Cancer Causes Control (2014) 25:395–403
DOI 10.1007/s10552-014-0340-3

ORIGINAL PAPER

Urinary lignans and inflammatory markers in the US National Health and Nutrition Examination Survey (NHANES) 1999–2004 and 2005–2008

Monika Eichholzer · Aline Richard · Holly L. Nicastro · Elizabeth A. Platz · Jakob Linseisen · Sabine Rohrmann

Received: 12 September 2013 / Accepted: 8 January 2014 / Published online: 25 January 2014

Abstract
Purpose Chronic inflammation has been implicated in the etiology of various chronic diseases. We previously found that certain urinary isoflavones are associated with markers of inflammation. In the present study, we examined the associations of serum C-reactive protein (CRP) and white blood cell (WBC) count with lignans, which are more frequent in the Western diet than isoflavones.
Methods Our analysis included 2,028 participants of NHANES 2005–2008 and 2,628 participants of NHANES 1999–2004 aged 18 years and older. The exposures of interest were urinary mammalian lignans (enterodiol and enterolactone). Outcome variables were two inflammatory markers (CRP [≤10 mg/L] and WBC [≥3.0 and ≤11.7 (1,000 cells/μL)]). Log-transformed CRP concentration and WBC count by log-transformed creatinine-standardized concentrations of mammalian lignans were used for linear regression.
Results Statistically significant inverse associations of urinary lignan, enterodiol, and enterolactone concentrations with circulating CRP and WBC counts were observed in the multivariate-adjusted models: In NHANES 2005–2008, per one-percent increase in lignan concentrations in the urine, CRP concentrations and WBC counts decreased by 8.1 % (95 % CI −11.5, −4.5) and 1.9 % (95 % CI −2.7; −1.2), respectively. Per one-percent increase in enterodiol and enterolactone, WBC counts decreased by 2.1 % (95 % CI −2.8, −1.3) and 1.3 % (95 % CI −1.9, −0.6), respectively. In NHANES 1999–2004, analogous results were 3.0 % (95 % CI −5.6, −0.3), 1.2 % (95 % CI −2.0; −0.4), 1.0 % (95 % CI −1.8, −0.2), and 0.8 % (95 % CI −1.4, 0.2).
Conclusions Mammalian lignans were inversely associated with markers of chronic inflammation. Due to the cross-sectional design, our findings require confirmation in prospective studies.

Keywords Mammalian lignans · C-reactive protein · White blood cell count · Cross-sectional study

M. Eichholzer (✉) · A. Richard · S. Rohrmann
Division of Cancer Epidemiology and Prevention, Institute of Social and Preventive Medicine, University of Zurich, Hirschengraben 84, 8001 Zurich, Switzerland
e-mail: monika.eichholzer@ifspm.uzh.ch

H. L. Nicastro
Cancer Prevention Fellowship Program, Nutritional Science Research Group, Division of Cancer Prevention, National Cancer Institute, Rockville, MD, USA

E. A. Platz
Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA

E. A. Platz
Sidney Kimmel Comprehensive Cancer Center, James Buchanan Brady Urological Institute, Johns Hopkins Medical Institutions, Baltimore, MD, USA

J. Linseisen
Institute of Epidemiology I, Helmholtz Zentrum München, Neuherberg, Germany

Introduction

Low-grade chronic inflammation has been postulated to play an important role in the development of chronic diseases, i.e., cardiovascular disease, type 2 diabetes mellitus, and various types of cancer [1]. C-reactive protein (CRP), which is produced in the liver in response to elevated cytokine levels in the context of an inflammatory stimulus, is used as biomarker of acute and chronic inflammation [2]. Similarly, elevated white blood cell (WBC) count is a

marker of systemic inflammation [3]. An increased risk of chronic diseases has been observed with elevated CRP levels and WBC counts [4–6]. CRP concentration and WBC count are easy to measure, and they do not have diurnal or seasonal variation [6]. However, they are very responsive to acute infections and are elevated in individuals with myocardial infarction, surgery, burns, malignancies, etc [6]. High circulating CRP concentrations have indeed been shown to be associated with the risk of some types of cancer [7] and also cancer survival [8]. Furthermore, elevated blood levels of CRP and WBC are associated with obesity, smoking, a lower level of physical activity, no or heavy alcohol intake (only CRP), and estrogen use [6, 9–19], all of which are cancer risk factors. Intakes of some nutrients or foods (n-3 polyunsaturated fatty acids, fiber, flavonoids, antioxidants, vegetables, fruit, and fish, etc.), on the contrary, have been reported to be inversely associated with serum CRP concentration [20–26].

Phytoestrogens are plant constituents found in many foodstuffs. They may act as weak estrogens [27], but also as antioxidants and anti-inflammatory agents, suggesting a possible CRP level- and WBC count-lowering role [28]. The main groups of phytoestrogens are lignans, isoflavones, and coumestans [27, 29]. The major lignans are matairesinol, secoisolariciresinol, pinoresinol, lariciresinol, and syringaresinol. The major isoflavones are genistein and daidzein. The mammalian lignans' enterolactone and enterodiol can be formed by the gut microflora from lignan plant precursors; and the isoflavones equol and *O*-DMA from daidzein [27].

Only a few studies exist on the association between lignans and CRP concentrations, and the results are not consistent [30–32] and need further investigation.

In previous studies, the health effects of phytoestrogens were mainly studied by estimating the dietary intake of these plant constituents by study participants or by testing dietary supplements or enriched foods. However, measuring the concentration of phytoestrogens in urine considers interindividual variations in microbial synthesis [33] and is independent of errors due to imprecise dietary assessments and databases.

In a recent publication, we have shown that higher excretion of certain soy isoflavonoids were associated with decreased CRP concentration and WBC counts in adults in the United States in the National Health and Nutrition Survey (NHANES) 2005–2008 [34]. However, the intake of soy products that are the major contributor to isoflavone intake is low in Western societies compared with Asian populations. The intake of lignans, on the other hand, is higher in Western societies. Therefore, we examined in the present study whether mammalian lignans, i.e., enterolactone and enterodiol, are associated with markers of inflammation. To be able to compare our results with the previous results by Nicastro et al. [34], we decided to show associations for the NHANES period 2005–2008 and 1999–2004 separately, as Nicastro et al. had analyzed the period 2005–2008. To our knowledge, this is the first study to investigate the association of urinary mammalian lignan concentration with circulating CRP and WBC counts.

Methods

Study population

NHANES is an on-going cross-sectional study representative of the population of the United States (US), which is carried out by the National Center for Health Statistics (NCHS). Data are released for two succeeding years (i.e., 1999/2000, 2001/2002, etc.). In NHANES, the health and nutritional status of adults and children in the US is assessed by interviews and physical examinations.

The NHANES interview includes demographic, socioeconomic, dietary, and health-related questions. The examination part consists of medical, dental, and physiological measurements, as well as laboratory tests. All data are anonymized. The sample for the survey represents the US population of all ages. A complex, multistage, probability sampling design is used to select participant representative of the civilian, non-institutionalized US population. To produce reliable statistics, NHANES oversamples individuals 60 years and older, African Americans, and Hispanics [35]. Furthermore, various subsamples weights for single two-year survey cycles are available for specified sampling fractions, which accounts for the additional probability of selection into the subsample component as well as the additional nonresponse [36].

Our analysis included data from the 2005–2006 and 2007–2008 NHANES cycles, as well as data from the 1999–2000, 2001–2002, and 2003–2004 NHANES cycles. The 2005–2008 cycles included data of 20,497 people, the 1999–2004 cycle data of 31,126 people. We restricted the analysis to participants 18+ years old ($n = 11{,}335$ and $n = 16{,}183$) to reduce variability in inflammation marker levels. In addition, we excluded pregnant women ($n = 351$ and $n = 687$). After excluding participants with missing information on urinary phytoestrogen levels, the sample size for this study consisted of 3,174 and 4,263 individuals. From these, all individuals reporting about acute infection ($n = 744$ and $n = 1{,}129$) were excluded. The final sample sizes from individuals with CRP concentration $\leq$10 mg/L or WBC counts $\geq$3.0 and $\leq$11.7 (1,000 cells/μL) were $n = 2{,}028$ and $n = 2{,}628$ (rationale for cut-off levels see [34]).

The NHANES study protocols were approved by National Center for Health Statistics (NCHS) Research

Ethics Review Board (ERB), and informed consent was obtained from all participants.

Measurements

Blood was drawn by venipuncture, and spot urine samples were collected at NHANES mobile examination centers. Urine specimens were processed, stored, and shipped to the Division of Environmental Health Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention for analysis (for details see NHANES Laboratory/Medical Technologists Procedures Manual). Vials were stored under appropriate frozen (−20 °C) conditions until shipment to National Center for Environmental Health for testing.

Urinary concentrations of lignans were measured by the Nutritional Biomarkers Branch, Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention. Comparable methods for the determination of lignans were used [37]. During the 1999–2002 surveys, analyses were performed by HPLC-APCI-MS building on the Barnes et al. [38] method for phytoestrogens. During the 2003–2004 surveys, they were analyzed by HPLC Electrospray Ionization MS [37] and during the 2005–2008 cycles by HPLC-APPI-MS/MS. Of the phytoestrogens measured in the five NHANES cycles 1999/2000, 2001/2002, 2003/2004, 2005/2006, and 2007/2008, lignans represented by enterodiol and enterolactone were used for the present analysis.

Urinary concentration of creatinine, used to correct urinary levels of analytes for urine dilution, was measured using Beckman Synchron CX3 Clinical Analyzer at the University of Minnesota [39]. Phytoestrogen concentrations were expressed in μg/g creatinine.

C-reactive protein was measured by latex-based nephelometry by the Immunology Division, Department of Laboratory Medicine, University of Washington Medical Center. The CRP limit of detection was 0.2 mg/L for the years 1999–2008, and 0.1 mg/L was assigned for CRP levels below detection limit. WBC count was determined using Beckman Coulter MAXM instruments in MECs with the Beckman Coulter method of counting and sizing.

Weight and height were measured by trained personnel. Body mass index (BMI) was calculated as weight in kilogram divided by squared height in meter. Age, sex, alcohol use, and smoking habits were self-reported and were assessed by interviews. Race/ethnicity, hormone use, and acute infection during the last 30 days (head/chest colds, stomach or intestinal illness, flu, pneumonia, or ear infection) were also part of an interview. Self-reported history of congestive heart failure, coronary heart disease, heart attack, or stroke was used to define prevalent cardiovascular disease (CVD) and self-reported history of a diagnosis of cancer (other than non-melanoma skin cancer) to define prevalent cancer. Participants were considered as being diabetic when they reported a history of a diagnosis of diabetes or were users of insulin or diabetic medication. Self-reported history of hypertension was assessed when the participants had been told at least twice by a doctor to have hypertension and/or when the participants were users of antihypertensive medication. History of kidney disease was assessed by the question whether ever a doctor or other health professional told the patient that he/she had weak or failing kidneys. Hormone therapy was assessed by questions concerning intake (pills) and patches of estrogen or estrogen/progestin.

Poverty–income ratio (PIR) was calculated as a proportion of the self-reported family income to the United States Census-based poverty threshold value for each calendar year adjusted for inflation and the age of the family reference person (a PIR value of 1 or greater indicates income above the poverty level, whereas a PIR value below value 1 indicates poverty). Women were considered as postmenopausal when they were 55+ years old.

Statistical analyses

Data were analyzed using survey methods in Stata statistical software version 11.2 (College Station, TX) to account for the complex NHANES sampling design. $p < 0.05$ was considered to be statistically significant (two-sided tests). Sampling weights were applied according NHANES guidelines to produce estimates that were representative of the non-institutionalized, civilian US population [36].

We used medians for levels of lignans and percentages for demographic variables. Log-transformend CRP concentration and WBC count by creatinine-standardized log-transformed concentrations of lignans were used for linear regression. In linear models, where both the dependent and the independent variables have been log-transformed, the dependent variable can be interpreted as percentage changes for a one-percent increase in the independent variable, while all other variables in the model are held constant. In the demographic-adjusted model, we adjusted for sex [40, 41], age (continuous) [40, 41], and race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic/Mexican American, other) [40, 42]. In the multivariable-adjusted model, we first adjusted additionally for BMI (continuous) [41, 43], alcohol consumption (no drinks, ≤1 drink per week, >1 drink per week) [41], menopausal status (yes/no) [44, 45], current hormone therapy (HT) use (yes/no) [44, 46], cigarette smoking status (never, former, or current smoking) [41, 47], PIR (<1 vs. ≥1) [41, 48], and chronic diseases (yes/no for kidney disease, cancer, hypertension, diabetes and CVD) [6, 49–52]. The covariates were chosen a priori based on known or suspected confounders of the relationship of lignans and inflammation.

Table 1 Baseline characteristics of individuals, aged 18+ years, with data on C-reactive protein (CRP) blood concentrations, white blood cell counts (WBC), and urinary lignans in NHANES 1999–2004 and 2005–2008[a]

	2005–2008			1999–2004		
	median	Q1	Q3	median	Q1	Q3
Phytoestrogens (μg/g creatinine)						
Lignans[b]	501.0	187.3	1,089.5	436.8	172.9	958.6
Enterodiol	41.5	15.9	105.0	37.0	14.8	84.2
Enterolactone	418.4	119.3	973.9	374.6	122.4	852.1
C-reactive protein (CRP; mg/L)	1.5	0.6	3.2	1.8	0.7	3.5
White blood cell count (WBC; 1,000 cells/μL)	6.8	5.6	8.0	6.7	5.6	8.0
Age (years)	49	34	64	46	32	65
	%			%		
Sex (women)	48.1			48.5		
Race						
Non-Hispanic white	72.7			73.8		
Non-Hispanic black	10.5			9.4		
Mexican American	7.4			7.3		
Other	9.3			9.5		
Poverty–income ratio (PIR)						
Below poverty (<1)	9.7			11.4		
At or above poverty (≥1)	84.2			81.7		
Missings	6.1			6.9		
Smoking history						
Current smoker	20.9			21.5		
Former smoker	25.5			24.3		
Never	51.9			52.3		
Missings	1.8			1.9		
Average number of alcoholic drinks/day—past 12 months						
0	38.0			36.1		
≤1	29.1			29.3		
>1	32.9			34.7		
BMI kg/m^2						
<18.5	2.1			1.5		
≥18.5–<25.0	32.8			33.2		
≥25.0–<30.0	34.5			34.6		
≥30	29.7			28.8		
Missings	0.8			2.0		
Hormone therapy in menopausal women	9.3			22.4		
Postmenopausal status in women	34.6			31.0		
Chronic diseases[c]						
History of diabetes	7.6			6.0		
History of hypertension	30.0			38.6		
History of cancer	8.1			6.6		
History of CVD	7.8			7.0		
History of kidney disease	1.2			0.6		

[a] Values are weighted except medians

[b] Enterodiol, enterolactone

[c] Self-reported

We compared the distributions of the above-mentioned covariates in individuals of our sample ($n = 2{,}028$ and $n = 2{,}628$ for the years 2005–2008 and 1999–2004, respectively) with those in individuals aged 18+ not included in our sample ($n = 9{,}307$ and $n = 13{,}555$ for the years 2005–2008 and 1999–2004, respectively) to evaluate differences between these two groups. Furthermore, we stratified the analyses by gender as a sub-analysis.

Results

Table 1 summarizes socioeconomic, lifestyle, and health characteristics of the study population by the two study periods 2005–2008 and 1999–2004. Of the middle-aged, mainly non-Hispanic whites, nearly half were smokers or former smokers, one-third were drinking more than one drink alcohol per day and about one-third had a history of hypertension, and less than 10 % were affected by a history of CVD, cancer, diabetes, or kidney disease. About one-third of the included women were postmenopausal. Comparing our sample with the non-included individuals in the same age range in the two study periods, the median level of CRP was similar (1.5 vs. 2.2 mg/L in 2005–2008 and 1.8 vs. 2.4 mg/L in 1999–2004) as well as the median WBC count (6.8 vs. 7.1 × 1,000 cells/μL) in 2005–2008 and 6.7 vs. 7.1 × 1,000 cells/μL in 1999–2004). Other demographic and lifestyle factors were similarly distributed between the two groups (data not shown).

Table 2 shows the associations of urinary lignans concentrations with CRP concentrations and WBC counts for the time periods 2005–2008 and 1999–2004, respectively. In both periods, we observed statistically significant inverse associations of urinary total lignan, enterodiol, and enterolactone levels with CRP concentrations and WBC counts, in the demographic-adjusted models. However, these associations were partly attenuated in the multivariable-adjusted models, such that enterodiol was not significantly associated with CRP concentration in the multivariable-adjusted models in the analysis of data of the 2005–2008 and the 1999–2004 cycles; the same was true for enterodiol and enterolactone in the analysis of data from 1999 to 2004. Per one-percent increase in urinary lignan concentrations, CRP concentrations and WBC counts decreased by 8.1 % (95 % CI −11.5, −4.5) and 1.9 % (95 % CI −2.7; −1.2), respectively, in the multivariable-adjusted models of the period 2005–2008. Per one-percent increase in enterodiol and enterolactone, WBC counts decreased by 2.1 % (95 % CI −2.8, −1.3) and 1.3 % (95 % CI −1.9, −0.6), respectively. Per one-percent increase of enterolactone, CRP concentrations decreased by 7.1 % (95 % CI −9.9, −4.3). In NHANES 1999–2004, per one-percent increase in lignan concentrations in urine resulted in a 3.0 % (95 % CI −5.6, −0.3) decrease in CRP concentration and 1.2 % decrease in WBC count (95 % CI −2.0; −0.4). Per one-percent increase in enterodiol and enterolactone, WBC counts decreased by 1.0 % (95 % CI −1.8, −0.2) and 0.8 % (95 % CI −1.4, 0.2), respectively. When analyses were stratified by gender, there were slightly higher, significant associations in women than in the total sample (NHANES 1999–2004). In NHANES 2005–2008, results were similar for men and women as well as for the total sample. There were not significant interactions between sex and any of the phytoestrogen (data not shown).

Discussion

In the present study, urinary mammalian lignans were significantly inversely related to serum CRP levels and WBC counts, suggesting a possible inverse association between lignans and inflammation. This was true for both time periods studied (NHANES 2005–2008 and NHANES 1999–2004). The associations were maintained after controlling for important factors that may have confounded the associations. To the best of our knowledge, the cross-sectional association of urinary lignans with CRP concentrations and WBC counts has not yet been examined in other studies. Our findings may be of interest for example in the context of prospective studies having shown a higher risk of developing cardiovascular diseases and cancer in those with elevated serum CRP [53].

We recently evaluated the association between urinary genistein, daidzein, *O*-DMA, and equol excretion and CRP levels, and WBC counts in adults in the United States in the National Health and Nutrition Survey (NHANES) 2005–2008, but no clear pattern emerged, although higher excretion of some soy isoflavonoids was associated with decreased CRP concentration and WBC counts [34]. In relation to lignans, Valentin-Blasini et al., using urinary phytoestrogen concentrations in NHANES 1999–2000, observed [40] the highest urinary concentrations of all phytoestrogens measured for enterolactone, and these values correlated with levels reported for other populations [54]. As already mentioned in the introduction, only a few studies investigated the association between lignans and CRP levels and the results are not consistent [30–32]. Hallund et al. [30] evaluated 2008 the effects of a plant lignan complex isolated from flaxseed, providing 500 mg/d of secoisolariciresinol diglucoside, on inflammatory markers. They found that daily consumption of a low-fat muffin enriched with a lignan complex for 6 weeks reduced CRP concentrations significantly compared to a low-fat muffin with no lignans added. Dodin et al. [31] evaluated in 2008 the effect of 40 g of flaxseed versus wheat germ placebo on markers of cardiovascular disease risk in healthy menopausal

Table 2 Association of phytoestrogen concentrations in urine with C-reactive protein (CRP) and white blood cell count (WBC) in serum in NHANES

	unadjusted model					age-, sex-, and race-adjusted model					multivariable-adjusted model[1]				
	Beta-coefficient	Standard error	Change in %[2]	95 % CI in %		Beta-coefficient	Standard error	Change in %[2]	95 % CI in %		Beta-coefficient	Standard error	Change in %[2]	95 % CI in %	
2005–2008															
CRP concentration															
Lignans	**−0.107**	0.022	−10.1	−14	−6.1	**−0.136**	0.022	−12.7	−16.5	−8.8	**−0.084**	0.019	−8.1	−11.5	−4.5
Enterodiol	**−0.042**	0.02	−4.2	−7.9	−0.3	**−0.052**	0.02	−5.1	−8.7	−1.3	−0.022	0.018	−2.1	−5.5	1.3
Enterloactone	**−0.092**	0.018	−8.8	−12	−5.5	**−0.114**	0.017	−10.8	−13.8	−7.7	**−0.074**	0.015	−7.1	−9.9	−4.3
WBC count															
Lignans	**−0.026**	0.005	−2.6	−3.5	−1.7	**−0.028**	0.005	−2.8	−3.7	−1.9	**−0.02**	0.004	−1.9	−2.7	−1.2
Enterodiol	**−0.024**	0.005	−2.3	−3.2	−1.5	**−0.025**	0.005	−2.5	−3.3	−1.6	**−0.021**	0.004	−2.1	−2.8	−1.3
Enterloactone	**−0.019**	0.004	−1.8	−2.6	−1	**−0.02**	0.004	−2	−2.7	−1.2	**−0.013**	0.004	−1.3	−1.9	−0.6
1999–2004															
CRP concentration															
Lignans	**−0.05**	0.015	−4.9	−7.6	−2.1	**−0.084**	0.015	−8.1	−10.7	−5.3	**−0.03**	0.014	−3	−5.6	−0.3
Enterodiol	−0.016	0.02	−1.6	−5.4	2.3	**−0.04**	0.02	−3.9	−7.6	−0.1	−0.012	0.018	−1.2	−4.6	2.4
Enterolactone	**−0.042**	0.013	−4.1	−6.5	−1.6	**−0.065**	0.013	−6.3	−8.7	−3.9	−0.022	0.012	−2.2	−4.4	0.2
WBC count															
Lignans	**−0.021**	0.004	−2.1	−2.8	−1.3	**−0.022**	0.004	−2.2	−2.9	−1.4	**−0.012**	0.004	−1.2	−2	−0.4
Enterodiol	**−0.015**	0.004	−1.5	−2.3	−0.7	**−0.017**	0.004	−1.7	−2.5	−0.9	**−0.01**	0.004	−1	−1.8	−0.2
Enterolactone	**−0.016**	0.003	−1.6	−2.2	−1	**−0.016**	0.003	−1.6	−2.2	−1	**−0.008**	0.003	−0.8	−1.4	−0.2

Bold values indicate significant values according to confidence intervals (CI) not including the value of 1

[1] Adjusted for age, race, sex, smoking status, alcohol consumption, poverty–income ratio, BMI, use of HT, menopause, CVD, cancer, hypertension, diabetes, and kidney disease

[2] Percentage change in CRP and WBC, per 1 % change in lignans

women. The values of CRP did not change significantly after 12 months of treatment. Pan et al. [32] explored the effects of lignans (360 mg flaxseed-derived secoisolariciresinol diglucoside daily) on inflammatory factors in type 2 diabetics, who have in general higher levels of these biomarkers. Baseline to follow-up concentrations of CRP increased significantly within the placebo group, but was comparatively unchanged in the lignan-supplemented group; a significant difference was observed between treatments. This effect was observed in women, but not in men. Potential explanations for these sex differences might be the relatively small sample of males, but also the possibility that postmenopausal women were more likely to be responsive to phytoestrogens [32]. In the present study, there were no significant interactions between sex and phytoestrogens, thus sex differences in NHANES 1999–2004 must be due to chance finding.

In the present study, we observed a significantly inverse association between urinary lignans and CRP concentrations and WBC counts.

However, the mechanisms of these associations are still unknown. One suggested effect could be that lignans and their metabolites, enterodiol and enterolactone, may affect CRP concentrations due to their antioxidant activity, which have been shown in different in vitro model systems [30]. For example, lignans have been shown to be able to lower lipid peroxidation and reduce deoxyribose oxidation and DNA strand breakage [55].

Our study has several strengths. For evaluating the associations of urinary lignans with CRP levels and WBC counts, we used data from a nationally representative sample of individuals (NHANES 2005–2008 and NHANES 1999–2004). Furthermore, urinary lignan values comprise phytoestrogen intake from all sources, which is very difficult to assess by a dietary survey. Urinary lignan measurements also take into account the capacity to produce enterolignans. In addition, the latex-based nephelometry used in the present study to assess serum CRP has a much lower detection limit than previously used methods.

This survey has also some important limitations. We adjusted our findings for alcohol intake, physical activity, smoking, BMI, age, gender, race, and poverty–income ratio to control confounding by sociodemographic factors and a healthy lifestyle. Residual confounding due to unmeasured or unknown factors is nevertheless a potentially important bias in our study. In a cross-sectional study of 468 US male health professionals [56], the main food groups for total lignan intake were tea and coffee (28 %), noncabbage, nonlettuce vegetables (11 %), alcoholic beverages (9 %), and bread, cereals, rice, and grains (7 %). Thus, residual confounding due to other dietary constituents in coffee, tea, vegetables, grains, etc. (dietary fiber, antioxidant vitamins, other flavonoids, etc.) cannot be excluded. Furthermore, most variables included in the multivariable models were self-reported. Recall and misclassification bias cannot be excluded. Our reduced sample of individuals due to missing information on phytoestrogen level is a further critical point. Nevertheless, the comparison of individuals of our sample with individuals of the same age range not included in our sample showed that the distribution of most demographic and lifestyle characteristics and the levels of CRP and WBC count were similar.

Additionally, CRP concentrations and WBC counts are affected by infection, acute, or chronic conditions. We excluded all individuals reporting acute infection and all participants with high circulating CRP (>10 mg/L) concentrations and WBC counts (<3.0 and >11.7 (1000 cells/μL)) from our analyses. In addition, we controlled for those with self-reported select chronic conditions, but there could still be confounding by medication. The potential impact of menstrual cycle variability on CRP levels, observed for example by Gaskins et al. [57] in the BioCycle Study; we were not able to assess in the present study. This may lead to under- or overestimation of the observed associations or be of no impact. Most probably, the latter is true because there is, to our knowledge, no evidence available that lignan intake/urinary excretion is related to the menstrual cycle.

In conclusion, our results indicate that in US adults mammalian lignans were associated with a beneficial profile of inflammatory markers (CRP, WBC), although causality cannot be inferred in these cross-sectional analyses. Our findings warrant further investigation in this field, on the one hand by using cellular and animal models to identify the exact biological mechanisms, and on the other hand by carrying out cohort and intervention studies in humans to establish causality of the observed associations.

Acknowledgments This work was supported by the Swiss Foundation for Nutrition Research (SFEFS; Zürich, Switzerland). We thank all individuals at the National Center for Health Statistics (NCHS) of the Centers for Disease Control and Prevention who were responsible for the planning and administering of NHANES.

Conflict of interest No potential conflicts of interest were disclosed.

References

1. Wu SH, Shu XO, Chow WH, Xiang YB, Zhang X, Li HL, Cai Q, Ji BT, Cai H, Rothman N, Gao YT, Zheng W, Yang G (2012) Soy food intake and circulating levels of inflammatory markers in Chinese women. J Acad Nutr Diet 112(7):996–1004. doi:10.1016/j.jand.2012.04.001
2. Touvier M, Fezeu L, Ahluwalia N, Julia C, Charnaux N, Sutton A, Mejean C, Latino-Martel P, Hercberg S, Galan P, Czernichow S (2013) Association between prediagnostic biomarkers of inflammation and endothelial function and cancer risk: a nested case-control study. Am J Epidemiol 177(1):3–13. doi:10.1093/aje/kws359

3. Kotani K, Sakane N (2012) White blood cells, neutrophils, and reactive oxygen metabolites among asymptomatic subjects. Int J Prev Med 3(6):428–431
4. Ridker PM, Hennekens CH, Buring JE, Rifai N (2000) C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Engl J Med 342(12): 836–843. doi:10.1056/NEJM200003233421202
5. Iida M, Ikeda F, Ninomiya T, Yonemoto K, Doi Y, Hata J, Matsumoto T, Kiyohara Y (2012) White blood cell count and risk of gastric cancer incidence in a general Japanese population: the Hisayama study. Am J Epidemiol 175(6):504–510. doi:10.1093/aje/kwr345
6. Pepys MB, Hirschfield GM (2003) C-reactive protein: a critical update. J Clin Invest 111(12):1805–1812. doi:10.1172/JCI18921
7. Siemes C, Visser LE, Coebergh JW, Splinter TA, Witteman JC, Uitterlinden AG, Hofman A, Pols HA, Stricker BH (2006) C-reactive protein levels, variation in the C-reactive protein gene, and cancer risk: the Rotterdam Study. J Clin Oncol 24(33): 5216–5222. doi:10.1200/JCO.2006.07.1381
8. Saito K, Kihara K (2011) C-reactive protein as a biomarker for urological cancers. Nat Rev Urol 8(12):659–666. doi:10.1038/nrurol.2011.145
9. Bazzano LA, He J, Muntner P, Vupputuri S, Whelton PK (2003) Relationship between cigarette smoking and novel risk factors for cardiovascular disease in the United States. Ann Intern Med 138(11):891–897
10. Wener MH, Daum PR, McQuillan GM (2000) The influence of age, sex, and race on the upper reference limit of serum C-reactive protein concentration. J Rheumatol 27(10):2351–2359
11. Visser M, Bouter LM, McQuillan GM, Wener MH, Harris TB (1999) Elevated C-reactive protein levels in overweight and obese adults. JAMA 282(22):2131–2135
12. Imhof A, Froehlich M, Brenner H, Boeing H, Pepys MB, Koenig W (2001) Effect of alcohol consumption on systemic markers of inflammation. Lancet 357(9258):763–767. doi:10.1016/S0140-6736(00)04170-2
13. Yu Z, Ye X, Wang J, Qi Q, Franco OH, Rennie KL, Pan A, Li H, Liu Y, Hu FB, Lin X (2009) Associations of physical activity with inflammatory factors, adipocytokines, and metabolic syndrome in middle-aged and older chinese people. Circulation 119(23): 2969–2977. doi:10.1161/CIRCULATIONAHA.108.833574
14. Nanri H, Nakamura K, Hara M, Higaki Y, Imaizumi T, Taguchi N, Sakamoto T, Horita M, Shinchi K, Tanaka K (2011) Association between dietary pattern and serum C-reactive protein in Japanese men and women. J Epidemiol 21(2):122–131
15. Zalokar JB, Richard JL, Claude JR (1981) Leukocyte count, smoking, and myocardial infarction. N Engl J Med 304(8): 465–468. doi:10.1056/NEJM198102193040806
16. Dixon JB, O'Brien PE (2006) Obesity and the white blood cell count: changes with sustained weight loss. Obes Surg 16(3): 251–257. doi:10.1381/096089206776116453
17. Aminzadeh Z, Parsa E (2011) Relationship between age and peripheral white blood cell count in patients with sepsis. Int J Prev Med 2(4):238–242
18. Pitsavos C, Chrysohoou C, Panagiotakos DB, Skoumas J, Zeimbekis A, Kokkinos P, Stefanadis C, Toutouzas PK (2003) Association of leisure-time physical activity on inflammation markers (C-reactive protein, white cell blood count, serum amyloid A, and fibrinogen) in healthy subjects (from the ATTICA study). Am J Cardiol 91(3):368–370
19. Pradhan AD, Manson JE, Rossouw JE, Siscovick DS, Mouton CP, Rifai N, Wallace RB, Jackson RD, Pettinger MB, Ridker PM (2002) Inflammatory biomarkers, hormone replacement therapy, and incident coronary heart disease: prospective analysis from the Women's Health Initiative observational study. JAMA 288(8): 980–987
20. Yoneyama S, Miura K, Sasaki S, Yoshita K, Morikawa Y, Ishizaki M, Kido T, Naruse Y, Nakagawa H (2007) Dietary intake of fatty acids and serum C-reactive protein in Japanese. J Epidemiol 17(3):86–92
21. Ma Y, Griffith JA, Chasan-Taber L, Olendzki BC, Jackson E, Stanek EJ 3rd, Li W, Pagoto SL, Hafner AR, Ockene IS (2006) Association between dietary fiber and serum C-reactive protein. Am J Clin Nutr 83(4):760–766
22. Esmaillzadeh A, Kimiagar M, Mehrabi Y, Azadbakht L, Hu FB, Willett WC (2006) Fruit and vegetable intakes, C-reactive protein, and the metabolic syndrome. Am J Clin Nutr 84(6):1489–1497
23. Zampelas A, Panagiotakos DB, Pitsavos C, Das UN, Chrysohoou C, Skoumas Y, Stefanadis C (2005) Fish consumption among healthy adults is associated with decreased levels of inflammatory markers related to cardiovascular disease: the ATTICA study. J Am Coll Cardiol 46(1):120–124. doi:10.1016/j.jacc.2005.03.048
24. Yang M, Chung SJ, Floegel A, Song WO, Koo SI, Chun OK (2013) Dietary antioxidant capacity is associated with improved serum antioxidant status and decreased serum C-reactive protein and plasma homocysteine concentrations. Eur J Nutr. doi:10.1007/s00394-012-0491-5
25. Chun OK, Chung SJ, Claycombe KJ, Song WO (2008) Serum C-reactive protein concentrations are inversely associated with dietary flavonoid intake in U.S. adults. J Nutr 138(4):753–760
26. Yang M, Chung SJ, Floegel A, Song WO, Koo SI, Chun OK (2013) Dietary antioxidant capacity is associated with improved serum antioxidant status and decreased serum C-reactive protein and plasma homocysteine concentrations. Eur J Nutr 52(8): 1901–1911. doi:10.1007/s00394-012-0491-5
27. Humfrey CD (1998) Phytoestrogens and human health effects: weighing up the current evidence. Nat Toxins 6(2):51–59. doi:10.1002/(SICI)1522-7189(199804)6:2<51:AID-NT11>3.0.CO;2-9
28. Patisaul HB, Jefferson W (2010) The pros and cons of phytoestrogens. Front Neuroendocrinol 31(4):400–419. doi:10.1016/j.yfrne.2010.03.003
29. Branca F, Lorenzetti S (2005) Health effects of phytoestrogens. Forum Nutr 57:100–111
30. Hallund J, Tetens I, Bugel S, Tholstrup T, Bruun JM (2008) The effect of a lignan complex isolated from flaxseed on inflammation markers in healthy postmenopausal women. Nutr Metab Cardiovasc Dis 18(7):497–502. doi:10.1016/j.numecd.2007.05.007
31. Dodin S, Cunnane SC, Masse B, Lemay A, Jacques H, Asselin G, Tremblay-Mercier J, Marc I, Lamarche B, Legare F, Forest JC (2008) Flaxseed on cardiovascular disease markers in healthy menopausal women: a randomized, double-blind, placebo-controlled trial. Nutrition 24(1):23–30. doi:10.1016/j.nut.2007.09.003
32. Pan A, Demark-Wahnefried W, Ye X, Yu Z, Li H, Qi Q, Sun J, Chen Y, Chen X, Liu Y, Lin X (2009) Effects of a flaxseed-derived lignan supplement on C-reactive protein, IL-6 and retinol-binding protein 4 in type 2 diabetic patients. Br J Nutr 101(8):1145–1149. doi:10.1017/S0007114508061527
33. Lampe JW, Atkinson C, Hullar MA (2006) Assessing exposure to lignans and their metabolites in humans. J AOAC Int 89(4): 1174–1181
34. Nicastro HL, Mondul AM, Rohrmann S, Platz EA (2013) Associations between urinary soy isoflavonoids and two inflammatory markers in adults in the United States in 2005–2008. Cancer Causes Control 24(6):1185–1196. doi:10.1007/s10552-013-0198-9
35. Mondul AM, Selvin E, De Marzo AM, Freedland SJ, Platz EA (2010) Statin drugs, serum cholesterol, and prostate-specific antigen in the National Health and Nutrition Examination Survey 2001–2004. Cancer Causes Control 21(5):671–678. doi:10.1007/s10552-009-9494-9
36. Johnson CL, Paulose-Ram R, Ogden C (2013) National health and nutrition examination survey: analytic guidelines, 1999-2010. Vital Health Stat 2(161):1–16

37. Penalvo JL, Lopez-Romero P (2012) Urinary enterolignan concentrations are positively associated with serum HDL cholesterol and negatively associated with serum triglycerides in U.S. adults. J Nutr 142(4):751–756. doi:10.3945/jn.111.150516
38. Barnes S, Coward L, Kirk M, Sfakianos J (1998) HPLC-mass spectrometry analysis of isoflavones. Proc Soc Exp Biol Med 217(3):254–262
39. National Center for Health Statistics (2004) National Health and Nutrition Examination Survey. 2001–2002 Data documentation, codebook, and frequencies
40. Valentin-Blasini L, Sadowski MA, Walden D, Caltabiano L, Needham LL, Barr DB (2005) Urinary phytoestrogen concentrations in the U.S. population (1999–2000). J Expo Anal Environ Epidemiol 15(6):509–523. doi:10.1038/sj.jea.7500429
41. Rybak ME, Sternberg MR, Pfeiffer CM (2013) Sociodemographic and lifestyle variables are compound- and class-specific correlates of urine phytoestrogen concentrations in the U.S. population. J Nutr 143(6):986S–994S. doi:10.3945/jn.112.172981
42. Kelley-Hedgepeth A, Lloyd-Jones DM, Colvin A, Matthews KA, Johnston J, Sowers MR, Sternfeld B, Pasternak RC, Chae CU (2008) Ethnic differences in C-reactive protein concentrations. Clin Chem 54(6):1027–1037. doi:10.1373/clinchem.2007.098996
43. Timpson NJ, Nordestgaard BG, Harbord RM, Zacho J, Frayling TM, Tybjaerg-Hansen A, Smith GD (2011) C-reactive protein levels and body mass index: elucidating direction of causation through reciprocal Mendelian randomization. Int J Obes (Lond) 35(2):300–308. doi:10.1038/ijo.2010.137
44. Kurzer MS (2003) Phytoestrogen supplement use by women. J Nutr 133(6):1983S–1986S
45. Davison S, Davis SR (2003) New markers for cardiovascular disease risk in women: impact of endogenous estrogen status and exogenous postmenopausal hormone therapy. J Clin Endocrinol Metab 88(6):2470–2478
46. Ridker PM, Hennekens CH, Rifai N, Buring JE, Manson JE (1999) Hormone replacement therapy and increased plasma concentration of C-reactive protein. Circulation 100(7):713–716
47. Frohlich M, Sund M, Lowel H, Imhof A, Hoffmeister A, Koenig W (2003) Independent association of various smoking characteristics with markers of systemic inflammation in men. Results from a representative sample of the general population (MONICA Augsburg Survey 1994/95). Eur Heart J 24(14):1365–1372
48. Alley DE, Seeman TE, Ki Kim J, Karlamangla A, Hu P, Crimmins EM (2006) Socioeconomic status and C-reactive protein levels in the US population: nHANES IV. Brain Behav Immun 20(5):498–504. doi:10.1016/j.bbi.2005.10.003
49. Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM (2001) C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. JAMA 286(3):327–334
50. Panichi V, Migliori M, De Pietro S, Taccola D, Bianchi AM, Norpoth M, Metelli MR, Giovannini L, Tetta C, Palla R (2001) C reactive protein in patients with chronic renal diseases. Ren Fail 23(3–4):551–562
51. Hage FG (2013) C-reactive protein and hypertension. J Hum Hypertens. doi:10.1038/jhh.2013.111
52. Adlercreutz H (2007) Lignans and human health. Crit Rev Clin Lab Sci 44(5–6):483–525. doi:10.1080/10408360701612942
53. Wang CS, Sun CF (2009) C-reactive protein and malignancy: clinico-pathological association and therapeutic implication. Chang Gung Med J 32(5):471–482
54. Valentin-Blasini L, Blount BC, Caudill SP, Needham LL (2003) Urinary and serum concentrations of seven phytoestrogens in a human reference population subset. J Expo Anal Environ Epidemiol 13(4):276–282. doi:10.1038/sj.jea.7500278
55. Adolphe JL, Whiting SJ, Juurlink BH, Thorpe LU, Alcorn J (2010) Health effects with consumption of the flax lignan secoisolariciresinol diglucoside. Br J Nutr 103(7):929–938. doi:10.1017/S0007114509992753
56. van der Schouw YT, Sampson L, Willett WC, Rimm EB (2005) The usual intake of lignans but not that of isoflavones may be related to cardiovascular risk factors in U.S. men. J Nutr 135(2):260–266
57. Gaskins AJ, Wilchesky M, Mumford SL, Whitcomb BW, Browne RW, Wactawski-Wende J, Perkins NJ, Schisterman EF (2012) Endogenous reproductive hormones and C-reactive protein across the menstrual cycle: the BioCycle Study. Am J Epidemiol 175(5):423–431. doi:10.1093/aje/kwr343