogens ($\beta = -0.18$; 95% confidence interval [CI], −0.22, −0.15), total lignan ($\beta = -0.15$; 95% CI, −0.18, −0.12), and enterolactone ($\beta = -0.15$; 95% CI, −0.19, −0.12). A decreased risk of having high CRP concentrations (≥3.0 mg/L) for quartile 4 vs quartile 1 was also found for total phytoestrogens (OR = 0.63; 95% CI, 0.53, 0.73), total lignan (OR = 0.64; 95% CI, 0.54, 0.75), and enterolactone (OR = 0.59; 95% CI, 0.51, 0.69).

Conclusion: Urinary total and individual phytoestrogens were significantly inversely associated with serum CRP in a nationally representative sample of the U.S. population.

Abbreviations: BMI, body mass index; CDC, Centers for Disease Control and Prevention; CI, confidence interval; CRP, C-reactive protein; HPLC, high-performance liquid chromatography; IQR, interquartile range; mg/L, milligrams per liter; MS/MS, tandem mass spectrometry; NCHS, National Center for Health Statistics; ng/mL, nanograms per milliliter; NHANES, National Health and Nutrition Examination Survey; OR, odds ratio; STAT3, signal transduces activator of transcription 3; TNF-α, tumor necrosis factor alpha

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Introduction

C-reactive protein (CRP) is an acute-phase reactant and its plasma concentrations rise rapidly in a cytokine-mediated response to tissue injury, infection, or inflammation [1]. In previous studies, CRP levels have been positively associated with the risk of developing or dying from cancer [2,3] and cardiovascular disease [4,5]. Inflammation has been linked to carcinogenesis [6]. Experimental studies have shown that an increased risk of some cancers associated with certain inflammatory diseases was likely mediated through the inhibition of apoptosis [7], prolonged activation of signal transducers, activator of transcription 3 (STAT3) [8], and the deactivation of tumor necrosis factor alpha (TNF-α) [9]. As for the potential mechanisms by which CRP is involved in cardiovascular risk, *in vitro* and animal studies have revealed that CRP may actively participate in plaque development through inducing monocyte adhesion to the endothelium [10] and promoting macrophage cholesterol accumulation [11]. Therefore, it is possible that reducing serum CRP levels may lower the risk of cancer and cardiovascular disease.

Phytoestrogens are a group of botanical bioactive compounds that are structurally similar to estrogen [12]. The biological effects of phytoestrogens, observed in experimental studies, are in part ascribed to the competition of these compounds with endogenous estrogen for binding to estrogen receptors [13,14]. There are 2 principal classes of phytoestrogens, isoflavones (genistein and daidzein) and lignans (pinoresinol and lariciresinol). The richest dietary source of isoflavones is soy products, kudzu root, and American groundnuts [15,16], whereas dietary lignans are primarily obtained from flax seed, green tea, and strawberries [17]. Both isoflavones and lignans are metabolized by gut bacteria to form their derivative compounds (equol and O-desmethylangolensin for isoflavones and enterodiol and enterolactone for lignans) [18]. A growing number of studies have shown that urinary concentrations of phytoestrogens are reliable, objective biomarkers of their dietary intakes [19,20].

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Intake of total and individual phytoestrogens and their biomarkers have been associated with a reduced risk of several cancers, cardiovascular disease, and other health conditions in some epidemiologic studies [21–24]. However, the biological mechanisms underlying these associations remain elusive. Epidemiologic studies evaluating the effect of dietary phytoestrogens on CRP levels have yielded inconsistent results, with both the CRP-lowering [25] and null [26] effects reported. It should be noted that most of those previous studies were conducted among specific groups of subjects (e.g., postmenopausal women). To date, little is known about the associations between dietary phytoestrogens and CRP levels in a large sample of the general population. Therefore, the present study was conducted to investigate this research question using data on urinary phytoestrogens and serum CRP, previously collected from the continuous National Health and Nutrition Examination Survey (NHANES) [27].

Subjects and methods

Study population

Data obtained from the NHANES for the years 1999–2010 were analyzed in this study. The data in this period of time were selected because 2010 is the most recent year for which data on urinary phytoestrogens were available at the time of the study. NHANES is an annual cross-sectional study initiated in 1999 by the Centers for Disease Control and Prevention to assess the health and nutritional status of the general U.S. population. Data collection and sampling procedures for NHANES have been described in detail elsewhere [28].

A total of 62,160 subjects enrolled in NHANES in 1999–2010 completed the personal interview and health examination. The subjects included in the statistical analysis were confined to those who were 40 years of age or older primarily because the present study was intended to offer insight on whether dietary intake of phytoestrogens alters the risk of cancer and cardiovascular diseases in part through their influence on inflammatory process. In addition, these 2 leading causes of death are not common among subjects younger than 40 years old [29]. Urinary phytoestrogens and serum CRP were measured among a subset of all participants to reduce participant burden and facilitate the scheduling of the interview and completion of the health examination. All subjects in the subsample were randomly selected from the pool of total participants to obtain a nationally representative sample, with subsample weights calculated to account for probability of being selected into the subsample and additional nonresponse [27]. Excluding subjects who were less than 40 years old and those who did not have data on urinary phytoestrogens and serum CRP resulted in 6009 subjects remaining for data analysis.

Approval of the present study by the Institutional Review Board of Indiana University was not applicable because the data analyzed are deidentified and available in the public domain.

Questionnaire data collection

NHANES participants were interviewed to collect data on demographic characteristics and lifestyle factors. Demographic variables relevant to this study included age, sex, race (non-Hispanic white, non-Hispanic black, and other race including multiracial), marital status (married or living with partner, widowed, divorced or separated, and never married), and education level (less than high school, high school graduate or equivalent, and more than high school). Lifestyle variables considered in this study were smoking status (never smokers [smoking 0 or <100 cigarettes in lifetime], former smokers [smoking ≥ 100 cigarettes in lifetime but not currently smoking], and current smoker), alcohol consumption (0, ≤ 1, and >1 drink/week), and dietary intake of energy and nutrients. The dietary intake of the subjects was assessed using a 24-hour dietary recall. Alcohol intake was determined with a comprehensive survey questionnaire. Body mass index (BMI; kg/m^2) was calculated from body height and weight measured during the health examination.

Laboratory measurements

Urinary phytoestrogens

Urinary concentrations of isoflavones (daidzein, genistein, equol, and O-desmethylangolensin) and lignans (enterodiol and enterolactone) were measured using high-performance liquid chromatography with tandem mass spectrometric detection [30]. The methods for the collection and analysis of urine samples for phytoestrogen concentrations have been described in detail elsewhere [31]. Briefly, spot urine specimens were collected at the mobile examination centers the morning after a recommended fast, processed, stored at -20°C and then shipped to the Division of Environmental Health Laboratory Sciences at the National Center for Health Statistics for analysis. Urine samples were amended with stable isotope-labeled internal standards to improve method accuracy and precision, incubated with a deconjugation enzyme to allow the quantification of individual phytoestrogens, extracted using solid-phase extraction to remove interferences and improve sensitivity, and then analyzed using negative ion mode electrospray ionization high-performance liquid chromatography with tandem mass spectrometric detection, an assay with a high degree of specificity for each of the analytes considered [31].

Serum CRP

The methods for the collection and analysis of blood samples for serum CRP have been described in detail elsewhere [32]. Briefly, blood samples were obtained from the subjects via venipuncture, processed, stored at lower than -20°C, and then shipped to the University of Washington in Seattle where serum CRP was quantified by latex-enhanced nephelometry [32].

Statistical analysis

Sample weights were applied to the data through the calculation of a 12-year weight variable according to the National Center for Health Statistics guidelines for combining 2 or more 2-year cycles of the continuous NHANES data to produce unbiased national estimates. Urinary excretion of total phytoestrogens was calculated by summing the individual phytoestrogens for both total isoflavones and total lignans. Demographic,

anthropometric, and lifestyle characteristics of subjects as well as their urinary concentrations of total and individual phytoestrogen were compared by the quartiles of serum CRP. Chi-square tests and analysis of variance were employed to compare differences in categorical and continuous variables across CRP quartiles, respectively. Because urinary phytoestrogens are continuous variables, their differences among subjects in different CRP quartiles were examined by analysis of variance.

Urinary phytoestrogens and serum CRP were log-transformed to improve the normality of their distributions before data analysis. As expected, the log-transformation resulted in a substantial improvement in the normality of all those variables. Linear regression was performed to determine the associations between total and individual phytoestrogens and CRP. Partial regression coefficients were estimated for changes in serum CRP (mg/L) per an interquartile range increase in urinary phytoestrogen (ng/mL). The interquartile ranges were 1.63, 2.09, 2.31, 2.38, 1.77, 3.36, 1.89, 1.94, and 2.19 ng/mL for total phytoestrogen, isoflavone, genistein, daidzein, equol, O-desmethylangolensin, total lignin, enterodiol, and enterolactone, respectively. Logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (CIs) for having serum CRP $\geq$ 3.0 mg/L in relation to urinary total and individual phytoestrogens. In the logistic regression, subjects were divided into quartiles, with those in the lowest quartile of total or each individual phytoestrogen serving as the reference group. The variables adjusted in the multivariable models of both the linear and logistic regressions were age, gender, race, education, BMI, smoking status, alcohol intake, and urinary creatinine. Urinary creatinine was included to control for variation in dilution effects derived from spot urine samples [20]. For both linear and logistic regressions, 3 models for the associations between urinary phytoestrogens and serum CRP were constructed: model 1 adjusted for creatinine level; model 2 additionally adjusted for age, gender, and race; and model 3 additionally adjusted for education, BMI, smoking status, and alcohol intake. The potential interactions of age, gender, BMI, education, smoking status, total energy intake, and sodium intake with urinary phytoestrogens in relation to serum CRP were tested and found to be not statistically significant. Marital status and intake of alcohol, total energy, sodium, fat, and calcium were examined as potential confounders but not included in the final models because they were not statistically significant in the model or did not substantively alter risk estimates (<10%). A 2-sided p value < 0.05 was considered statistically significant. SPSS Version 23 (IBM, Armonk, NY) was used for all statistical analyses.

Results

Characteristics of study subjects by the quartiles of serum CRP levels are shown in Table 1. Subjects in the highest quartile were more likely to be older, female, non-Hispanic black, obese, less educated, current smokers, and nondrinkers than those in

Table 1. Characteristics of subjects by quartiles of serum C-reactive protein (mg/L) in the continuous national health and nutrition examination survey, 1999–2010.

	Serum C-reactive protein (mg/L)				
Characteristics	Quartile 1 0.1–0.8 $n = 1801$	Quartile 2 0.9–2.1 $n = 1847$	Quartile 3 2.2–4.9 $n = 1777$	Quartile 4 5.0–296.0 $n = 1763$	p value
Age, mean (SD)	54.9 (11.9)	57.3 (12.2)	57.7 (12.2)	56.8 (12.0)	<0.0001
Gender (%)					
Male	52.6	52.1	48.7	36.8	<0.0001
Female	47.4	47.9	51.3	63.2	
Race/ethnicity (%)					
Non-Hispanic white	77.8	76.8	75.2	73.5	<0.0001
Non-Hispanic black	7.4	8.5	9.0	13.7	
Other	14.8	14.7	15.8	12.8	
BMI, mean (SD)	25.5 (4.4)	27.9 (5.1)	29.7 (5.7)	32.4 (7.8)	<0.0001
Education (%)					
Less than high school	15.5	17.8	22.2	23.6	<0.0001
High school graduate or equivalent	25.7	24.6	27.1	26.7	
More than high school	58.8	57.6	50.7	49.8	
Smoking status (%)					
Never smoker	51.2	50.9	48.5	46.1	<0.0001
Former smoker	32.4	32.4	29.7	28.9	
Current smoker	16.4	16.8	21.8	25.0	
Alcohol intake (%)					
0 drinks/week	28.7	34.9	37.3	41.3	<0.0001
≤1 drink per week	37.2	35.6	36.1	37.3	
>1 drink per week	34.1	29.6	26.6	21.4	
Urinary phytoestrogens (ng/mL), mean (SD)					
Total phytoestrogens	2266 (6223)	1613 (3010)	1550 (3666)	1243 (4159)	<0.0001
Total isoflavones	909 (3437)	640 (2153)	562 (2324)	488 (1602)	<0.0001
Genistein	227 (966)	167 (720)	135 (615)	127 (407)	<0.0001
Daidzein	482 (2029)	339 (1268)	296 (1336)	254 (824)	<0.0001
Equol	80 (717)	51 (392)	54 (446)	49 (407)	0.24
O-desmethylangolensin	124 (695)	86 (408)	81 (510)	61 (463)	0.004
Total lignans	1358 (4145)	973 (1912)	987 (2689)	756 (2166)	<0.0001
Enterodiol	161 (603)	120 (336)	174 (1087)	188 (1800)	0.27
Enterolactone	1197 (3864)	854 (1730)	813 (1967)	568 (1057)	<0.0001

BMI = body mass index.

Table 2. Multiple regression analysis of serum C-reactive protein on urinary excretion of total and individual phytoestrogens among 6009 subjects in the continuous national health and nutrition examination survey, 1999–2010[a].

	Serum C-reactive protein (mg/L)		
Urinary phytoestrogen (ng/mL)	Model 1[b]	Model 2[c]	Model 3[d]
Total phytoestrogen			
β (95% CI)	−0.28 (−0.31, −0.24)	−0.31 (−0.35, −0.28)	−0.18 (−0.22, −0.15)
p value	<0.0001	<0.0001	<0.0001
Total isoflavone			
β (95% CI)	−0.14 (−0.18, −0.11)	−0.16 (−0.20, −0.13)	−0.11 (−0.14, −0.07)
p value	<0.0001	<0.0001	<0.0001
Genistein			
β (95% CI)	−0.11 (−0.15, −0.07)	−0.13 (−0.17, −0.09)	−0.08 (−0.12, −0.05)
p value	<0.0001	<0.0001	<0.0001
Daidzein			
β (95% CI)	−0.13 (−0.17, −0.09)	−0.14 (−0.18, −0.10)	−0.10 (−0.14, −0.07)
p value	<0.0001	<0.0001	<0.0001
Equol			
β (95% CI)	−0.08 (−0.12, −0.04)	−0.10 (−0.14, −0.06)	−0.05 (−0.09, −0.02)
p value	<0.0001	<0.0001	0.003
O-desmethylangolensin			
β (95% CI)	−0.17 (−0.22, −0.13)	−0.20 (−0.24, −0.15)	−0.14 (−0.18, −0.10)
p value	<0.0001	<0.0001	<0.0001
Total lignan			
β (95% CI)	−0.24 (−0.28, −0.20)	−0.27 (−0.31, −0.24)	−0.15 (−0.18, −0.12)
p value	<0.0001	<0.0001	<0.0001
Enterodiol			
β (95% CI)	−0.13 (−0.16, −0.09)	−0.15 (−0.18, −0.12)	−0.07 (−0.10, −0.04)
p value	<0.0001	<0.0001	<0.0001
Enterolactone			
β (95% CI)	−0.23 (−0.27, −0.20)	−0.26 (−0.29, −0.22)	−0.15 (−0.19, −0.12)
p value	<0.0001	<0.0001	<0.0001

CI = confidence interval.
[a]β is the partial regression coefficient that indicates changes in serum C-reactive protein (mg/L) per an interquartile range increase in urinary phytoestrogens (ng/mL).
[b]Model 1: adjustment for creatinine level.
[c]Model 2: additional adjustment for age, gender, and race.
[d]Model 3: additional adjustment for education, body mass index, smoking status, and alcohol intake.

other 3 quartiles (*p* values for differences in all of these variables across the quartiles were <0.0001). Urinary concentrations of total and all individual phytoestrogens (except equol and enterodiol) monotonically decreased with increasing quartiles of serum CRP concentrations (all $p < 0.0001$). The association of both equol and enterodiol with CRP concentration levels were not statistically significant.

The results of multivariable linear regression analysis are presented in Table 2. After adjusting for confounders, urinary concentrations of total and all individual phytoestrogens were inversely associated with serum concentrations of CRP (all $p < 0.0001$, except equol, for which $p = 0.003$). For the fully adjusted model 3, the largest reduction in serum CRP (mg/L) associated with an interquartile range increase in urinary phytoestrogens (ng/mL) was observed for total phytoestrogens ($\beta = -0.18$; 95% CI, −0.22, −0.15), followed by total lignan ($\beta = -0.15$; 95% CI, −0.18, −0.12) and enterolactone ($\beta = -0.15$; 95% CI, −0.19, −0.12).

The results of multivariable logistic regression analysis are displayed in Table 3. Higher urinary concentrations of total and all individual phytoestrogens (except enterodiol) were associated with a reduced risk of having high serum concentrations of CRP (≥3.0 mg/L) independent of all confounders adjusted in model 3 (*p* values for the trends for all phytoestrogens were ≤0.01). Similar to the results of the linear regression analysis, the strongest inverse associations were observed for total phytoestrogens (OR = 0.63; 95% CI, 0.53, 0.73), total lignan (OR = 0.64; 95% CI, 0.54, 0.75), and enterolactone (OR = 0.59; 95% CI, 0.51, 0.69). These ORs for having high concentrations of CRP were determined by comparing values for subjects in quartile 4 of each of those phytoestrogens to those in quartile 1 of the respective phytoestrogen. A comparison of the risk estimates obtained from the 3 fitted models in both the linear and logistic regression analyses show that the strength of the inverse associations between urinary phytoestrogens and serum CRP was generally weaker in the fully adjusted model 3 than in models 1 and 2, suggesting the confounding effects of education, BMI, cigarette smoking, and/or alcohol intake. When the regression analysis was restricted to subjects aged 50 years or older, the results described above materially remain unchanged, with an exception that the association between urinary equol and serum CRP became weaker and no longer statistically significant in the multiple linear regression analysis.

Discussion

The present study revealed significant inverse associations of urinary concentrations of total and individual phytoestrogens with serum concentrations of CRP in a nationally representative sample of the U.S. population. Furthermore, subjects with higher urinary concentrations of total and individual phytoestrogens (except enterodiol) were less likely to have high serum concentrations of CRP (≥3.0 mg/L). These significant associations were independent of established or suspected confounders.

Table 3. ORs (95% CIs) for high concentrations of serum C-reactive protein in relation to quartiles of urinary concentrations of total and individual phytoestrogens in the continuous national health and nutrition examination survey, 1999–2010[a].

	Quartile of urinary phytoestrogens (ng/mL)				
	Q1	Q2	Q3	Q4	*p* trend
Total phytoestrogen					
Subjects with CRP ≥ and < 3 mg/L	780/1017	744/1055	633/1163	542/1254	
Concentrations (median)	159	484	1019	2797	
Model 1	Reference	0.88 (0.77–1.00)	0.66 (0.57–0.75)	0.50 (0.43–0.58)	<0.0001
Model 2	Reference	0.83 (0.72–0.95)	0.60 (0.52–0.70)	0.45 (0.39–0.53)	<0.0001
Model 3	Reference	0.88 (0.76–1.02)	0.67 (0.58–0.78)	0.63 (0.53–0.73)	<0.0001
Total isoflavone					
Subjects with CRP ≥ and < 3 mg/L	718/1078	696/1104	671/1124	614/1183	
Concentrations (median)	23	69	181	834	
Model 1	Reference	0.90 (0.78–1.03)	0.83 (0.73–0.96)	0.72 (0.62–0.83)	<0.0001
Model 2	Reference	0.89 (0.77–1.02)	0.81 (0.71–0.94)	0.69 (0.60–0.79)	<0.0001
Model 3	Reference	0.86 (0.74–1.00)	0.75 (0.65–0.88)	0.73 (0.63–0.86)	0.002
Genistein					
Subjects with CRP ≥ and < 3 mg/L	695/1106	701/1093	673/1124	629/1167	
Concentrations (median)	4	15	43	214	
Model 1	Reference	0.98 (0.85–1.12)	0.90 (0.78–1.03)	0.80 (0.70–0.92)	0.001
Model 2	Reference	0.96 (0.84–1.11)	0.89 (0.78–1.03)	0.78 (0.67–0.90)	<0.0001
Model 3	Reference	0.95 (0.82–1.11)	0.85 (0.73–0.99)	0.80 (0.69–0.94)	0.010
Daidzein					
Subjects with CRP ≥ and < 3 mg/L	707/1094	710/1084	641/1156	641/1156	
Concentrations (median)	9	31	95	435	
Model 1	Reference	0.97 (0.85–1.11)	0.81 (0.71–0.93)	0.80 (0.70–0.92)	0.003
Model 2	Reference	0.95 (0.83–1.09)	0.80 (0.70–0.92)	0.77 (0.67–0.89)	0.001
Model 3	Reference	0.94 (0.81–1.09)	0.77 (0.66–0.90)	0.79 (0.67–0.92)	0.009
Equol					
Subjects with CRP ≥ and < 3 mg/L	682/1077	667/1087	664/1094	616/1138	
Concentrations (median)	2	5	10	28	
Model 1	Reference	0.95 (0.83–1.09)	0.90 (0.78–1.03)	0.78 (0.67–0.90)	<0.0001
Model 2	Reference	0.94 (0.82–1.07)	0.90 (0.78–1.03)	0.77 (0.66–0.89)	<0.0001
Model 3	Reference	0.92 (0.80–1.07)	0.91 (0.78–1.06)	0.76 (0.65–0.89)	0.001
O-desmethylangolensin					
Subjects with CRP ≥ and < 3 mg/L	743/1064	697/1041	604/1154	599/1168	
Concentrations (median)	0.3	2	8	71	
Model 1	Reference	0.93 (0.82–1.07)	0.73 (0.63–0.83)	0.71 (0.62–0.81)	<0.0001
Model 2	Reference	0.93 (0.81–1.07)	0.71 (0.62–0.82)	0.67 (0.58–0.77)	<0.0001
Model 3	Reference	0.91 (0.78–1.05)	0.71 (0.61–0.82)	0.72 (0.62–0.84)	0.002
Total lignan					
Subjects with CRP ≥ and < 3 mg/L	789/1008	727/1073	639/1157	544/1251	
Concentrations (median)	63	272	670	1836	
Model 1	Reference	0.85 (0.74–0.97)	0.68 (0.59–0.77)	0.50 (0.44–0.58)	<0.0001
Model 2	Reference	0.80 (0.70–0.92)	0.63 (0.55–0.72)	0.46 (0.40–0.53)	<0.0001
Model 3	Reference	0.87 (0.75–1.00)	0.73 (0.63–0.85)	0.64 (0.54–0.75)	<0.0001
Enterodiol					
Subjects with CRP ≥ and < 3 mg/L	738/1058	649/1147	659/1137	649/1144	
Concentrations (median)	6	27	64	202	
Model 1	Reference	0.79 (0.69–0.91)	0.78 (0.68–0.90)	0.75 (0.65–0.86)	0.004
Model 2	Reference	0.77 (0.67–0.88)	0.76 (0.66–0.87)	0.69 (0.60–0.79)	<0.0001
Model 3	Reference	0.87 (0.75–1.01)	0.81 (0.70–0.95)	0.87 (0.75–1.02)	0.37
Enterolactone					
Subjects with CRP ≥ and < 3 mg/L	805/1004	720/1075	651/1141	522/1269	
Concentrations (median)	30	200	573	1630	
Model 1	Reference	0.83 (0.73–0.95)	0.69 (0.61–0.79)	0.48 (0.41–0.55)	<0.0001
Model 2	Reference	0.80 (0.70–0.92)	0.66 (0.58–0.76)	0.45 (0.39–0.51)	<0.0001
Model 3	Reference	0.83 (0.72–0.96)	0.76 (0.65–0.88)	0.59 (0.51–0.69)	<0.0001

OR = odds ratio, CI = confidence interval, CRP = C-reactive protein.
[a]Model 1: adjustment for creatinine level; model 2: additional adjustment for age, gender, and race; model 3: additional adjustment for education, body mass index, smoking status, and alcohol intake.

Our observation that serum concentrations of CRP significantly decreased with increasing urinary concentrations of phytoestrogens is overall consistent with the results of some previous studies [25,33–36]. In a randomized, double-blind, placebo-controlled dietary intervention trial, 117 post-menopausal women were asked to consume either isoflavone-enriched (50 mg/day) or placebo cereal bars for 8 weeks, with a washout period of 8 weeks between the crossover. The isoflavone supplementation resulted in a significant reduction in plasma CRP concentrations but did not alter the levels of other inflammatory biomarkers of cardiovascular disease risk. Specifically, the OR (95% CI) for CRP > 1 mg/L for isoflavone compared with placebo was 0.43 (0.27, 0.69) [25]. In another dietary intervention study, 60 patients with subclinical hypothyroidism were randomly assigned to receive either low-dose (2 mg/day) or high-dose (16 mg/day) phytoestrogen supplementation for 8 weeks and then crossed over after an 8-week washout period. The

high-dose phytoestrogen supplementation significantly reduced CRP levels in this patient population [33].

However, the potential beneficial effect of phytoestrogen intake on CRP levels were not confirmed in some other randomized intervention trials [26,37]. For example, one study tested the effect of isoflavone supplementation with a dose of 114 mg/day among 56 postmenopausal women with a history of breast cancer [26], and another study evaluated changes in CRP concentrations after 1-month supplementation of high-dose isoflavones (73 mg/day) in comparison with the low-dose intervention (10 mg/day) among 41 postmenopausal women and men with hypercholesterolemia [37]. To date, the majority of published studies on the associations between phytoestrogen intake and CRP levels have been intervention trials. These studies have advantages over observational studies largely because confounding can be eliminated or minimized if a randomization procedure is successfully implemented at the time of group assignment. However, randomized intervention trials are subject to several weaknesses. Dietary intake of phytoestrogens from a normal Western diet is approximately 2 mg/day [33], but most previous randomized trials adopted a dose of isoflavones that is far beyond this amount. Other weaknesses of those studies include small sample size ($n < 150$ for most trials), inadequate compliance of subjects to intervention measures, and dropout during follow-up.

There are several possible mechanisms by which high intake of phytoestrogens might decrease CRP levels in human populations. It has been reported that phytoestrogens possess antioxidant properties [38,39]. Previous studies have shown that total antioxidant capacity or an increased intake of antioxidants was inversely associated with blood levels of CRP [40,41]. In addition, it has been found that orally delivered estrogen preparations resulted in increased levels of CRP [42,43]. Therefore, competitive binding of phytoestrogens to estrogen receptors may counteract the promoting effects of endogenous or pharmacological estrogens on CRP levels. It is also possible that intake of phytoestrogens in Western populations is a surrogate of an overall healthy diet or another nutrient(s) that is truly associated with a reduction in CRP levels. This possibility is indirectly supported by the overall attenuation of our observed inverse association between urinary phytoestrogens and serum CRP after adjustment for socioeconomic and lifestyle factors (education, BMI, cigarette smoking, and alcohol consumption). However, caution should be exercised because the potential mechanisms discussed above need to be further clarified by additional studies.

There are several strengths in the present study. A major strength is that it is among the first to investigate the associations between urinary phytoestrogens and serum CRP in a large representative sample of the U.S. population. Most previous studies published to date on this topic have been randomized trials carried out among specific population subgroups (e.g., postmenopausal women or men with hypercholesterolemia), which limits extrapolation of the results obtained from those studies to the general population. Another major strength of the present study is that urinary concentrations of phytoestrogens measured as biomarkers of their dietary intake are free from recall bias that is frequently inherent in questionnaire-based dietary assessment. In addition, urinary phytoestrogen concentrations are capable of capturing dietary intake of total and individual phytoestrogens from both food sources and soy additives to processed foods as well as their metabolites produced by gut bacteria (e.g., equol and O-desmethylangolensin, which are not associated with dietary intake) [44]. Finally, measuring urinary lignans allows us to estimate their dietary intake. It is not possible to determine lignan intake using dietary assessment instruments because no reliable food composition database on this group of compounds is available at this time [19].

The present study also has several weaknesses. Because it is a cross-sectional study, it is not possible for us to make any causal inference on the observed inverse associations between urinary phytoestrogens and serum CRP. Urinary concentrations of phytoestrogens were determined for only one point in time and therefore might not reflect the usual dietary intake of study subjects if within-person variation is substantial. Nevertheless, a British study has shown a significant, strong correlation between phytoestrogen concentrations in spot urine and those in serum ($r > 0.80$) [19]. Spot, rather than 24-hour, urine samples were collected from NHANES participants primarily for feasibility reasons. Measuring phytoestrogens in spot urine is a potential weakness because the concentrations of these compounds are affected by urine dilution. To control for variation in urine dilution, concentrations of total and each individual phytoestrogen were normalized to urinary creatinine. This is a commonly used method to address this methodological issue [20,45] because the excretion of creatinine by glomerular filtration physiologically occurs at a relatively constant rate [46]. Furthermore, only a modest correlation between dietary intake of total and individual phytoestrogens and their respective concentrations in urine ($r = 0.29$–0.54 for isoflavone; $r = 0.40$ for lignan) were observed in most validation studies [19,47,48].

We are aware that 2 other studies have investigated the effect of urinary excretion of some phytoestrogens on serum levels of CRP among participants in NHANES [35,36]. Eichholzer et al. evaluated the association between urinary lignans and serum CRP [35], and Nicastro et al. examined the association between urinary isoflavones and serum CRP [36]. Compared with those studies, the present study offers more robust and comprehensive evidence that urinary concentrations of total phytoestrogens, total isoflavones, total lignans, and individual phytoestrogens were inversely associated with serum CRP. First, the present study investigated the associations between urinary phytoestrogens and serum CRP among a larger number of participants in the NHANES over a longer period of time (6009 subjects in 1999–2010) than Eichholzer et al.'s study (2628 subjects in 1999–2004 and 2028 subjects in 2005–2008) [35] and Nicastro et al.'s study (1683 subjects in 2005–2008) [36]. Therefore, our study has substantially expanded and strengthened the findings of those 2 previous studies. Second, the present study evaluated the associations between urinary phytoestrogens and serum CRP among NHANES participants of a more biologically relevant age range. Both previous studies analyzed data collected from the participants aged 18 years or older. As mentioned previously, it is more methodologically appropriate to evaluate the associations between urinary phytoestrogens and serum CRP among

subjects aged 40 years or older because such an analysis of middle-aged or older subjects could help to elucidate whether inflammation is involved in biological mechanisms linking dietary intake of phytoestrogens to the risk of cancer and cardiovascular diseases, major chronic conditions with low incidence rates among subjects aged 40 years or younger.

In summary, the present study found that higher urinary concentrations of total and individual phytoestrogens were associated with reduced serum concentrations of CRP, with the largest reduction observed for total phytoestrogens, lignan, and enterolactone. A growing body of evidence indicates that inflammation is involved in the occurrence of cancer and cardiovascular disease [49], which is supported by an increased risk of these diseases associated with high CRP levels in epidemiological studies [50,51]. Previous studies have overall suggested that increased phytoestrogen consumption confers a beneficial effect on some cancers and cardiovascular disease through its influence on inflammation-mediated pathogenesis [52,53]. Therefore, the findings of the present study offer a novel biological basis for using phytoestrogens as a potential bioactive agent for the prevention of these life-threatening diseases.

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