Urinary phytoestrogen levels and frailty in older American women of the National Health and Nutrition Examination Survey (NHANES) 1999-2002: a cross-sectional study

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Urinary Phytoestrogen Levels and Frailty in Older American Women of the National Health and Nutrition Examination Survey (NHANES) 1999–2002: A Cross-Sectional Study

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Abstract

Background/Aims: A deficit of various hormones during the process of aging and/or a heightened inflammatory state may be causally linked to the development of frailty. Phytoestrogens as weak estrogens, antioxidants, and anti-inflammatory agents may counteract this process. ***Methods:*** In a cross-sectional study including two cycles of the National Health and Nutrition Examination Survey (NHANES, i.e. 1999–2002), logistic regression was used to analyze the association between urinary concentrations of isoflavones and lignans and frailty in 600 females aged 50 years or older (median age 66.5 years). Participants were classified as 'frail' (meeting 3 or more of the 5 frailty criteria), 'prefrail' (meeting 1 or 2 of the criteria), or 'robust' (meeting none of the criteria). Four percent were frail. ***Results:*** For all of the phytoestrogens considered, the unadjusted OR were lower than 1 but generally not statistically significant aside from the association with O-desmethylan-golensin (O-DMA) (OR = 0.76; 95% CI 0.61–0.92). Multivariate analysis did not attenuate this finding (OR = 0.74; 95% CI 0.61–0.90). ***Conclusions:*** This first analysis of the relationship between phytoestrogens and frailty revealed an inverse association between urinary O-DMA levels and frailty in women. However, the number of frail women was low. Although this finding may be confounded or biased, it seems worthwhile to intensify research on the potential preventive effects of O-DMA.

Introduction

Frailty is characterized by a decreased resistance to stress due to cumulative declines in multiple interrelated physiologic systems [1, 2]. This results in an increased risk of falls, disability, hospitalization, institutionalization, and mortality [2]. Frailty is the consequence of impairment of multiple subsystems, such as sarcopenia [3], nutritional deficiencies [4], hormonal declines [5], or inflammation [6], and knowledge of the pathophysiological mechanisms of frailty remains limited.

The most widely used definition of frailty is the one by Fried et al. [2], which includes unintentional weight loss, self-reported exhaustion, poor grip strength, slow walking speed, or low physical activity. Frailty is rarely observed in the absence of (subclinical) chronic disease. Unintentional weight loss may be the consequence of catabolic processes in the course of an underlying disease or it may be related to low energy and nutrient intakes. Accordingly, the characteristics of 65- to 79-year-old women becoming frail during follow-up in the Women's Health Initiative study were: high and low body weight, smoking, depression, cardiovascular disease (CVD), diabetes, and chronic obstructive pulmonary disease [7]. Furthermore, in the InCHIANTI study [8] more than half of the frail participants had insufficient intakes of energy, protein, calcium, iron, zinc and the vitamins A, D, E, C, and/or folate. In this cross-sectional study a low energy intake was associated with exhaustion, and a low nutrient score of proteins and vitamins was associated with weakness.

However, frailty is not a unidirectional process [9]. It is possible to recover from frailty to prefrailty and nonfrailty conditions, respectively [10]. This makes the adoption of preventive measures attractive. Accordingly, trials investigating the effect of hormone replacement therapy (HRT) in women on muscle mass and strength suggest a direct relationship with levels of plasma estradiol, but their results remain conflicting [11]. There is even evidence that usual-dose estrogen may accelerate muscle loss [12] and that higher levels of endogenous estradiol are directly associated with frailty [3]. It is therefore of interest to examine the effect of natural, weak estrogens on the risk of frailty in elderly women, without the health risks associated with the use of HRT.

Phytoestrogens are naturally occurring plant constituents found in many human foodstuffs. As their name implies, phytoestrogens may act as weak estrogens [13]. In addition, they may act as antioxidants and anti-inflammatory agents [14]. There are three main groups of phytoestrogens, i.e. isoflavones (in soybeans and other legumes), lignans (in flaxseed and berries), and coumestans (in soybean sprouts) [13]. The major soy isoflavones are daidzin and genistin, the glycoside conjugates of daidzein and genistein [15]. After ingestion, daidzein can be metabolized by gut bacteria into diverse compounds such as equol and O-desmethylangolensin (O-DMA). About 30–35% of the Western population and up to 60% of vegetarians and Asians possess the ability to produce equol, but these percentages vary between studies, depending, among other things, on the urine

equol concentration used as the cutoff and the equol intake via milk products [16–19]. Equol is 100 times more estrogenic than daidzein [20]. Several animal products such as cheese, eggs, and milk also contain equol [18, 21]. The major human lignans are enterolactone and enterodiol, formed from plant precursors by the gut microflora [13].

So far, the health effects of phytoestrogens have often been studied by measuring the dietary intake of these compounds by study participants. However, measurement of the concentration of phytoestrogens in urine takes into account interindividual variations in microbial synthesis [22] and is independent of errors due to imprecise dietary assessments and databases. To determine whether frailty, based on a modified index of Fried et al. [2; see also 23], is associated with urinary phytoestrogen concentrations of isoflavones, lignans, genistein, daidzein, equol, equol of equol producers, O-DMA, enterolactone, and enterodiol, we used the data of women aged 50 years or older from the National Health and Nutrition Survey (NHANES) 1999–2002. To our knowledge, this is the first study to investigate the association between these urinary phytoestrogen levels and frailty.

Methods

Study Design
NHANES is an on-going cross-sectional study representative of the population of the USA, which is being conducted by the National Center for Health Statistics (NCHS). Data are released for 2 successive years (i.e. 1999/2000, 2001/2002, etc.). NHANES is a program of studies designed to assess the health and nutritional status of adults and children in the USA. The survey is unique in that it combines interviews and physical examinations.

The NHANES interview includes demographic, socioeconomic, dietary, and health-related questions. The examination component consists of medical, dental, and physiological measurements, as well as laboratory tests administered by highly trained medical personnel. All data are anonymized.

The sample for the survey is selected to represent US populations of all ages. A complex, multistage, probability sampling design is used to select participants representative of the civilian, noninstitutionalized US population. To produce reliable statistics, NHANES oversamples individuals aged 60 years or older, African-Americans, and Hispanics.

Participants (n = 21,004) were available from the 1999/2000 and 2001/2002 NHANES cycles. After exclusion of participants younger than 50 years, our sample consisted of 4,983 individuals. In a further step, all men were excluded, leaving 2,551 women in our data set. Of these women, 617 had available data on urinary phytoestrogen measurements. After defining frailty variables, finally 600 women were included in our analyses.

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The NHANES study protocols are in accordance with the guidelines put forth in the Declaration of Helsinki, and all procedures involving human subjects were approved by NCHS Research Ethics Review Board. Written informed consent was obtained from all subjects.

Measurement of Urinary Phytoestrogen Concentration

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 [24]. Vials were stored under appropriate frozen (−20°C) conditions until shipment to the National Center for Environmental Health for testing. An HPLC-MS/MS method was used to determine urinary phytoestrogen concentrations in NHANES [12]. The methods were adapted, which resulted in improved selectivity, sensitivity, and precision for the quantitative detection of phytoestrogens in human urine and serum. The method uses enzymatic deconjugation of the phytoestrogens followed by solid-phase extraction and reverse-phase HPLC to resolve the analytes. The phytoestrogens are detected using a Sciex API III heated nebulizer-atmospheric pressure chemical ionization (HN-APCI) interface coupled with MS/MS. The following phytoestrogens were measured in both NHANES cycles (1999/2000 and 2001/2002): daidzein, genistein, equol, O-DMA, enterodiol, and enterolactone. In our analyses, isoflavones were represented by daidzein, genistein, equol, and O-DMA. Lignans consisted of enterodiol and enterolactone.

The creatinine concentration was analyzed using a Jaffé rate reaction with a CX3 analyzer (Beckmann Instruments, Brea, Calif., USA) [25]. In our analyses, we used creatinine to standardize urinary phytoestrogen concentrations. Phytoestrogen concentrations were expressed as micrograms per gram of creatinine.

Assessment of Frailty

We based our analyses on the frailty score developed by Fried et al. [2], which was modulated to the data available in NHANES III by Wilhelm-Leen et al. [23] and to the data available in NHANES 1999–2002. This adapted score comprised the following 5 items: (1) low body weight for height, defined as a BMI ≤18.5; (2) slow walking, defined as the slowest quintile by sex in a timed 20-ft walk; (3) weakness, defined as present if participants answered 'some difficulty', 'much difficulty', or 'unable to do' when asked how much difficulty they have 'lifting or carrying something as heavy as ten pounds (like a sack of potatoes or rice)'; (4) exhaustion, defined as present if participants answered 'some difficulty', 'much difficulty', or 'unable to do' when asked how much difficulty they have 'walking from one room to the other on the same level', and (5) low physical activity, defined as present if participants answered 'less active' when asked: 'Compared with most women your age, would you say that you are more active, less active or about the same?'

In our analysis we included participants with valid information on at least 3 of the 5 frailty symptoms. One item was missing for 60 women, and 2 items were missing for 115 women. In total, all of the participants with 1 or 2 components missing emerged as belonging to the subgroup 'robust' (see below).

Women meeting 3 or more of the 5 criteria were classified as 'frail', those with 1 or 2 criteria were classified as 'prefrail', and those meeting none of the criteria were classified as 'robust'. In addition, the frailty variable was dichotomized into 'frails' (3 or more frailty criteria) versus 'nonfrails' (0–2 frailty criteria) for the purposes of our analyses [2, 23].

Other Explanatory Variables

Age, sex, race/ethnicity, poverty income ratio (PIR), cigarette smoking, and use of HRT were assessed using a questionnaire. Weight was measured to the nearest 0.01 kg using an electronic digital scale while the participant was wearing a paper shirt and paper pants and foam slippers. Height was measured to the nearest 0.1 cm using a stadiometer. The BMI was calculated as the weight in kilograms divided by the square of the height in meters. A self-reported history of congestive heart failure, coronary heart disease, heart attack, or stroke was used to define prevalent CVD, and a self-reported history of a diagnosis of cancer (other than nonmelanoma skin cancer) was used to define prevalent cancer. Participants were considered to be diabetic if they reported a history of a diagnosis of diabetes or were users of insulin or diabetic medication. A self-reported history of hypertension was assessed if the participants had been told at least twice by a doctor that they had hypertension and/or if the participants took medication.

Statistical Analysis

Analyses were performed using STATA version 11 (Stata Corporation, College Station, Tex., USA), and $p < 0.05$ was considered statistically significant (two-sided tests). Sampling weights were applied according NHANES guidelines to produce estimates that were representative of the noninstitutionalized, civilian US population [26].

We used medians to compare phytoestrogen levels in the categories of frailty (robust, prefrail, and frail) and percentages for demographic variables. Differences between groups were evaluated using a univariate t statistic or χ^2 test, respectively. All significance tests were two-sided using $p < 0.05$ as the level of statistical significance.

Frailty was dichotomized into frail and nonfrail (prefrail and robust) for logistic regression, which was used to calculate OR and 95% CI. We did combine prefrails with robusts and not with frails because we were not able to fully distinguish between robusts and prefrails due to missing data. Creatinine-standardized phytoestrogen concentrations were continuous variables. Besides the unadjusted model, we adjusted in a first model for age (≥50–59, 60–69, 70–79, and >80; equal results were found when we considered age as a continuous variable) and race/ethnicity (non-Hispanic black, non-Hispanic white, Mexican-American, and other). With the multivariable model, we wanted to evaluate potential confounders that influence frailty. Thus, besides age and race/ethnicity, we also included the PIR [which is the proportion of the self-reported family income to the US 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 an income above the poverty level, whereas a PIR value below 1 indicates poverty)], smoking history (former, current, or never), current HRT (yes or no), and chronic diseases, i.e. CVD, hypertension, diabetes, and cancer. Furthermore, we performed a subanalysis for equol concentration in equol producers. Equol producers were defined as having an equol concentration over the detection limit. Moreover, we conducted a sensitivity analysis comparing the prevalence of frailty symptoms and the distribution of frailty scores in the women of our sample (n = 600) with those in women aged 50 years or older not included in our sample (n = 1,951) to evaluate differences between these two groups of women.

Results

Tables 1 and 2 summarize the biochemical, lifestyle, and clinical variables of the participants with data on urinary phytoestrogen concentrations and frailty. Of the 600 women (unweighted n) included in the present analysis, 64.4% belonged to the category robust, 31.6% belonged to the category prefrail, and 4.0% belonged to the category frail. Comparing our sample to the nonincluded women in the same age range (n = 1,951), 70.1% belonged to the category robust, 27.1% belonged to the category prefrail, and 2.9% belonged to the category frail. The prevalence of frailty symptoms in the nonincluded sample was distributed as follows: weakness, 43.3%; slow walking, 19.3%; exhaustion, 18.3%; low physical activity, 20.1%, and low BMI, 1.6% (see table 1 for a comparison with the included women).

In relation to phytoestrogens, a higher percentage of frail women versus prefrail and robust women had a somehow lower urine concentration of lignans, equol, O-DMA, enterodiol, and enterolactone. The opposite was true for isoflavones, daidzein, and genistein. Among all women, 63.6% were equol producers. The equol concentration in frail equol producers was slightly higher than in robust women.

Moreover, a higher percentage of frails versus prefrails/robusts were HRT users. A history of CVD, diabetes, and hypertension, but not of cancer, was more often noted among frail women than prefrail and robust women (for statistically significant differences, see the p values in table 2).

Table 3 presents the associations between phytoestrogen concentrations and frailty. Unadjusted OR were below 1 for all phytoestrogens considered, but neither was statistically significant, with the exception of O-DMA (OR = 0.76; 95% CI 0.61–0.92). Models adjusting for age and race/ethnicity revealed similar results, again with a significantly reduced risk of frailty with increasing O-DMA urinary levels (OR = 0.76; 95% CI 0.62–0.92). The latter finding was not attenuated in multivariate analysis (OR = 0.74; 95% CI 0.61–0.90). Considering age as a continuous variable did not affect the results.

Discussion

To the best of our knowledge, this is the first study to investigate the association between urinary phytoestrogen concentrations and frailty. In our cross-sectional study, representative of noninstitutionalized US women aged 50

Table 1. Prevalence of frailty symptoms and distribution of frailty scores (unweighted n = 600) in women aged 50 years or older from NHANES 1999–2002

	n[a]	%[b]
Frailty score [23]		
0	371	64.4
1	150	22.2
2	57	9.4
3–5	22	4.0
Frailty score components		
Weakness	175	33.7
Slow walking	121	28.3
Exhaustion	56	11.6
Low physical activity	99	16.1
Low BMI	9	1.8

[a] Unweighted. [b] Weighted.

years or older, 64.4% were robust, 31.6% were prefrail, and 4.0% were frail. The low percentage of frails observed in the present analysis most probably reflects the rather low age group considered. Furthermore, some of the women may not yet have experienced menopause (no data on menopausal status were available), which is of importance for musculoskeletal health [27]. In addition, there may be an underestimation of the proportion of frails since NHANES requires participants to come in for examination. A considerable number of frails might have been physically unable to participate, and not all of the frail women might have used the alternative of a home examination. Accordingly, the prevalence of frailty in the community-dwelling population of Western countries has been estimated to be about 6–20% using various definitions of frailty [28, 29]. In comparison, in the first study estimating the prevalence of frailty in Taiwan, 4.9% of seniors aged 65–103 years were frail, 40% were prefrail, and 55.1% were nonfrail (no criterion of frailty) [28], i.e. the prevalence of frailty was lower than in Western countries. Moreover, we confirmed previous observations showing a higher risk of frailty for subjects with chronic disease such as diabetes [30], hypertension [31], or a history of CVD [32]. The urinary phytoestrogen concentrations in the present study were comparable to those in previous studies in the US population [20] but lower than, for example, those in people living in Japan or Vietnam [33].

We observed no significant association between frailty and urinary levels of isoflavones, lignans, genistein, enterodiol, enterolactone, daidzein, or equol in women. There was one exception: increasing urinary O-DMA lev-

Table 2. Age-standardized baseline characteristics by frailty status in women aged 50 years or older from NHANES 1999–2002

	Frail[a]			Prefrail[b] and robust[c]			p value
	median	Q1	Q3	median	Q1	Q3	
Phytoestrogens, µg/g creatinine							
Isoflavones[d]	116.8	28.4	423.6	104.1	46.2	339.9	0.033
Lignans[e]	394.2	107.6	766.0	476.6	192.7	1,038.7	0.145
Daidzein	62.6	14.7	214.7	48.3	16.4	169.6	0.057
Equol	5.0	2.4	11.8	7.8	3.7	17.3	0.024
Equol among equol producers	14.1	6.0	38	11.9	6.7	24.1	0.046
Genistein	46.2	11.7	115.8	25.0	10.0	89.6	0.081
O-DMA	1.3	0.4	7.4	2.9	0.6	19.8	0.021
Enterodiol	40.1	10.5	100.0	44.0	18.3	93.9	0.569
Enterolactone	316.9	58.7	417.2	402.4	133.3	944.6	0.003
Age, years	69	57	75	66	60	75	0.525
BMI	30.8	25.4	35.8	28.1	24.0	32.3	0.091

	%			%			
Race							0.885[g]
Non-Hispanic white	74.4			76.6			
Non-Hispanic black	11.3			10.1			
Mexican-American	6.6			3.6			
Other	7.8			9.8			
PIR							0.176[g]
Below poverty	16.3			15.2			
At or above poverty	81.8			73.6			
Missing	1.9			11.2			
Smoking history							0.81[g]
Current smoker	20.9			18.6			
Former smoker	23.8			21.8			
Never	55.4			60.0			
HRT users	37.3			27.2			0.532
Chronic diseases[f]							
History of diabetes	32.6			10.4			0.057
History of hypertension	59.9			46.4			0.176
History of cancer	10.4			14.8			0.506
History of CVD	35.6			11.5			0.004

Values are weighted and age-standardized, except for medians. [a] Participants meeting 3 or more of the 5 criteria of the frailty score. [b] Participants meeting 1 or 2 of the criteria. [c] Participants meeting none of the criteria. [d] Daidzein, equol, genistein, and O-DMA. [e] Enterodiol and enterolactone. [f] Self-reported. [g] χ^2 test.

els were associated with significantly lower odds of frailty in the multivariate analysis.

O-DMA is an intestinal bacterial metabolite of daidzein. Not all individuals harbor bacteria capable of metabolizing daidzein to O-DMA, i.e. individuals can be classified as O-DMA producers and nonproducers. About 80–95% of individuals of soy-consuming populations are O-DMA producers. Factors that may influence an individual's ability to produce O-DMA include the composition of the gut microflora, endogenous hormones, host genetics, and nonmodifiable host factors (e.g. decreasing levels with increasing age) [20, 34, 35]. O-DMA is less structurally similar to 17b-estradiol than its precursor compound daidzein and may exhibit different biological actions than daidzein.

Several biological actions of O-DMA have been observed in vitro, e.g. effects on cancer cells, binding to estrogen, androgen, and progesterone receptors, osteoclast formation, influencing immunological markers, and radical scavenging activity [36]. Furthermore, Low et al. [37]

Table 3. Associations between phytoestrogen concentrations and frailty in women aged 50 years or older from NHANES 1999–2002

Phytoestrogens, µg/g creatinine	Unadjusted OR (95% CI)	Age, race/ethnicity OR (95% CI)	Multivariable adjusted[a] OR (95% CI)
Isoflavones[b]	0.79 (0.56–1.12)	0.80 (0.57–1.12)	0.82 (0.62–1.08)
Lignans[c]	0.71 (0.47–1.07)	0.70 (0.46–1.06)	0.68 (0.46–1.01)
Daidzein	0.83 (0.61–1.15)	0.84 (0.61–1.15)	0.86 (0.66–1.12)
Genistein	0.95 (0.71–1.27)	0.95 (0.71–1.28)	0.97 (0.77–1.21)
O-DMA	0.76 (0.62–0.92)	0.76 (0.62–0.92)	0.75 (0.61–0.92)
Enterodiol	0.81 (0.51–1.27)	0.82 (0.51–1.31)	0.79 (0.52–1.22)
Equol among equol producers	0.87 (0.55–1.36)	0.89 (0.59–1.34)	0.97 (0.64–1.46)
Equol	0.85 (0.61–1.18)	0.86 (0.62–1.19)	0.88 (0.63–1.23)
Enterolactone	0.81 (0.61–1.07)	0.81 (0.61–1.07)	0.80 (0.60–1.07)

Frailty was dichotomized into nonfrail and frail. [a] Adjusted for age, race, PIR, HRT, smoking history, and chronic diseases (CVD, hypertension, diabetes, and cancer). [b] Daidzein, equol, genistein, and O-DMA. [c] Enterodiol and enterolactone.

stratified analyses of plasma sex hormones by presence or no presence of detectable urinary O-DMA concentrations in women aged 45–74 years who participated in EPIC-Norfolk. O-DMA-producing women had 6% higher plasma estradiol concentrations after adjustment for potential confounding factors. Overall, O-DMA could affect the risk of frailty via various mechanisms. It is not just a marker for aging as adjustment for age did not substantially modify the effect estimate in our study.

The interpretation of the inverse association of O-DMA with frailty in women is complex. Firstly, the cross-sectional study design does not allow for establishment of the temporal sequence of events. Frailty could result from low O-DMA concentrations, but frailty is also known for an increased risk of malnutrition and this could affect the dietary intake of soy products or products containing soy. The major sources of phytoestrogens in the US diet are doughnuts, pancakes, waffles, and bread, all of which contain added soy [20]. Secondly, O-DMA may be seen as an indicator of high soy consumption, and other bioactive compounds and nutrients in soy may play a role in the prevention of frailty [8, 38–42]. Thirdly, there is some evidence that the intestinal microflora of O-DMA producers may transform additionally polyphenolic dietary compounds, such as quercetin and kaempferol, which may reveal specific biological effects [36]. Finally, people with a high soy food intake, such as Asian people or vegetarians, may consume a more balanced diet than Americans consuming soy mainly through fortified foods, such as pancakes and doughnuts [41]. An unbalanced diet in our study population could be one of the reasons why we

did not observe associations between most phytoestrogens and frailty in the present study. Furthermore, it is possible that, because this was a low-soy-consumption population, isoflavonoide and other phytoestrogen levels were too low or there was insufficient variation in the exposure to detect associations. Moreover, in the present study, the urine samples for the determination of phytoestrogen levels were spot urines, and measurements in spot urines may not be representative of the habitual nutritional intake of phytoestrogens. However, in a previous analysis of NHANES III data, urinary and serum levels of phytoestrogens showed a good correlation (daidzein r = 0.72) [43]. In addition, given the low prevalence of frails, the power of this study is limited and further studies are warranted to illuminate the associations of phytoestrogens with frailty. On the other hand, the comparison of the women of our sample with women of the same age range not included in our sample showed that the distribution of frailty prevalence was similar.

Conclusion

Even though the observed negative association between O-DMA and frailty in women of the present study may be the result of various confounders, it may be worthwhile to intensify research on the potential preventive effects of O-DMA. It should be taken into consideration that a low urinary concentration of O-DMA may be the result of a low intake of soy and/or of not being able to harbor the necessary intestinal microflora to produce these compounds.

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