# The association between urinary phytoestrogen excretion and components of the metabolic syndrome in NHANES

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# The association between urinary phytoestrogen excretion and components of the metabolic syndrome in NHANES

**Tristan Struja · Aline Richard · Jakob Linseisen · Monika Eichholzer · Sabine Rohrmann**

**Abstract**
*Background* Metabolic syndrome is a major risk factor for cardiovascular diseases, which are still the major cause of death in developed countries.
*Methods* We cross-sectionally studied the association between urinary phytoestrogen excretion and metabolic cardiovascular risk factors. Hence, we used data from the National Health and Nutrition Examination Survey from 1999 to 2004 with 1,748 participants, who had urine levels of isoflavones and lignans measured. Geometric means of waist circumference, blood pressure, fasting glucose, HDL cholesterol, and triglyceride levels were computed by quartiles of isoflavone or lignan urinary excretion. Outcome was assessed as the presence of metabolic syndrome according to NCEP-ATP III criteria. The association between phytoestrogen concentration and the metabolic syndrome was calculated using logistic regression analyses.
*Results* Plasma triglyceride and HDL cholesterol levels were lower in participants in the highest quartile of lignan excretion compared with the lowest (both $P < 0.01$). However, blood pressure, waist circumference, and plasma glucose levels did not differ significantly between extreme quartiles. The presence of metabolic syndrome was lower with increasing levels of urinary lignans (OR 0.48, 95 % CI 0.28; 0.80 top vs. bottom quartile), especially when separately computed for the excretion of enterolactone (OR 0.47, 95 % CI 0.28; 0.78). There was no significant association between isoflavone excretion and any component of the metabolic syndrome.
*Conclusions* Our study shows that an increasing excretion of lignans, especially enterolactone, might be associated with a decreased presence of the metabolic syndrome.

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## Introduction

Over the past years, the metabolic syndrome has become a major issue to the healthcare systems of developed countries. The worldwide prevalence of the common sequel diabetes has increased from 153 million people in 1980 to 347 million in 2008. However, the steep increase is alarming not only in Western countries, but also in Oceania and South America [1]. Thus, the World Health Organization assumes that deaths from diabetes will increase by two-thirds from 2008 to 2030 [2].

For quite some time, phytoestrogens have become an object of interest as additional treatment option of the metabolic syndrome [3]. Phytoestrogens are secondary plant products and have been named because of their structural and functional similarity to the 17β-estradiol. They have hormonal activities by either agonistically or antagonistically binding to the estrogen receptor and can be divided into four groups: coumestans, lignans, isoflavones, and stilbenes, among which isoflavones and lignans have raised great interest as their dietary intake has been well documented [4]. Isoflavones belong to the large class of

flavonoids, and their main dietary components are daidzein, genistein, formononetin, and biochanin A, of which daidzein and genistein are the ones most widely studied. They can be found in numerous vegetables, fruits, legumes, flaxseed, and especially soybeans [5]. They are also hidden in industrial products, i.e., baked goods, being produced with soy flour [6]. The most abundant lignans are lariciresinol, pinoresinol, syringaresinol, and medioresinol. A long time, only matairesinol and secoisolariciresinol were investigated, and thus, lignan uptake was largely underestimated in Western diets. Lignans can be found in berries, cereals, and especially flaxseed [7]. Isoflavones and lignans are mostly consumed as glycoside and metabolized by bacteria in the large intestine into their biologically active forms. Although the binding capacity to the estradiol receptors is much lower than for their endogenous counterparts, it is assumed that due to the much higher serum levels, they provide a strong biological effect in the human body [8]. The exact ways how phytoestrogens influence the metabolism is still unclear. On the one hand, they seem to have a certain influence on hypothalamic neurons decreasing food intake and increasing activity levels. On the other hand, the activation of estrogen receptor α seems leading to a lower adipogenesis and increases the number of expressed glucose transporters on muscle cells [9].

It has been well documented that the amount of these hormonally active components in a Western diet can easily be assessed by measuring urinary concentrations [5, 10, 11].

It has not only been shown that phytoestrogens seem to lower the risk of breast [12] and prostate [13] cancer, but in numerous studies, it was also possible to show a beneficial effect on cardiovascular risk factors as reviewed by Cederroth [9]. Several clinical studies have been conducted to answer the question whether phytoestrogens may improve cardiovascular parameters. It has been reported that isoflavone consumption correlated with higher HDL and lower BMI [14–16]. A Taiwanese study on postmenopausal women comparing the effects of isoflavones and estrogens observed a reduction in fasting glucose and insulin levels with a daily intake of 100 mg isoflavones [17]. On the other hand, there have also been some studies reporting no effects of phytoestrogens on some parameters of the metabolic syndrome [18–20].

These favorable effects have already been taken into account by the US Food and Drug Administration, which issued a health claim regarding the beneficial effects of soy proteins on coronary heart disease [21]. On the other hand, the Natural Health Products Directorate of Health Canada stated in August 2009 that there was not enough evidence to support any health claims, and further research in this field is warranted [22]. Overall, it is still unclear which impact phytoestrogens have on cardiovascular health as numerously reviewed. It seems as if the ability of a hosts gut flora to convert daidzein into equol, a feature only common to one-third of the human population, is an important factor. Up-to-date it is not clear which bacteria are responsible for this effect, and whether or not it can be changed lastingly [7, 9, 23].

The objective of this study was to assess a possible association between urinary excretion of isoflavones and lignans, assuming that a higher excretion is associated with a lower presence of the metabolic syndrome. The data were derived from the National Health and Nutrition Examination Survey (NHANES) conducted between 1999 and 2004. Previously, Peñalvo and Lopez–Romero have reported similar results on lipids in this survey. We extended this analysis by looking not only at lipids but also at other components of the metabolic syndrome [24].

## Methods

### Study population

All data were derived from the United States NHANES, a cross-sectional study conducted by the Centers for Disease Control and Prevention and under the supervision of the National Center for Health Statistics representing the civilian, non-institutionalized US population at least two months old from the years 1999 to 2004. After its beginning in the early 1960s, it has become a continuous program examining a nationally representative sample of approximately 5,000 persons per year in mobile examination centers (MEC) traveling throughout the country. To ensure the production of reliable data with minimal sample sizes, the study is designed to over-sample persons older than 60 years, African Americans and Hispanics [25].

The NHANES study protocols accord with the guidelines laid down in the Declaration of Helsinki and were approved by National Center for Health Statistics (NCHS) Research Ethics Review Board (ERB), and written informed consent was obtained from all participants [26].

Our analysis is based on the NHANES samples 1999/2000, 2001/2002, and 2003/2004. In these surveys with a total of 31,126 participants, phytoestrogen concentrations in the urine were measured in one-third of the population older than 6 years of age, leaving 7,226 [27–29].

Of these participants, we excluded all individuals younger than 20 years of age ($n = 2{,}985$) and pregnant women as this may interact with the possible estrogenic effects of the phytoestrogens, leaving 4,032 individuals. Because plasma glucose levels and serum lipid levels are depending on nutritional intake and fasting status, we additionally excluded participants fasting less than 8 h, leaving 2,668 participants. Furthermore, we only included persons from the NHANES morning sessions as triglyceride

levels were only measured then. This left 1,748 participants for the final analysis. We compared the distributions (unweighted) of the above-mentioned covariates in participants of our sample with those without morning sample weights to evaluate differences between these two groups.

### General data collection

The participants were first interviewed at home with questions on sex, age, race/ethnicity, income, and education by a standardized questionnaire, followed by an extensive physical examination including blood and urine samples at the MEC. Sensitive habits such as alcohol and illicit drug use, sexual behavior, smoking, and current health status were assessed in a computer-assisted self-interview. A 24-h recall was used to assess the dietary intake of nutrients [26].

Height, weight, and waist circumference were measured during the examination procedure. Body mass index (BMI) was calculated by dividing weight in kilograms by height in meters squared. Blood pressure was measured using an automated electronic heart rate and blood pressure monitor from Colin (STBP-780). Urine creatinine was measured in a Jaffé reaction with a CX 3 analyzer from Beckman Coulter [30].

### Urinary phytoestrogens

A spot urine sample was obtained at the MEC in collection cups (borosilicate glass or polypropylene or standard specimen cup) and was deep-frozen at −20 °C right away. Phytoestrogen concentration was measured by two different methods. In the survey years 1999–2002, samples were analyzed by a HPLC–APCI–MS technique as reported by Barnes et al. [30, 31]. In the following years, the analysis was performed at the CDC's Division of Laboratory of Sciences by a HPLC–electrospray ionization MS [32]. These two methods are comparable, and they both incorporated an enzymatic deglucoronidation before a solid-phase extraction was conducted. Afterwards, a reverse-phase HPLC was performed to resolve the components. A MS with internal isotope-labeled standards was used to assure the proper accuracy and the detection limit. A quality protocol according to the Westgard rules was used [33].

No differences between the two different HPLC methods for urinary phytoestrogens [27, 29] were reported in the NHANES protocols, and it has furthermore been assessed by Peñalvo et al. [24], yielding no biologically reasonable differences (NHANES 1999–2002 vs. 2003–2004).

### Plasma glucose

The blood samples were drawn into sodium fluoride vials. They were centrifugated immediately, and plasma was frozen at −70 °C. Plasma glucose was measured indirectly in a coupled enzymatic reaction assessing the absorbance of a by-product [30].

### Serum lipids

Cholesterol blood samples were drawn into EDTA vials. Total cholesterol was measured indirectly in a coupled enzymatic reaction. In NHANES 1999–2000, HDL cholesterol was assessed by a heparin–manganese precipitation method to remove apolipoprotein B containing particles. In NHANES 2001–2004, HDL cholesterol was assessed directly in serum with a reagent blocking apolipoprotein B non-reactive. Both methods during years 1999–2002 showed undesirable bias and were adjusted according to Solomon Park Research Laboratories (Kirkland, WA) quality controls. Serum LDL levels were calculated according to the Friedewald formula [34, 35]. Triglycerides were also measured indirectly in a coupled enzymatic reaction [34]. In particular, triglycerides levels are influenced by diet, so we excluded persons not fasting for at least 8 h.

### Metabolic syndrome

Several proposals for a clinical diagnosis of the metabolic syndrome have been made during the past 15 years. We based our calculations on the revised model proposed by the National Cholesterol Education Program—Adult Treatment Panel III (NCEP-ATP III) from 2004 [36] because of its clinical practicability. Furthermore, it does not require a single predisposing cause and/or medical condition, i.e., impaired fasting glucose or insulin resistance testing, to diagnose a metabolic syndrome, but is rather based on the simple scoring of easily assessable clinical parameters (at least three out of five). Additionally, this definition has been verified by a large number of studies and has widely been accepted not only in the USA but worldwide.

According to the NCEP-ATP III definition, these five criteria made up the score for the metabolic syndrome: waist circumference $\geq$102 cm in men or $\geq$88 cm in women; systolic blood pressure $\geq$130 mmHg or use of antihypertensives or diastolic blood pressure $\geq$85 mmHg; plasma triglycerides $\geq$1.7 mmol/L or use of cholesterol-lowering medication; plasma HDL cholesterol $<$1.00 mmol/L (men) or $<$1.30 mmol/L (women); fasting plasma glucose level $\geq$5.6 mmol/L or use of glucose-lowering medication.

### Statistical analysis

The statistical analysis was performed using STATA software version 11.2 (College Station, Texas). Urinary

phytoestrogen concentrations were standardized to creatinine concentration and expressed as μg/g creatinine. Furthermore, they were computed as geometric means. The study population was separated into quartiles of urinary phytoestrogen concentration and weighted to take into account over-sampling, refusal, selection probabilities, and differences to the general US population [25] rendering data representative. We computed means of waist circumference, systolic and diastolic blood pressure as well as circulating levels of triglycerides, HDL, and glucose by quartiles of urinary excretion of isoflavones and lignans. Test for trend over quartiles was performed using the means of creatinine-standardized phytoestrogen quartiles as a continuous variable and performing a linear regression. Logistic regression was used to examine the association of urinary concentrations of isoflavones and lignans (in quartiles) with every single dichotomized variable (yes or no) that defines metabolic syndrome: central obesity, hypertension, elevated triglycerides, low HDL cholesterol, hyperglycemia, and the metabolic syndrome itself. Based on our working hypothesis, we defined confounders and created three logistic linear models to study the association between the metabolic syndrome and geometric means of urinary phytoestrogen levels. Model 1 was an unadjusted model. Model 2 was adjusted by age, sex, race/ethnicity, poverty income ratio (below poverty, at or above poverty [25]), and educational level. Model 3 was furthermore adjusted for smoking (never, former, or current), alcohol consumption (drinks per day 0, ≤1, or >1), menopausal status, use of hormone replacement therapy (HRT; yes or no), and BMI. The analysis yielded no major differences between the different models; hence, only results of model 3 are shown. Test for trend was conducted using the means of the creatinine-standardized phytoestrogen quartiles as a continuous variable and performing a logistic regression with the desired outcome term and the new phytoestrogen variable. All significance tests were two-sided, and $P < 0.05$ was considered to be statistically significant.

## Results

In our study population, 29.1 % met the criteria of metabolic syndrome. Among the participants with the metabolic syndrome, 92.1 % had a large waist circumference, 76.4 % had hypertension, 75.4 % had elevated triglyceride concentrations, 70.1 % had low HDL cholesterol concentrations, and 21.7 % had elevated glucose concentrations. The mean age of the participants was 46 years. People with a metabolic syndrome were older than those without (51 years, 95 % CI 50; 53 compared to 43 years, 95 % CI 42; 45). The geometric mean of total urinary excretion of lignans was 12.9 μg/g creatinine (95 % CI 12.7; 13.0), whereas geometric mean of total excretion of isoflavones was 4.9 μg/g creatinine (95 % CI 4.8; 5.0) (Table 1).

Roughly, 12 % of the participants lived in poverty. Almost 20 % had not attained a high school degree, whereas 54 % achieved a higher education than high school. More participants with metabolic syndrome had an education above high school level than those without metabolic syndrome (54.8 vs. 48.9 %). Smoking was more common among those without metabolic syndrome (27.1–22.2 %), whereas the proportion of former smokers was higher among participants with metabolic syndrome (24.7 %) than among those without the metabolic syndrome (23.5 %); 12.1 % of the female participants were HRT users as defined as estrogen intake in women older than 50 years. HRT rate was higher in women with a metabolic syndrome (16.9 %) than in those without (10.3 %). The mean daily intake of selected nutrients did not differ appreciably between participants with and without the metabolic syndrome, but it has to be taken into account that only one 24-h dietary recall has been used to assess a participant's diet.

There was no statistically significant difference in systolic or diastolic blood pressure between the highest and lowest quartiles of isoflavone excretion, but systolic blood pressure tended to decrease with increasing lignan excretion (Table 2). This corresponds with results of the logistic regression model such that we observed no significant association between isoflavone excretion and hypertension, but lignans were inversely associated with hypertension (fourth vs. first quartile: OR 0.62, 95 % CI 0.40; 0.96) (Table 3).

There was neither a difference in plasma glucose levels between the extreme quartiles of isoflavones nor lignan excretion (Table 2). There was also no statistically significant association between elevated fasting glucose levels (≥5.6 mmol/L) and urinary phytoestrogens concentrations (Table 3).

We observed a slightly, but statistically significantly lower mean waist circumference in participants in the lowest quartile of isoflavone excretion compared with participants in the highest quartile (98.1 vs. 97.5 cm, $P < 0.05$), but the difference was not significant between extreme quartiles of lignan excretion (Table 2). However, using logistic regression analysis, there was no statistically significant association of central obesity with isoflavone or lignan excretion (Table 3), not even if stratified by sex (data not shown).

There was a statistically significant difference in HDL cholesterol concentration between the first and fourth quartile of isoflavones (1.27 vs. 1.34 mmol/L, $P < 0.05$) and lignans (1.27 vs. 1.37 mmol/l, $P < 0.01$) (Table 2). In the multivariable logistic model (Table 3), the odds of having low HDL cholesterol concentrations were

**Table 1** General characteristics of the study population; NHANES 1999–2004

| | Total ($n$ = 1,748) | | | No metabolic syndrome[a,b] ($n$ = 1,239) | | | Metabolic syndrome[a,b] ($n$ = 509) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mean | 95 % CI | | Mean | 95 % CI | | Mean | 95 % CI | |
| Participants included (no.) | 1,748 | | | 1,196 | | | 552 | | |
| Sex, women (%) | 51.4 | | | 50.2 | | | 54.9 | | |
| Age (years) | 45.57 | 44.60 | 46.53 | 43.51 | 42.37 | 44.65 | 51.38 | 49.54 | 53.23 |
| Isoflavones (μg/g creatinine)[c] | 4.85 | 4.76 | 4.95 | 4.88 | 4.76 | 4.99 | 4.79 | 4.62 | 4.96 |
| Lignans (μg/g creatinine)[d] | 12.85 | 12.74 | 12.95 | 12.95 | 12.82 | 13.07 | 12.57 | 12.38 | 12.77 |
| Dietary intake of selected nutrients[e] | | | | | | | | | |
| Total saturated fatty acids (gm) | 27.70 | 26.75 | 28.64 | 27.39 | 26.29 | 28.49 | 28.56 | 26.72 | 30.41 |
| Total polyunsaturated fatty acids (gm) | 17.54 | 16.90 | 18.19 | 17.79 | 17.03 | 18.56 | 16.85 | 15.84 | 17.86 |
| Dietary fiber (gm) | 15.40 | 14.81 | 15.99 | 15.50 | 14.81 | 16.19 | 15.13 | 14.14 | 16.12 |
| Total Folate (μg) | 398.06 | 384.62 | 411.51 | 404.26 | 390.00 | 418.52 | 380.89 | 361.10 | 400.68 |
| Vitamin C (mg) | 86.77 | 80.49 | 93.05 | 87.04 | 79.71 | 94.37 | 86.03 | 75.82 | 96.23 |
| Calcium (mg) | 870.91 | 831.05 | 910.77 | 877.31 | 831.41 | 923.21 | 853.17 | 795.05 | 911.30 |
| Magnesium (mg) | 279.53 | 272.12 | 286.94 | 282.90 | 275.06 | 290.74 | 270.21 | 257.00 | 283.41 |
| Race (%) | | | | | | | | | |
| Non-Hispanic white | 72.0 | | | 71.5 | | | 73.3 | | |
| Non-Hispanic black | 10.8 | | | 11.8 | | | 8.1 | | |
| Mexican American | 7.5 | | | 7.5 | | | 7.5 | | |
| Other | 9.7 | | | 9.2 | | | 11.0 | | |
| Poverty income ratio (PIR) (%) | | | | | | | | | |
| Below poverty | 12.3 | | | 12.1 | | | 13.1 | | |
| At or above poverty | 81.0 | | | 81.2 | | | 80.5 | | |
| Missings | 6.6 | | | 6.7 | | | 6.3 | | |
| Education (%) | | | | | | | | | |
| <High school | 19.8 | | | 18.6 | | | 23.2 | | |
| High school | 26.2 | | | 24.8 | | | 30.0 | | |
| >High school | 54.1 | | | 56.6 | | | 46.8 | | |
| Average number of alcoholic drinks/day—past 12 months (%) | | | | | | | | | |
| 0 | 31.7 | | | 28.3 | | | 41.3 | | |
| ≤1 | 33.1 | | | 32.8 | | | 33.8 | | |
| >1 | 35.2 | | | 38.8 | | | 24.9 | | |
| Smoking history (%) | | | | | | | | | |
| Current smoker | 25.8 | | | 27.1 | | | 22.2 | | |
| Former smoker | 23.8 | | | 23.5 | | | 24.7 | | |
| Never | 50.4 | | | 49.4 | | | 53.1 | | |
| Body mass index (kg/m$^2$) | 28.4 | 27.9 | 28.8 | 26.9 | 26.5 | 27.2 | 32.7 | 32.0 | 33.4 |
| Waist circumference *(cm)* | 97.2 | 96.1 | 98.2 | 92.9 | 92.0 | 93.8 | 109.5 | 108.0 | 111.0 |
| Plasma glucose (mmol/L) | 5.6 | 5.5 | 5.7 | 5.3 | 5.3 | 5.4 | 6.4 | 6.1 | 6.6 |
| Systolic blood pressure (mmHg) | 122.2 | 121.1 | 123.4 | 119.5 | 118.1 | 121.0 | 129.9 | 128.3 | 131.4 |
| Diastolic blood pressure (mmHg) | 71.7 | 70.8 | 72.7 | 71.0 | 70.0 | 72.0 | 73.9 | 72.4 | 75.3 |
| HDL cholesterol (mmol/L) | 1.32 | 1.30 | 1.35 | 1.41 | 1.38 | 1.43 | 1.10 | 1.07 | 1.13 |
| Triglycerides (mmol/L) | 1.71 | 1.57 | 1.85 | 1.36 | 1.24 | 1.48 | 2.69 | 2.40 | 2.98 |
| Use of HRT in women (%) | 12.1 | | | 10.3 | | | 16.9 | | |

Exclusion due to limited data of phytoestrogen levels: participants younger than 20 years old, pregnancy, fasting less then 8 h

[a] Metabolic syndrome: one point for each of the following: systolic blood pressure ≥130 mmHg or use of antihypertensives or diastolic blood pressure ≥85 mmHg; plasma triglycerides ≥1.7 mmol/L or use of cholesterol-lowering medication; plasma HDL cholesterol <1.00 mmol/L (men) or <1.30 mmol/L (women); waist circumference >102 cm (men) or >88 cm (women); plasma glucose level ≥5.6 mmol/L or use of glucose-lowering medication; range 0–5 points, diagnosis of metabolic syndrome met with ≥3 points

[b] Values are adjusted for age, race/ethnicity, education, income, smoking, alcohol categories, BMI

[c] Geometric mean in μg/g creatinine: daidzein, equol, genistein, o-demethylangolesin

[d] Geometric mean in μg/g creatinine: enterodiol, enterolactone

[e] From 24-h recalls

**Table 2** Linear regression of means of metabolic syndrome parameters; participants aged ≥20 years old, NHANES 1999–2004

| Quartiles of urine concentration | Systolic blood pressure | | Diastolic blood pressure | | Plasma glucose | | Waist circumference | | HDL | | Triglycerides | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean (mmHg) | 95 % CI | Mean (mmHg) | 95 % CI | Mean (mmol/L) | 95 % CI | Mean (cm) | 95 % CI | Mean (mmol/L) | 95 % CI | Mean (mmol/L) | 95 % CI |
| Isoflavones (μg/g creatinine)[a] | | | | | | | | | | | | |
| <44.4 | 124.7 | 122.4, 126.9 | 71.6 | 69.9, 73.4 | 5.72 | 5.57, 5.86 | 98.1 | 97.6, 98.7 | 1.27 | 1.22, 1.31 | 2.09 | 1.66, 2.52 |
| 44.39–109.8 | 124.5 | 122.5, 126.4 | 70.2 | 68.2, 72.1 | 5.85 | 5.67, 6.03 | 98.1 | 97.5, 98.7 | 1.31 | 1.24, 1.38 | 1.69 | 1.53, 1.86 |
| 109.8–314.9 | 126.3 | 124.7, 127.9 | 71.6 | 70.2, 73.0 | 5.86 | 5.65, 6.07 | 97.8 | 96.9, 98.7 | 1.31 | 1.27, 1.35 | 1.70 | 1.53, 1.87 |
| >314.9 | 125.3 | 123.3, 127.2 | 72.1 | 70.7, 73.6 | 5.76 | 5.62, 5.89 | 97.5 | 97.0, 98.0 | 1.34 | 1.30, 1.39 | 1.56 | 1.46, 1.66 |
| *P*-trend | 0.49 | | 0.30 | | 0.87 | | <0.05 | | <0.05 | | <0.05 | |
| Lignans (μg/g creatinine)[b] | | | | | | | | | | | | |
| <153,030 | 127.0 | 125.0, 129.0 | 72.3 | 70.5, 74.2 | 5.65 | 5.50, 5.80 | 98.5 | 97.9, 99.2 | 1.27 | 1.23, 1.32 | 2.04 | 1.82, 2.26 |
| 153,030–372,512 | 124.7 | 122.8, 126.6 | 70.6 | 69.2, 72.0 | 5.98 | 5.80, 6.16 | 97.8 | 97.0, 98.6 | 1.29 | 1.25, 1.33 | 1.90 | 1.58, 2.22 |
| 372,513–794,500 | 125.2 | 123.4, 127.1 | 71.4 | 69.6, 73.2 | 5.68 | 5.54, 5.82 | 97.6 | 97.0, 98.3 | 1.30 | 1.25, 1.36 | 1.68 | 1.41, 1.94 |
| >794,500 | 123.8 | 121.6, 126.0 | 71.3 | 69.6, 72.9 | 5.87 | 5.63, 6.10 | 97.5 | 96.8, 98.2 | 1.37 | 1.31, 1.43 | 1.46 | 1.33, 1.59 |
| *P*-trend | 0.07 | | 0.73 | | 0.54 | | 0.09 | | <0.01 | | <0.001 | |

Values are weighted and age-standardized, for definition of variables see "Methods" section

[a] Daidzein, equol, genistein, o-demethylangolesin

[b] Enterodiol, enterolactone

significantly lower in participants with highest levels of isoflavone (OR 0.65, 95 % CI 0.46; 0.92) and lignan (OR 0.64, 95 % CI 0.41; 0.99) excretion compared with lowest levels.

Circulating levels of triacylglycerols differed between extreme quartiles of isoflavones (first quartile 2.09 vs. fourth quartile 1.56 mmol/L, $P < 0.05$) and lignan excretion (2.04 vs. 1.46 mmol/L, $P < 0.001$) (Table 2). There was no statistically significant association between isoflavone excretion and having elevated triglyceride concentration (Table 3), but lignan excretion was statistically significantly inversely associated with the odds of having elevated triglyceride concentration (fourth vs. first quartile: OR 0.39, 95 % CI 0.28; 0.56).

In the multivariable logistic model (Table 3), only high urinary lignan concentrations were related to decreased odds of metabolic syndrome (top vs. bottom quartile: OR 0.48, 95 % CI 0.28; 0.80). To further differentiate the responsible agent, we computed values for enterodiol and enterolactone separately and observed a statistically significantly inverse association between enterolactone excretion and odds of metabolic syndrome (OR 0.47, 95 % CI 0.28; 0.78), but not for enterodiol excretion (OR 0.73, 95 % CI 0.44; 1.20). The test for heterogeneity between the two lignans was not statistically significant ($P > 0.05$). We also computed the results adjusted for the dietary nutrients shown in Table 1. There were no appreciable differences. We further compared the means of waist circumference, blood pressure, glucose, and HDL levels of our sample (morning sessions) with the means of the non-included participants from the afternoon sessions ($n = 920$), yielding no particular difference. Just the levels of urinary lignans and isoflavones were roughly 100 μg/g creatinine lower in the afternoon sessions.

## Discussion

In this cross-sectional study of the general US population, we observed a statistically significant association between urinary excretion of lignans and the metabolic syndrome, such that participants with high excretion of these lignans, in particular enterolactone, had lower odds of having the metabolic syndrome compared with participants with low lignan concentrations. When looking at single components of the metabolic syndrome, an increase in HDL cholesterol over quartiles of both isoflavones and lignans was observed as well as a decrease in triglyceride concentration and a lower waist circumference.

To the best of our knowledge, we conducted the first study on urinary phytoestrogen excretion and their effects on all parameters of the metabolic syndrome. So far, most studies only examined single components of the metabolic

**Table 3** Multivariable logistic association between quartiles of phytoestrogen concentration and parameters of metabolic syndrome; participants aged ≥20 years old, NHANES 1999–2004

| | Quartiles of phytoestrogen concentration[a] | | | | | | | Test for trend |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | | 3 | | 4 | | |
| | Odds ratio | Odds ratio | 95 % CI | Odds ratio | 95 % CI | Odds ratio | 95 % CI | *P* value |
| Hypertension | | | | | | | | |
| Isoflavones[b] | 1.00 | 0.83 | 0.51–1.36 | 0.82 | 0.55–1.20 | 0.87 | 0.56–1.36 | 0.58 |
| Lignans[c] | 1.00 | 0.81 | 0.54–1.22 | 0.73 | 0.48–1.09 | 0.62 | 0.40–0.96 | 0.045 |
| Plasma glucose level | | | | | | | | |
| Isoflavones[b] | 1.00 | 0.73 | 0.31–1.71 | 1.36 | 0.62–2.95 | 1.09 | 0.62–2.95 | 0.45 |
| Lignans[c] | 1.00 | 1.84 | 0.89–3.80 | 1.01 | 0.37–2.80 | 1.98 | 0.79–4.95 | 0.56 |
| Waist circumference | | | | | | | | |
| Isoflavones[b] | 1.00 | 0.99 | 0.58–1.69 | 1.33 | 0.57–3.13 | 0.90 | 0.48–1.67 | 0.99 |
| Lignans[c] | 1.00 | 0.61 | 0.28–1.33 | 0.52 | 0.28–0.96 | 0.86 | 0.42–1.76 | 0.61 |
| HDL | | | | | | | | |
| Isoflavones[b] | 1.00 | 0.87 | 0.57–1.32 | 0.89 | 0.54–1.43 | 0.65 | 0.46–0.92 | 0.03 |
| Lignans[c] | 1.00 | 0.73 | 0.52–1.03 | 0.59 | 0.39–0.89 | 0.64 | 0.41–0.99 | 0.06 |
| Triglycerides | | | | | | | | |
| Isoflavones[b] | 1.00 | 0.84 | 0.58–1.21 | 0.97 | 0.66–1.42 | 0.77 | 0.54–1.11 | 0.20 |
| Lignans[c] | 1.00 | 0.66 | 0.41–1.05 | 0.46 | 0.33–0.65 | 0.39 | 0.28–0.56 | <0.001 |
| Metabolic syndrome[d] | | | | | | | | |
| Isoflavones[b] | 1.00 | 1.04 | 0.69–1.56 | 1.00 | 0.62–1.61 | 0.81 | 0.55–1.18 | 0.24 |
| Lignans[c] | 1.00 | 0.67 | 0.45–1.00 | 0.57 | 0.37–0.86 | 0.48 | 0.28–0.80 | 0.02 |
| Enterodiol | 1.00 | 0.75 | 0.47–1.18 | 0.70 | 0.45–1.10 | 0.73 | 0.45–1.20 | 0.12 |
| Enterolactone | 1.00 | 0.60 | 0.40–0.89 | 0.58 | 0.38–0.86 | 0.47 | 0.28–0.78 | 0.003 |

Values are adjusted for age, sex, race/ethnicity, education, income, smoking, alcohol categories, BMI, menopause status, and HRT

[a] Geometric mean in μg/g creatinine

[b] Daidzein, equol, genistein, o-demethylangolesin

[c] Enterodiol, enterolactone

[d] According to NCEP-ATP III definition: waist circumference ≥102 cm (men) or ≥88 cm (women); systolic BP ≥130 or diastolic BP ≥85 mmHg or use of medication; triglycerides ≥1.7 mmol/L or use of medication; HDL <1.00 mmol/L (men) or <1.30 mmol/L (women); glucose ≥5.6 mmol/L or use of medication

syndrome or used dietary questionnaires [37] to estimate phytoestrogen intake. There is only one randomized controlled study [38] using lignans, which showed a slight decrease in diastolic blood pressure and triacylglycerol only in men. Additionally, we included both men and women, whereas most previous research has only been conducted among women [15, 39, 40].

NHANES has only one 24-h dietary recall, and it is known that the elimination half-life of phytoestrogens ranges from 7 to 36 h, depending on the actual component [41–44]. This has led to concerns whether urinary excretion concentrations were valid biomarkers of dietary phytoestrogen intake, especially as these nutrients are to be consumed only on an irregular basis in Western societies [6]. To assure its reliability, several studies have been conducted with spot urine [5, 10, 11, 45], stating that measuring spot urinary phytoestrogen concentrations is a valid approach for estimating dietary phytoestrogen exposure.

One must be aware that the interpretation of our results is limited by the cross-sectional design, not allowing for the differentiation between cause and effect. To test whether phytoestrogen consumption affects the risk of metabolic syndrome, longitudinal studies with preferably multiple measurements would better reflect actual disease status (e.g., levels of glucose and cholesterol) and phytoestrogen levels. Although NHANES offers a large population sample reducing the chance for selection bias, only a subsample of one-third of the participants were tested for urinary phytoestrogens, thus, reducing sample size. However, large samples would be needed to detect statistically significant differences as large reviews of several studies [7, 9, 46, 47] showed only small effects of phytoestrogens in human populations. For example, a meta-analysis found a mean reduction of only 0.10 mmol/L of LDL cholesterol over all studies. HDL and triglyceride levels did not differ significantly after a lignan supplementation [48]. Hence, a

large randomized controlled trial would be necessary to draw a final conclusion. Persons prone to developing a metabolic syndrome might also be missed. This issue should best be addressed by a cohort study. Furthermore, we did not asses the excretion of other phytoestrogens such as stilbenes or coumestans. Although their actual impact on cardiovascular disease is not clear [7], they may have influenced our results.

Although we adjusted our results for potential differences in income/wealth of the study population and educational level, we did not see a difference between these models (models 1 and 2). This might be due to rather basic poverty income ratio and educational levels used by NHANES. A more elaborate data set on this issue would help to further delineate the social classes and their actual dietary habits and lifestyle. Consequently, we cannot exclude that richer households are the ones that not only lead a healthier lifestyle [49] but also are the ones consuming more vegetable products and having subsequently a higher phytoestrogen intake [50, 51]. Although we made adjustments for smoking status, it could be possible that former smokers changed their lifestyle vigorously after cessation, leading to an overall healthier life compared with never smokers.

It is well known that Asians have a higher intake of soy food than found in Western societies. So far, NHANES does not over-sample Americans of Asian descent [52], which is not going to be integrated until the 2011 surveys. This would warrant further studies as it is known that the amount of excreted metabolites varies greatly even among different Asian populations, although their diets lead to a higher consumption of phytoestrogenic nutrients, in particular isoflavones [53, 54]. Their possible increased consumption of soy products may lead to significant effects of isoflavones on the parameters we have observed. Based on the current NHANES data, we do not know of a comprehensive comparison of these three populations. In general, urinary levels of isoflavones of our sample were lower than those measured in Vietnamese and Japanese samples, whereas levels of lignans of our sample were higher compared to the samples of the Asian study [45]. However, the comparison between different studies is difficult because many different assessment methods are being used (e.g., study design, units, and measurements) making interpretation difficult. A future analysis of the NHANES 2011–2014 might reveal interesting insights, especially if the dietary intake of phytoestrogens in the Asian population is compared with the Caucasian. So far, a conclusive statement cannot be made.

Elevated plasma glucose concentrations are the hallmark of a beginning insulin resistance or the sign of a manifest type 2 diabetes mellitus leading to manifold complications, e.g., cardiovascular disease, diabetic retinopathy, or polyneuropathy [55]. It is commonly assumed that this underlying insulin resistance is a basic etiologic cause leading to the manifestation of the metabolic syndrome [56]. Several experimental trials with rodents have shown that the administration of phytoestrogens increases excretion of insulin by pancreatic β-cells and decreases peripheral insulin resistance by increasing glucose uptake in skeletal muscles [57–59]. There are also trials in humans presenting similar effects [17, 60, 61]. In contrast to these results, we could not detect any association of phytoestrogens excretion on plasma glucose levels in our analysis. We speculate this could be due to the small biological impact of phytoestrogens as consumed in a habitual diet, as already observed by others [20, 62].

Participants with high isoflavone or lignan excretion had lower waist circumference compared with those with low phytoestrogen excretion. However, there was no association between phytoestrogen excretion and risk of being centrally obese. Although several studies showed promising results on this topic [14, 19, 37, 63], there are contradictory results, too [15, 18, 38]. To the best of our knowledge, there is no comprehensive meta-analysis up-to-date.

There are several studies showing no effects of phytoestrogens on blood pressure as reviewed by Rosero et al. [64]. There is also increasing evidence that HRT in postmenopausal women is not able to lower blood pressure [65], which further supports this conclusion, as phytoestrogens are supposed to induce a similar state in postmenopausal women. In our study, we observed lower odds of hypertension among participants with high lignan excretion, but mean blood pressure values did not differ by quartiles of lignan excretion. Thus, taking the results of previous studies into account, we speculate that our observation in the logistic regression model might be due to residual confounding as mentioned above.

As previously published by Peñalvo and colleagues for the same set of data [24], we also observed a positive association between HDL cholesterol concentration and excretion of lignans and isoflavones. These effects are contradictory to two extensive meta-analyses of randomized controlled trials [48, 66], which reported no effects of flaxseed and lignan supplements or soy proteins, respectively, on HDL cholesterol concentrations. We also observed a significant decrease in triglyceride concentration with increasing levels of lignan excretion; an effect already observed by Peñalvo et al. [24]. This, however, is not consistent with the meta-analyses mentioned earlier [48], which did not show any statistically significant effects of lignans on triglyceride levels. To the best of our knowledge, there are no further data concerning this issue, and the differences found might be attributed to chance. Clinically, increases in HDL cholesterol are considered to

be more important because low HDL cholesterol is seen as major risk factor in atherosclerosis besides high levels of LDL cholesterol and triglycerides [67]. This HDL increasing association might even have clinical impact as most medications against dyslipidemia, except niacin, decrease LDL cholesterol and triglyceride concentrations but only increase HDL cholesterol slightly [67]. Other interventions such as HRT have shown to increase HDL cholesterol [68] but do have side effects that are not desired in a public being widely interested in alternative medical treatment [69].

Many authors consider the metabolic syndrome an accumulation of potent cardiovascular risk factors. Whether insulin resistance or obesity is the underlying cause of the metabolic syndrome is still a subject of debate [56]. Nevertheless, manifestation of diabetes mellitus type 2 increases the risk of cardiovascular diseases dramatically. At the end, these multiple risk factors have a much higher influence on cardiovascular disease than a single factor would have. There was no association between isoflavone excretion and presence of a metabolic syndrome in our study population, in contrast to de Kleijn and colleagues [37]. On the other hand, we observed statistically significant lower odds of metabolic syndrome with increasing amounts of urinary lignans, as already observed by others, too [37, 38]. To further analyze this effect, we computed results for both enterodiol and enterolactone, of which only enterolactone had a statistically significant impact. We assume this effect is mostly due to the beneficial effects of lignans on triglycerides and on HDL.

In summary, our study on the metabolic syndrome and its relation to the urinary excretion of phytoestrogens shows that a higher excretion of lignans, especially enterolactone, might be associated with the metabolic syndrome, such that we conclude that a high consumption of foods rich in lignans may protect from this complex of diseases—an association that has only been shown previously by de Kleijn, but for dietary-assessed phytoestrogens instead of urinary excretion [37]. This might be due to a healthier lifestyle but has been taken into account by adjusting for these confounders. However, further studies are needed to confirm our cross-sectional observation.

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