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Association of urinary phytoestrogens with hormone-related cancers and cancer biomarkers: NHANES 1999–2010

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Abstract

Phytoestrogens may have potential effects on hormone-related cancers (HRC) and cancer biomarkers, but the findings have been inconsistent so far. Participants from the National Health and Nutrition Examination Survey 1999–2010 with information on the levels of urinary phytoestrogens, serum cancer biomarkers and cancer history were included. Sampling-weighted logistic regression models examined the association between urinary phytoestrogens concentrations (creatinine-standardised and log-transformed) and HRC, followed by stratified analyses by race/ethnicity, age and menopausal status for different gender. Correlation analyses between phytoestrogens and cancer biomarkers were performed. Of the total 8844 participants, there were 373 with HRC. We observed total isoflavone and enterodiol excretion were positively associated with HRC, especially in non-Hispanic white female subpopulations ($P_{trend} < 0{\cdot}05$). Similar association also existed in the total isoflavones and enterodiol levels with breast cancer. Whereas the highest concentration of total isoflavones was significantly linked to a reduced prevalence of HRC (OR = 0·40, 95 % CI: 0·19, 0·84) in white males and of prostate cancer (OR = 0·40, 95 % CI: 0·18, 0·86). Among twenty-four participants with HRC, urinary equol concentration was positively correlated with CA15.3. Also, an inverse correlation of total prostate-specific antigens (PSA) and positive correlation of the PSA ratio with urinary enterolactone were detected in thirteen prostate cancer patients. Our findings indicated that higher concentrations of total isoflavones and enterodiol were positively associated with HRC. Urinary certain phytoestrogen excretion may affect serum cancer biomarker levels in cancer patients. But further prospective studies are needed to provide stronger evidence.

Key words: **Phytoestrogens: Hormone-related cancers: Cancer biomarkers: Urinary biomarkers: NHANES**

Hormone-related cancers (HRC) are greatly influenced by hormone levels and generally respond to hormone regulation, which plays an indispensable role in tumour growth[1]. Six cancer types, including breast, ovarian, endometrial, thyroid, prostate and testicular cancer, are usually referred to as HRC since they share the same carcinogenic mechanism[2]. Worldwide, there were estimated 5 million newly diagnosed cases of HRC in 2020, accounting for more than a quarter of new cancer cases[3]. As the most frequently diagnosed cancer in the great majority of countries, HRC have become the leading cause of cancer death in over 100 countries[3].

Considering the side effects of traditional hormone therapy for HRC, the use of phytochemicals with chemoprophylaxis is of great concern[4]. Phytoestrogens (mainly isoflavones and lignans), abundant in several edible and/or medicinal plants belonging mostly to the *Leguminosae* family, seem to have hormone-like and anti-hormone effects due to their structural similarity to the natural estrogen estradiol (e.g. 17β-estradiol) as well as other steroid hormones and steroid hormone antagonists[5] and are known to modulate multiple molecular targets in cancer cells[6,7]. Several existing meta-analyses summed up the evidence for the relationship between dietary soy isoflavones and lignans intake and risk of the aforementioned specific HRC among observational studies[8–12]. Specifically, the present results supported an association of isoflavone intake with a reduced risk of breast cancer in Asians but not in Westerners

Abbreviations: HRC, hormone-related cancer; NHANES, National Health and Nutrition Examination Survey; O-DMA, O-desmethylangolensin; PSA, prostrate-specific antigen.

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as well as a more likely link of lignan intake with breast cancer in post-menopausal women but not with prostate cancer[8,11,12]. Soy foods are regularly consumed as part of a normal diet in Asian countries, whereas soy protein is used as a functional ingredient (e.g. to promote water retention or as a bleaching agent) in many Western foods[13]. Higher intake (over 35–63 mg/d) of isoflavones in Asians tends to be more than ten times of the higher intake (over 0·03–4 mg/d) in Westerners[14], leading to the extreme difficulty in accurately quantifying isoflavone intake when exposure is so low particularly in the West. Meanwhile, some metabolites from isoflavones, such as O-desmethylangolensin (O-DMA) and equol, vary more markedly in urine excretion and exert more biological activity than the parent compound of soy isoflavones[15]. Of note, Americans have been found to be less capable of producing equol from isoflavone than Asians[16]. However, most studies assessed phytoestrogens intake based on dietary questionnaires, and it is far from sufficient to assess the phytoestrogens intake given the metabolic and absorption levels of these compounds. Urinary phytoestrogens excretion is also correlated to their dietary intake estimated by the FFQ[17–19]. Therefore, the excretion of phytoestrogens in urine is considered a better measure of bioavailable phytoestrogens than an assessment of dietary soy intake[17]. To our knowledge, studies assessing the associations of urinary phytoestrogens with the risk of breast and prostate cancer have yielded inconsistent findings[20–28]. However, there are still no studies that systematically and comprehensively investigate the relationship between urinary phytoestrogens (total isoflavones and total lignans) and HRC.

Studies manifested the role of phytoestrogens on cancer biomarkers were ambiguous. Although individual randomised controlled trials might advocate for the efficacy of phytoestrogens (diets rich in soy and linseed or soy/isoflavones supplements) in reducing prostate-specific antigens (PSA) concentrations, a meta-analysis of randomised controlled trials indicated that the consumption of soy products or isoflavones did not affect the PSA concentrations among men with prostate cancer diagnosis or a clinically identified risk of prostate cancer[29]. Further, only a few case reports showed the therapy with over-the-counter phytoestrogens or fermented soy influenced subsequent levels of cancer biomarkers[30,31].

Therefore, we aimed to collect cross-sectional data from National Health and Nutrition Examination Survey (NHANES) to estimate the association between urinary phytoestrogens and HRC and concentrations of serum cancer biomarkers among Americans.

Materials and methods

Study design and data source

We selected adult participants (≥ 20 years) from the NHANES, the design and content of which can be obtained from https://wwwn.cdc.gov/nchs/nhanes/default.aspx[32,33]. Each survey participant completed a household interview followed by a standardised physical examination in a mobile examination centre. The NHANES has been approved by the NCHS Ethics Review Board and written informed consent has been obtained from all participants.

In this secondary analysis, data and information on phytoestrogens and history of cancer were both available during 1999–2010 in continuous NHANES[34]. From 1999 to 2010, 62 160 participants completed the household interview, of which 59 367 participated in the mobile examination centre. We examined the integrity of variables interested and included 9755 participants aged ≥ 20 years with accessible urinary phytoestrogens and creatinine and history of cancer. After the further exclusion of participants who were pregnant (n 411) and who had cancers other than HRC (n 500), 8844 participants were included finally as investigative samples.

Ascertainment of hormone-related cancers

Participants were questioned, 'have you ever been told by a doctor or other health professional that you had cancer or a malignancy of any kind?' in the household interview. If they answered 'yes', they would be asked to offer the type of cancer. Up to three types of cancer were registered. When they offer more than 3, enter 'more than 3 kinds (code 66)' as the fourth response. HRC were defined as cancers of breast (code 14), ovary (code 28), prostate (code 30), testis (code 36), thyroid (code 37) and corpus uteri (code 38) according to any of the three responses. If they had more than one of these cancers, they were regarded as subjects with multiple HRC.

Measurement of urinary phytoestrogen and creatinine

A minimum of 5 ml of urine specimens were collected from subjects in standard urine collection cups on the morning after a recommended fast at the mobile examination centre, transferred to specimen vials within 4 h of collection and stored frozen in borosilicate glass or polypropylene vials or specimen cups. Teflon-coated stoppers were used to plug vials, and the vials were sealed with an aluminum seal. The specimens were then labelled and frozen immediately to −20°C and stored on dry ice for shipping. Subsequently, spot 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. The test principle utilises HPLC-atmospheric pressure chemical ionisation-tandem MS (HPLC-APCI-MS/MS) in NHANES 1999–2002[35], HPLC-electrospry ionisation-tandem MS (HPLC-ESI-MS/MS) in NHANES 2003–2004[36] and HPLC-atmospheric pressure photoionisation-tandem MS (HPLC-APPI-MS/MS) in NHANES 2005–2010[37] for the quantitative detection of six phytoestrogens, including four isoflavones (genistein, daidzein, equol and O-DMA), as well as two lignans (enterodiol and enterolactone). The comparison of three laboratory methods for testing urinary phytoestrogens was shown in Supplementary Table S1. The detection limits were constant for all of the analytes in the data set (0·2 ng/ml for genistein, 0·4 ng/ml for daidzein, 0·06 ng/ml for equol, 0·2 ng/ml for O-DMA, 0·04 ng/ml for enterodiol and 0·1 ng/ml for enterolactone). Cross-over studies comparing samples analysed by different methods demonstrated high-correlation coefficients ($r > 0·99$) and regression slopes approximately equal to 1 and intercepts close to 0.

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There was a change in the instrumentation and method for urine creatinine from 2006 to 2007. Prior to 2007, the urine creatinine was performed on the Beckman CX3 using a Jaffe reaction, while from 2007 forward, the urine creatinine was performed on the Roche ModP using an enzymatic (creatinase) method[38]. Therefore, urine creatinine data from 2005 to 2006 need to be adjusted for comparison with data from 2007 forward. The following equations are recommended:

For urine creatinine < 75:

$$Adjusted\ creatinine = \left[\left(1 \cdot 02 \times \sqrt{unadjusted\ creatinine}\right) - 0 \cdot 36\right]^2 \quad (1)$$

For urine creatinine 75 to < 250:

$$Adjusted\ creatinne = \left[\left(1 \cdot 05 \times \sqrt{unadjusted\ creatinine}\right) - 0 \cdot 74\right]^2 \quad (2)$$

For urine creatinine ≥ 250:

$$Adjusted\ creatinine = \left[\left(1 \cdot 01 \times \sqrt{unadjusted\ creatinine}\right) - 0 \cdot 10\right]^2 \quad (3)$$

Measurement of cancer biomarkers

Considering that cancer biomarker levels are closely related to the onset and progression of cancer, we attempt to explore the relationship between urinary phytoestrogen excretion and cancer biomarkers. Out of 4412 women samples, only 678 serum from women aged over 20 years from NHANES 2001 to 2002 were tested for cancer antigen 125 (CA125, considered as biomarkers for ovarian cancer), cancer antigen 15·3 (CA15.3, considered as biomarkers for breast cancer) and human epididymal secretory protein E4 (HE4, considered as biomarkers for ovarian cancer)[39]. Among 4432 men samples, total and free PSA (, considered as biomarkers for prostate cancer) were measured by the Access Hybritech Assay (Beckman Access, Fullerton CA) in 2159 men aged 40 years and older from NHANES 2001–2010[40]. PSA ratio is calculated by rounding the percentage of free PSA divided by total PSA. We have used preset cut-off values for the definition of abnormal biomarkers: CA125 ≥ 35 kU/l, CA15.3 ≥ 25 000 mU/ml, HE4 ≥ 70 pM, total PSA > 4 ng/mL and PSA ratio < 15 %. Due to free PSA tending to have no specific diagnosis value, the median value (0·27 ng/ml) was utilised. Ultimately, the associations of biomarkers for males and females with urinary phytoestrogens were analysed.

Covariates

Age, gender, race, education, marital status, poverty income ratio, physical activity intensity and smoking status were self-reported by participants in a household interview. Poverty income ratio was calculated by dividing family income by the poverty guidelines specific to family size, as well as the appropriate year and state[41] and was categorised into poorer (0·00–1·30), average (> 1·30–3·50) and richer (> 3·50 and above) based on Supplemental Nutrition Assistance Program eligibility[34]. Different types of physical activity in a typical week were asked before the physical examination, in the home, by trained interviewers using the Computer Assisted Personal Interviewing system. According to the increase in physical exertion, breathing and heart rate, physical activity intensity was categorised as vigorous, moderate and inactive. Based on the participants' responses to the question of whether they smoked at least 100 cigarettes during their lifetime and whether they were currently smoking, they were categorised as non-smoker, former smokers and current smokers. During the mobile examination centre interview, 24-hour dietary recall was administered to calculate alcohol intake, total energy intake and fat intake. Alcohol intake was defined as no drinking (0 g/d), moderate drinking (0–14 g/d for females and 0–28 g/d for males) and heavy drinking (≥ 14 g/d for females and ≥ 28 g/d for males)[42]. Measurements of height, weight and blood pressure were performed following a standardised protocol. BMI was calculated as weight in kilograms divided by height in meters squared (kg/m^2, < 25, 25 to 29·9, ≥ 30)[32,33]. Hypertension was described as having a diagnosis by a doctor or other health professional with high blood pressure, taking antihypertensive medicines or having systolic blood pressure level of ≥ 130 mm Hg or diastolic blood pressure level of ≥ 80 mm Hg as per the 2017 ACC/AHA Hypertension Guideline[43]. Indicators such as HbA1c, fasting plasma glucose, 2-hour glucose (oral glucose tolerance test), HDL-cholesterol, LDL-cholesterol and TAG were measured in NHANES laboratory[34]. Diabetes was described as any participant recognised with diabetes, taking insulin, taking diabetes pills, having a HbA1c level of ≥ 6·5 %, a fasting plasma glucose level of ≥ 126 mg/dl or an oral glucose tolerance test level of ≥ 200 mg/dl[44]. Dyslipidaemia was characterised as having a physician's diagnosis, or currently taking lipid-lowering prescribed medicine, or having an abnormal lipid profile that includes an HDL-cholesterol level of < 40 mg/dl, an LDL-cholesterol level of ≥ 110 mg/dl or a TAG level of ≥150 mg/dl based on guidelines from the third report of the National Cholesterol Education Program Adult Treatment Panel III[45].

Statistical analyses

All statistical analyses accounting for the complex, multistage, stratified and cluster-sampling design (including oversampling of certain subgroups) of NHANES were conducted using survey modules of SAS software version 9.4 (SAS Institute). Sample weights were allowed to combine to obtain national estimates that reflect the true relative proportions of particular subgroups in the USA population as a whole.

Urinary phytoestrogens were classified into four isoflavones and two lignans. The isoflavone concentration was the sum of daidzein, genistein, O-DMA and equol concentration. The lignan concentration was the sum of enterodiol and enterolactone concentrations. Six standardised phytoestrogen concentrations (μg/g creatinine) were calculated by dividing each original phytoestrogen concentration by the adjusted creatinine level. The distribution of all urinary phytoestrogens was severely right-skewed with a kurtosis coefficient from 13·9 to 29·1 using Kurtosis test method. Hence, standardised phytoestrogens were log-transformed to accommodate subsequent analyses treating

phytoestrogens as continuous variables. Finally, log-transformed data were utilised as categorical variables or continuous variables for subsequent statistical analysis.

Weighted means or geometric means (for urinary phytoestrogens) and proportions of baseline characteristics among participants with and without HRC were compared by using sampling-weighted ANOVA for continuous variables and Rao–Scott chi-square tests for categorical variables. For HRC analysis, sampling-weighted multivariate logistic regression models were performed to calculate the OR and 95 % CI for the overall and gender-stratified association between urinary phytoestrogens in quartile categories and HRC with the adjustment for covariates. *P* value for linear trend was calculated after treating classified urinary phytoestrogens as a continuous variable. Stratified analyses were further performed by race/ethnicity (White and non-White) and age (≤ 60 years and > 60 years) among females and males, respectively, as well as menopausal status (premenopausal and postmenopausal) only in females. Specifically, we also explored the association of urinary phytoestrogens with major cancer types, primarily female breast and prostate cancer. We calculated the duration of HRC by subtracting the age at HRC diagnosis from age at baseline and performed association analyses of urinary phytoestrogens with HRC based on the duration of HRC (within 5 years or over 5 years). Restricted cubic splines were conducted to assess the non-linear relationship of urinary phytoestrogens to HRC. For biomarker analysis, the weighted geometric means of female cancer biomarkers, CA125, CA15.3 and HE4, and male cancer biomarkers, total PSA, free PSA and PSA ratio were calculated to carry out partial correlation analyses with urinary phytoestrogens. A level of < 0·05 for adjusted two-sided *P* values was considered statistically significant.

Results

Characteristics of study population

A flow chart of dataset selection and subject screening process is outlined in Supplementary Fig. S1. Among 8844 participants aged 20 years and older in this study, the prevalence rate of HRC was 4·2 %. One hundred and forty-seven had breast cancer and 145 had prostate cancer. The number of subjects with uterine corpus cancer, ovarian cancer, thyroid cancer and testicular cancer was 43, 29, 13, and 5, respectively. Participants with HRC were more likely to be elder, female, non-Hispanic white, divorced/separated/widowed, physically inactive, former smokers, with hypertension, diabetes and dyslipidaemia and with lower total energy intake and lower fat intake, when compared with those without HRC (*P* < 0·05), as shown in Table 1. But neither female or male cancer biomarkers differed between those with and without HRC (*P* > 0·05). Compared with subjects with the lowest concentration of total urinary phytoestrogens, subjects with the highest concentration were more likely to be elder, female, non-Hispanic white, well-educated, married/living with partner, high-earning, non-smokers, free of obesity and dyslipidaemia, and with lower total energy intake and lower fat intake (*P* < 0·05; online Supplementary Table S2).

Distribution of urinary phytoestrogens

The median (P25, P75) of total urinary phytoestrogens was 711·2 (314·6, 1520·1) μg/g creatinine in all participants. Among which the excretion of lignan (mainly enterolactone) was approximately four times higher than that of isoflavone. As for isoflavone metabolites, the excretion of O-DMA (median (P25, P75): 3·3 (0·7, 19·4) μg/g creatinine) and equol (median (P25, P75): 7·0 (3·3, 14·5) μg/g creatinine) was much lower than that of maternal compound such as daidzein (median (P25, P75): 50·8 (18·3, 176·9) μg/g creatinine). For urinary phytoestrogens, participants with HRC had higher concentrations of total phytoestrogens, isoflavones (daidzein, genistein, O-DMA, equol) and lignans (enterodiol and enterolactone) than those without HRC (All *P* < 0·001; Table 2).

Overall association between urinary phytoestrogens and hormone-related cancers

The multivariable-adjusted OR for HRC by urinary phytoestrogens are shown in Fig. 1. Participants with higher concentration of urinary daidzein and enterodiol had HRC (*P* for trend < 0·05). Simultaneously, total isoflavones were associated with the augmented prevalence of HRC at Q3 levels (OR = 1·81, 95 % CI: 1·20, 2·73) but showed insignificant effects at Q4 levels. As shown in Supplementary Fig. S2, there was a significantly non-linear trend for the dose–response relationship between urinary lignans (mainly enterodiol) and HRC ($P_{\text{non-linear}}$ = 0·033). When the concentration of lignans was above the first quartile (P25, a certain level (ln(Concentration of lignans) = 7·5, i.e., concentration of lignans is approximately 1800 μg/g creatinine)), the higher the concentration, the higher the prevalence of HRC.

Association between urinary phytoestrogens and hormone-related cancers by gender

We further conducted analyses on the association between urinary phytoestrogens and HRC among females and males. Higher concentrations of total isoflavone (*P* for trend = 0·010), daidzein (*P* for trend = 0·006), equol (*P* for trend = 0·027) and enterodiol (*P* for trend = 0·001) were positively associated with HRC among female participants (Fig. 2(a)). Compared with the lowest quartile (Q1) level, Q3 level of equol was positively associated with HRC among female participants, whereas only the highest concentration of equol had an association with a lower prevalence of HRC among males (Fig. 2(b)). For the purpose of focusing on specific subpopulations to investigate further associations, we carried out race/ethnicity- and age-stratified analyses among female and male participants, respectively (Fig. 3 and Fig. 4). We observed a higher prevalence of HRC conferred by a higher concentration of total isoflavone and enterodiol in urine among the White women (*P* for trend < 0·05) but not among the non-White women (Fig. 3(a)). As for the younger women, the highest concentration of total urinary phytoestrogens was linked with a higher prevalence of HRC (OR = 3·30, 95 % CI: 1·39, 7·81) (Fig. 3(b)). Compared with postmenopausal women, total phytoestrogens, isoflavones and lignans were more positively associated with HRC in premenopausal women (online Supplementary Table S3).

Table 1. Baseline characteristics of 8844 participants with and without hormone-related cancers from NHANES 1999–2010

	All participants (*n* 8844)		With HRC (*n* 373)		Normal (*n* 8471)		
	n	%	*n*	%	*n*	%	*P*-value
Age, years							
Mean	46·0		64·5		45·3		< 0·001
SD	0·3		1·3		0·3		
Gender, %							< 0·001
Male	4432	49·2	152	28·7	4280	50·0	
Female	4412	50·8	221	71·3	4191	50·0	
Race/ethnicity, %							< 0·001
NH White	4177	69·4	234	80·9	3943	69·0	
NH Black	1841	11·7	80	10·8	1761	11·8	
Mexican American	1865	8·4	33	2·7	1832	8·6	
Other	961	10·5	26	5·7	935	10·6	
Education, %							0·474
Below high school	2710	18·8	106	21·5	2604	18·7	
High school	2094	25·4	94	27·0	2000	25·3	
College or above	4028	55·8	173	51·5	3855	56·0	
Marital status, %							< 0·001
Married or living with partner	5296	64·3	223	65·9	5073	64·2	
Divorced, separated or widowed	1965	18·5	129	30·0	1836	18·0	
Never married	1447	17·3	15	4·1	4132	17·7	
PIR, %							0·530
0–1·30	2418	20·4	84	17·4	2334	20·5	
1·31–3·50	3109	36·3	129	37·3	2980	36·1	
3·51–	2591	43·3	123	45·3	2468	43·2	
BMI, %							0·665
< 25	2658	33·2	118	33·2	2540	33·2	
25–30	2975	32·3	123	30·0	2852	32·4	
> 30	3069	34·6	123	36·9	2946	34·5	
Physical activity, %							0·004
Vigorous	2316	30·1	52	18·1	2264	30·5	
Moderate	2173	27·4	101	30·8	2072	27·3	
Inactive	4182	42·5	206	51·1	3976	42·2	
Smoking status, %							0·001
Non-smoker	4664	52·2	179	52·2	4485	52·3	
Current smoking	2028	24·2	54	14·3	1974	24·6	
Former smokers	2146	23·6	139	33·5	2007	23·2	
Alcohol intake, %							0·132
No drinking	6223	70·0	263	75·2	5960	69·8	
Moderate drinking	945	10·8	48	10·4	897	10·8	
Heavy drinking	1308	19·2	46	14·4	1262	19·4	
	Mean	SD	Mean	SD	Mean	SD	
Hypertension, %	4253	44·5	250	65·4	4003	43·7	< 0·001
DBP, mm Hg	71·0	0·3	69·0	0·7	71·1	0·3	0·001
SBP, mm Hg	122·2	0·3	129·3	1·2	122·0	0·3	< 0·001
Diabetes, %							
n	1362		86		1276		< 0·001
%	11·6		22·4		11·2		
HbA1c, %	5·5	0·0	5·8	0·1	5·5	0·0	< 0·001
Fasting glucose, mg/dl	103·6	0·7	114·3	3·4	103·2	0·6	< 0·001
OGTT, mg/dl	116·1	2·1	148·6	8·6	115·1	2·0	< 0·001
Dyslipidaemia, %							
n	4668		221		4447		0·020
%	53·5		61·5		53·2		
HDL-c, mg/dl	52·9	0·3	56·0	1·3	52·8	0·3	< 0·001
LDL-c, mg/dl	115·6	0·8	110·9	4·7	115·8	0·8	0·019
TAG, mg/dl	139·7	2·4	160·2	22·3	139·0	2·4	0·006
TEI, kcal/d	2205·5	15·4	1771·7	56·9	2221·5	16·0	< 0·001
Fat intake, g/d	83·1	0·7	66·8	2·7	83·7	0·8	< 0·001
	n	%	*n*	%	*n*	%	
Female cancer biomarkers (*n* 678)							
CA125 ≥ 35 U/ml, %	42	6·5	1	8·4	41	6·5	0·814
CA15·3 ≥ 25 000 mU/ml, %	10	1·0	/		10	1·0	/
HE4 ≥ 70 pM, %	12	1·6	1	2·6	11	1·5	0·727
Male cancer biomarkers (*n* 2159)							
Total PSA > 4 ng/ml, %	185	5·9	1	4·1	184	6·0	0·685
Free PSA < median, %	986	49·0	11	44·7	975	49·3	0·794
PSA ratio < 15 %, %	167	7·0	3	19·0	164	6·9	0·289

Abbreviations: CA125, cancer antigen 125; CA15.3, cancer antigen 15.3; HRC, hormone-related cancer; NHANES, National Health and Nutrition Examination Survey; NH, non-Hispanic; DBP, diastolic blood pressure; SBP, systolic blood pressure; OGTT, oral glucose tolerance test; ; TEI, total energy intake; HDL-c, HDL-cholesterol; HE4, human epididymal secretory protein E4; LDL-c, LDL-cholesterol; NHANES, National Health and Nutrition Examination Survey; PIR, poverty income ratio; PSA, prostate-specific antigen; SD, standard deviation.

Data were expressed as the weighted mean ± SD or number of participants (weighted proportion). Sampling-weighted Rao-Scott chi-square tests were performed for categorical variables and sampling-weighted ANOVA methods were performed for continuous variables. A level of < 0·05 for two-sided *P* values was considered statistically significant.

Table 2. Distribution of urinary phytoestrogens in participants with and without hormone-related cancers from NHANES 1999–2010

Urinary phytoestrogens, μg/g creatinine	All participants (*n* 8844)		With HRC (*n* 373)		Normal (*n* 8471)		*P*-value
	Median	P_{25}, P_{75}	Median	P_{25}, P_{75}	Median	P_{25}, P_{75}	
Total phytoestrogen	711·2	314·6, 1520·1	1115·5	495·0, 2199·4	703·6	306·7, 1492·8	< 0·001
Isoflavone	103·3	43·9, 334·2	171·8	69·6, 444·2	99·8	43·2, 329·8	< 0·001
Daidzein	50·8	18·3, 176·9	78·3	34·3, 257·8	49·9	17·9, 175·1	< 0·001
Genistein	23·3	9·0, 79·6	36·2	13·3, 117·6	23·1	8·8, 78·3	< 0·001
O-DMA	3·3	0·7, 19·4	6·5	1·2, 37·2	3·3	0·7, 18·9	< 0·001
Equol	7·0	3·3, 14·5	9·7	4·7, 16·8	6·9	3·3, 14·3	< 0·001
Lignan	436·1	153·1, 1001·7	739·0	286·8, 1538·3	429·6	151·2, 984·3	< 0·001
Enterodiol	43·2	16·4, 106·7	82·5	34·9, 158·5	42·2	16·0, 103·8	< 0·001
Enterolactone	351·4	94·1, 883·1	572·5	177·1, 1344·4	347·7	92·7, 867·6	< 0·001

Abbreviations: HRC, hormone-related cancers; NHANES, National Health and Nutrition Examination Survey; O-DMA, O-desmethylangolensin.
Data were expressed as the weighted median (P_{25}, P_{75}). Sampling-weighted ANOVA methods was tested for the distribution difference of urinary phytoestrogens between participants with and without HRC. A level of < 0·05 for two-sided *P* values was considered statistically significant.

In the analysis limited to male subjects, the excretion of total isoflavones (mainly equol) at Q4 level was negatively associated with HRC among those who were White (Fig. 4(a)). Notably, the highest level of enterodiol (OR = 2·78, 95 % CI: 1·29, 5·98) seemed to be associated with the higher prevalence of HRC in elder males (Fig. 4(b)).

With respect to the main specific cancer type, we observed a similarly positive correlation between daidzein and enterodiol in female breast cancer (online Supplementary Fig. S3). However, the highest concentration of total isoflavones (OR = 0·40, 95 % CI: 0·21, 0·80), mainly equol (OR = 0·38, 95 % CI: 0·16, 0·93), was significantly linked to a reduced prevalence of prostate cancer compared to the lowest concentration (online Supplementary Fig. S4). Also, for HRC with different years of diagnosis, we found that the daidzein and total lignans were more positively associated with HRC over 5 years than HRC within 5 years. The positive association of urinary enterodiol was more pronounced in HRC within 5 years.

Urinary phytoestrogens and cancer biomarkers

For female's cancer biomarker analysis, firstly, we observed participants with HRC had higher serum HE4 concentrations than those without HRC. The concentration of HE4 was more likely to be correlated with the well-known cancer risk factors (online Supplementary Table S5). Further, we explored the correlation of three female cancer biomarkers with urinary phytoestrogens. Notably, the positive correlation between serum CA15.3 and urinary equol was found among females with HRC in the partial correlation analysis ($r = 0·448$, $P = 0·032$) (Table 3).

Similarly, 2159 male participants with information on PSA were included in our study. The PSA ratio was correlated with race/ethnicity and marital status and was lower in participants with HRC (online Supplementary Table S6). Generally, no significant correlations between urinary phytoestrogens and serum total/free PSA and PSA ratio were observed in partial correlation analyses (Table 4). However, among patients with prostate cancer, higher concentration of urinary enterolactone was forcefully correlated with decreased total PSA ($r = -0·979$, $P = 0·021$) and elevated PSA ratio ($r = 0·636$, $P = 0·036$).

Discussion

Although estimates of total phytoestrogens among different studies were not the same (such as isoflavones mainly calculated from the sum of daidzein and genistein or the sum of daidzein, genistein and equol), the study herein firstly comprehensively evaluated the role of total phytoestrogens excretion (the sum of isoflavones including daidzein, genistein, O-DMA and equol and lignans including enterodiol and enterolactone) in HRC. In this study of 8844 participants from the continuous NHANES, we firstly observed higher concentrations of isoflavones (mainly daidzein) and enterodiol were positively associated with female HRC, which appeared even more pronounced in non-Hispanic white and breast cancer. Whereas, the highest concentration of total isoflavones was significantly linked to a reduced prevalence of HRC in white males and of prostate cancer. Among participants with HRC, urinary equol concentration was strikingly positively correlated with serum CA15·3. Inverse correlation of total PSA and positive correlation of the PSA ratio with urinary enterolactone were detected in prostate cancer patients.

To the best of our knowledge, the findings of previous studies in western populations on the relationship between urinary phytoestrogens and risk of breast cancer were conflicting[20,22,23,46]. A nested case–control study within Multiethnic Cohort Study established in America prospectively identified invasive postmenopausal breast cancer cases through linkage to the cancer registries and showed no effects of urinary phytoestrogens excretion on breast cancer risk but the risk varies slightly among different races[23], suggesting the existence of a regional and ethnic disparity between different studies was necessitated for consideration. An earlier small-scale case–control study in Australia reported a substantial reduction in breast cancer risk among women with a high excretion of phytoestrogens, particularly the equol and the enterolactone[46]. However, two other nested case–control studies from European Prospective into Cancer-Norfolk (EPIC-Norfolk) study implied that higher concentrations of urinary isoflavones were associated with a marginally increased risk of female breast cancer, but total lignans were not[20,22], which is consistent with our results, even though the dietary source of isoflavones might be quite different in these

Urinary phytoestrogens	Range, μg/g creatinine	N (HRCs/non−HRCs)	OR (95% CI)	*P* for trend
Total phytoestrogen				0·466
Q1	1·03–314·58	55/2214	1·00 (Ref)	
Q2	314·59–711·17	89/2221	1·16 (0·69–1·94)	
Q3	711·18–1520·08	108/2155	1·02 (0·59–1·77)	
Q4	1520·09–142862·61	121/1881	1·25 (0·77–2·04)	
Total isoflavone				0·065
Q1	0·77–43·89	74/2327	1·00 (Ref)	
Q2	43·90–103·27	80/2043	1·23 (0·69–2·18)	
Q3	103·28–334·21	111/2097	1·81 (1·20–2·73)	
Q4	334·22–111751·63	108/2004	1·43 (0·87–2·35)	
Daidzein				0·001
Q1	0·08–18·28	66/2306	1·00 (Ref)	
Q2	18·29–50·83	90/2030	1·76 (1·10–2·81)	
Q3	50·84–176·94	109/2091	2·20 (1·47–3·30)	
Q4	176·95–67500·00	108/2044	1·82 (1·12–2·98)	
Genistein				0·242
Q1	0·06–8·98	67/2206	1·00 (Ref)	
Q2	8·99–23·30	89/2088	1·59 (0·93–2·73)	
Q3	23·31–79·61	98/2101	1·50 (0·87–2·61)	
Q4	79·62–37687·50	119/2076	1·53 (0·87–2·70)	
O−DMA				0·358
Q1	0·02–0·73	76/2371	1·00 (Ref)	
Q2	0·74–3·33	98/2164	1·06 (0·65–1·75)	
Q3	3·34–19·35	89/1995	1·06 (0·61–1·85)	
Q4	19·36–34166·67	110/1941	1·26 (0·75–2·11)	
Equol				0·388
Q1	0·01–3·34	88/2417	1·00 (Ref)	
Q2	3·35–6·98	87/2233	0·78 (0·48–1·27)	
Q3	6·99–14·47	110/1939	1·40 (0·85–2·31)	
Q4	14·48–19392·16	88/1882	1·03 (0·62–1·71)	
Total lignan				0·487
Q1	0·12–153·06	69/2167	1·00 (Ref)	
Q2	153·07–436·13	70/2205	1·10 (0·64–1·87)	
Q3	436·14–1001·71	101/2161	0·94 (0·57–1·56)	
Q4	1001·72–142797·70	133/1938	1·22 (0·71–2·11)	
Enterodiol				<0·001
Q1	0·01–16·40	67/2393	1·00 (Ref)	
Q2	16·41–43·15	75/2234	0·83 (0·47–1·48)	
Q3	43·16–106·67	106/2086	1·44 (0·83–2·51)	
Q4	106·68–40909·09	125/1758	2·01 (1·22–3·31)	
Enterolactone				0·682
Q1	0·08–94·14	75/2079	1.00 (Ref)	
Q2	94·15–351·43	69/2235	1·26 (0·72–2·20)	
Q3	351·44–883·05	95/2193	0·84 (0·49–1·46)	
Q4	883·06–130888·95	134/1964	1·23 (0·71–2·12)	

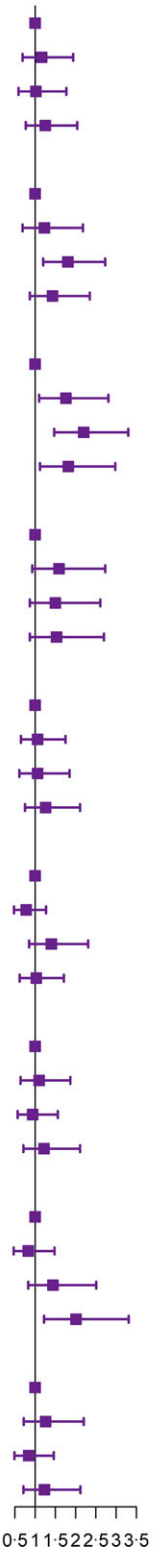

Fig. 1. Forest plot for the association between urinary phytoestrogens and HRC from NHANES 1999–2010. Adjusted for age (continuous), gender (female or male), race/ethnicity (non-Hispanic White, non-Hispanic Black, Mexican American or other), education level (below high school, high school or college or above), marital status (married/living with partner, divorced/separated/widowed or never married), poverty income ratio (0–1·30, 1·31–3·50 or 3·51–), BMI (< 25, 25–30 or > 30 kg/m^2), physical activity (vigorous, moderate or inactive), smoking status (non-smoker, current smoking or former smokers), alcohol intake (no drinking, moderate drinking or heavy drinking), hypertension (yes or no), diabetes (yes or no), dyslipidaemia (yes or no), total energy intake (continuous) and fat intake (continuous). HRC, hormone-related cancer; NHANES, National Health and Nutrition Examination Survey; O-DMA, O-desmethylangolensin.

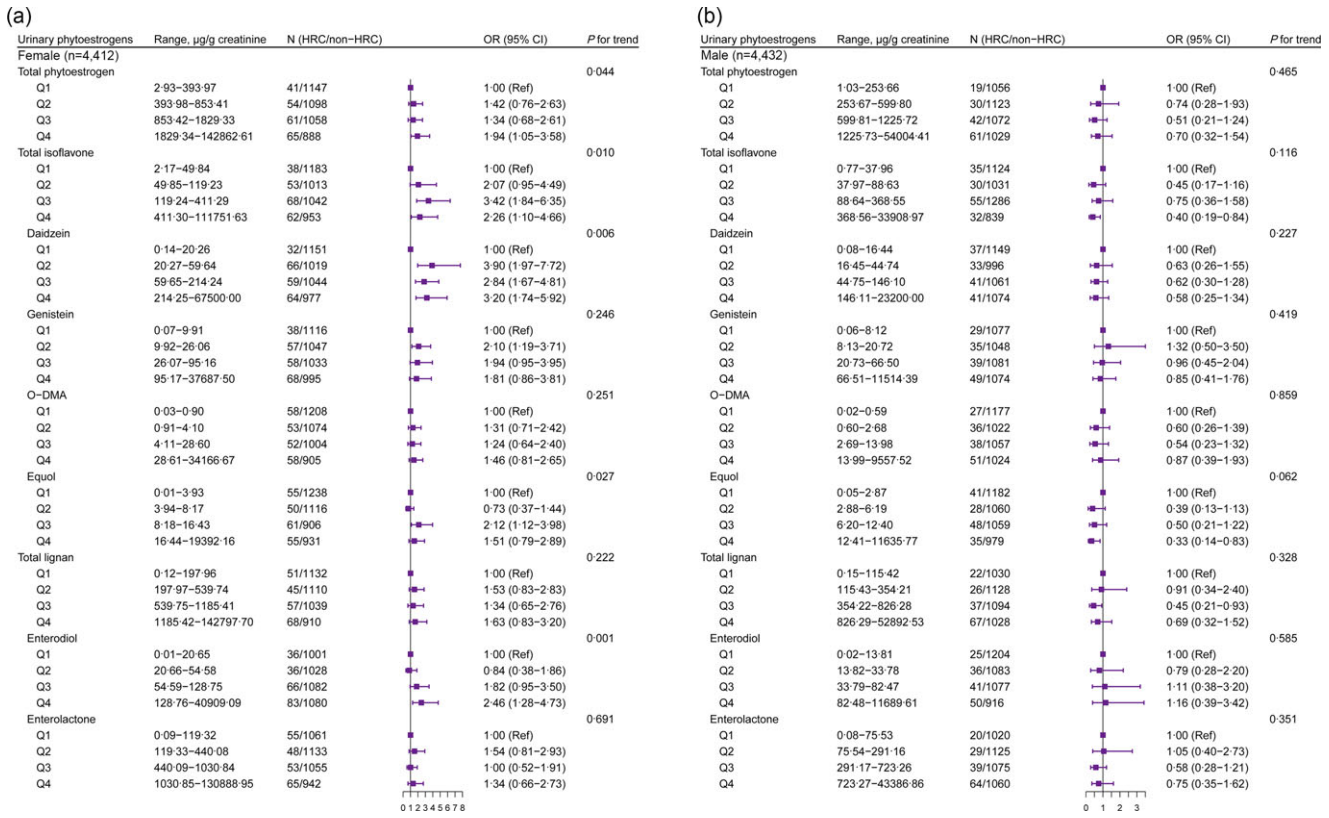

Fig. 2. Gender-stratified association between urinary phytoestrogens and HRC from NHANES 1999–2010. Adjusted for age (continuous), race/ethnicity (non-Hispanic White, non-Hispanic Black, Mexican American or other), education level (below high school, high school or college or above), marital status (married/living with partner, divorced/separated/widowed or never married), poverty income ratio (0–1·30, 1·31–3·50 or 3·51–), BMI (< 25, 25–30 or > 30 kg/m^2), physical activity (vigorous, moderate or inactive), smoking status (non-smoker, current smoking or former smokers), alcohol intake (no drinking, moderate drinking or heavy drinking), hypertension (yes or no), diabetes (yes or no), dyslipidaemia (yes or no), total energy intake (continuous) and fat intake (continuous). HRC, hormone-related cancer; NHANES, National Health and Nutrition Examination Survey; O-DMA, O-desmethylangolensin.

two studies. For the EPIC-Norfolk study, approximately 3 % subjects were classified as soya consumers with 38·5 % of their isoflavone intake deriving from vegetable dishes and milks, whereas among non-soya subjects, bread and bread rolls were the main food sources of isoflavones, accounting for 81 %[47]. A study on dietary assessment and food sources from NHANES found that 44·8 % of isoflavones were derived from legumes[48]. Meanwhile, after unit conversion, urinary phytoestrogen concentrations were slightly lower in our data from NHANES than those in the EPIC-Norfolk study (e.g. median for genistein: 2·64 μg/mmol creatinine *v.* 5·71 μg/mmol creatinine). Phytoestrogens have been suggested to promote carcinogenesis on account of their estrogenic effects and the role in potentially modulating enzymes known to regulate estrogen levels[49,50], which may explain the results we observed, but it remains to be seen whether this effect will emerge due to such low exposure levels of phytoestrogens in Western women. On the contrary, there were two Chinese studies leading to the suggestion that urinary isoflavones might be linked to a decreased risk of breast cancer[21,24]. The lower incidence of breast cancer that has been observed in Asian populations may be attributed to a higher intake of phytoestrogen-rich food, higher concentrations of phytoestrogen biomarkers and more production of equol relative to western populations[20]. It is difficult to assess phytoestrogens in western countries, and the limited evidence of an association between urinary isoflavones and breast cancer warrants further study in subsequent examinations of breast cancer risk and phytoestrogen biomarkers. Regarding prostate cancer, a protective effect of urinary daidzein excretion was found in American populations[28]. As a metabolite of daidzein formed by intestinal microbiota, equol is considered to be the most bioactive phytoestrogens among soybean isoflavones. The findings of a Jamaican study showed that when compared with males who have no detectable concentrations of equol, equol producers were at a reduced risk of prostate cancer[27]. Consistently, equol seems to possess the potential to reduce the risk of prostate cancer in our study, as well as the total isoflavones. Studies have also observed that equol exerts its inhibitory effect on prostate cancer cells through androgen receptor signaling, Akt/FOXO3a pathway and so on[51,52]. Thus, phytoestrogenic metabolites may be more valuable than parent compounds in prostate cancer prevention and treatment. In the future, a more comprehensive evaluation of phytoestrogens and its metabolites is warranted.

Furthermore, it has been suggested that some phytoestrogens like genistein may not be antiestrogens but agonists under the circumstance of relatively lower circulating levels of mammalian estrogen[20], which may have been the state of the

(a)

Urinary phytoestrogens	Range, μg/g creatinine	N (HRC/non-HRC)	OR (95% CI)	*P* for trend
NH white (n=2,045)				
Total phytoestrogen				0·051
Q1	2·93–432·12	27/486	1·00 (Ref)	
Q2	432·13–930·28	32/490	1·20 (0·60–2·39)	
Q3	930·29–1963·08	38/488	1·50 (0·72–3·11)	
Q4	1963·09–112595·38	48/436	1·95 (0·93–4·08)	
Total isoflavone				0·015
Q1	2·17–53·92	19/493	1·00 (Ref)	
Q2	53·93–124·80	36/480	2·40 (0·99–5·83)	
Q3	124·81–426·41	46/479	3·61 (1·75–7·44)	
Q4	426·42–111751·63	44/448	2·50 (1·16–5·36)	
Daidzein				0·011
Q1	0·14–21·83	16/517	1·00 (Ref)	
Q2	21·84–62·57	45/456	5·13 (2·23–11·79)	
Q3	62·58–219·14	36/482	2·99 (1·47–6·08)	
Q4	219·15–67500·00	48/445	3·91 (1·84–8·29)	
Genistein				0·267
Q1	0·07–10·11	20/485	1·00 (Ref)	
Q2	10·12–27·21	40/476	2·48 (1·19–5·16)	
Q3	27·22–98·82	37/472	2·12 (0·89–5·06)	
Q4	98·83–37687·50	48/467	2·03 (0·81–5·09)	
O-DMA				0·131
Q1	0·04–1·08	32/523	1·00 (Ref)	
Q2	1·09–4·91	37/473	1·73 (0·85–3·51)	
Q3	4·92–33·81	35/474	1·57 (0·78–3·19)	
Q4	33·82–11634·62	41/430	1·76 (0·89–3·48)	
Equol				0·011
Q1	0·05–4·69	29/461	1·00 (Ref)	
Q2	4·70–9·59	34/492	1·02 (0·47–2·21)	
Q3	9·60–18·59	40/454	2·51 (1·22–5·17)	
Q4	18·59–12000·00	42/493	1·82 (0·92–3·61)	
Total lignan				0·115
Q1	0·12–228·51	32/494	1·00 (Ref)	
Q2	228·52–599·99	32/485	1·66 (0·84–3·28)	
Q3	600·00–1325·76	34/472	1·64 (0·76–3·54)	
Q4	1325.77–47080·00	47/449	1·91 (0·89–4·13)	
Enterodiol				0·002
Q1	0·01–24·82	27/520	1·00 (Ref)	
Q2	24·83–61·85	28/494	1·17 (0·51–2·69)	
Q3	61·86–137·47	40/462	1·89 (0·87–4·13)	
Q4	137·48–17400·00	50/424	2·92 (1·40–6·08)	
Enterolactone				0·731
Q1	0·09–130·69	35/474	1·00 (Ref)	
Q2	130·70–492·41	33/486	1·40 (0·71–2·78)	
Q3	492·42–1165·30	32/481	1·24 (0·60–2·54)	
Q4	1165·31–43075·75	45/459	1·21 (0·55–2·67)	

Urinary phytoestrogens	Range, μg/g creatinine	N (HRC/non-HRC)	OR (95% CI)	*P* for trend
Non-NH white (n=2,367)				
Total phytoestrogen				0·475
Q1	5·36–324·62	14/586	1·00 (Ref)	
Q2	324·63–713·65	20/596	2·17 (0·68–6·86)	
Q3	713·66–1509·15	26/589	1·53 (0·46–5·15)	
Q4	1509·16–142862·61	16/520	1·77 (0·63–4·98)	
Total isoflavone				0·261
Q1	3·82–40·92	19/600	1·00 (Ref)	
Q2	40·93–104·18	16/582	1·07 (0·49–2·35)	
Q3	104·19–375·02	23/582	1·54 (0·53–4·48)	
Q4	375·03–63415·10	18/527	1·43 (0·69–2·97)	
Daidzein				0·477
Q1	0·15–17·88	14/605	1·00 (Ref)	
Q2	17·89–51·21	24/557	1·43 (0·64–3·17)	
Q3	51·22–197·10	22/576	1·54 (0·51–4·67)	
Q4	197·11–50790·53	16/553	1·35 (0·62–2·95)	
Genistein				0·077
Q1	0·07–9·16	17/603	1·00 (Ref)	
Q2	9·17–23·52	15/560	0·76 (0·28–2·05)	
Q3	23·53–85·26	22/576	1·78 (0·62–5·11)	
Q4	85·27–11895·09	22/552	1·77 (0·77–4·09)	
O-DMA				0·265
Q1	0·03–0·66	20/618	1·00 (Ref)	
Q2	0·67–2·72	21/537	0·62 (0·19–2·01)	
Q3	2·73–20·49	15/317	0·92 (0·26–3·26)	
Q4	20·50–34166·67	20/519	1·52 (0·58–3·97)	
Equol				0·154
Q1	0·01–2·66	21/581	1·00 (Ref)	
Q2	2·67–5·55	19/581	1·32 (0·50–3·48)	
Q3	5·56–10·91	23/562	1·36 (0·45–4·10)	
Q4	10·92–19392·16	13/567	2·11 (0·76–5·86)	
Total lignan				0·326
Q1	0·39–161·53	18/577	1·00 (Ref)	
Q2	161·54–433·32	11/576	0·82 (0·26–2·56)	
Q3	433·33–966·06	30/592	1·70 (0·55–5·27)	
Q4	966·07–142797·70	17/546	1·32 (0·51–3·47)	
Enterodiol				0·112
Q1	0·02–15·53	17/614	1·00 (Ref)	
Q2	15·54–41·12	17/591	2·29 (0·74–7·06)	
Q3	41·13–107·56	23/578	2·55 (0·81–8·10)	
Q4	107·57–40909·09	19/508	1·99 (0·88–4·47)	
Enterolactone				0·395
Q1	0·18–98·67	22/544	1·00 (Ref)	
Q2	98·68–340·72	7/585	0·40 (0·14–1·18)	
Q3	340·73–821·96	28/601	1·28 (0·50–3·28)	
Q4	821·97–130888·95	19/561	1·10 (0·49–2·50)	

(b)

Urinary phytoestrogens	Range, μg/g creatinine	N (HRC/non-HRC)	OR (95% CI)	*P* for trend
<=60 y (n=3,020)				
Total phytoestrogen				0·018
Q1	3·67–351·74	19/830	1·00 (Ref)	
Q2	351·75–774·45	17/759	1·69 (0·65–4·34)	
Q3	774·46–1742·85	19/754	1·27 (0·39–4·09)	
Q4	1742·86–142862·61	20/602	3·30 (1·39–7·81)	
Total isoflavone				0·053
Q1	2·17–47·73	12/829	1·00 (Ref)	
Q2	47·74–115.84	20/710	4·49 (1·52–13·24)	
Q3	115·85–379·89	25/729	4·97 (2·08–11·85)	
Q4	379·90–111751·63	18/677	3·58 (1·31–9·81)	
Daidzein				0·055
Q1	0·14–19·45	12/813	1·00 (Ref)	
Q2	19·46–55·57	20/709	3·99 (1·37–11·65)	
Q3	55·58–199·09	24/722	4·16 (1·84–9·41)	
Q4	199·10–67500·00	19/701	3·69 (1·43–9·50)	
Genistein				0·674
Q1	0·07–9·40	14/783	1·00 (Ref)	
Q2	9·41–24·38	19/721	2·45 (1·00–5·97)	
Q3	24·39–85·46	18/739	1·04 (0·29–3·68)	
Q4	85·47–37687·50	24/702	1·80 (0·61–5·29)	
O-DMA				0·057
Q1	0·03–0·82	20/837	1·00 (Ref)	
Q2	0·83–3·78	17/746	1·70 (0·62–4·68)	
Q3	3·79–26·64	15/720	1·92 (0·59–6·21)	
Q4	26·65–12338·27	23/642	2·75 (1·07–7·06)	
Equol				0·012
Q1	0·01–3·73	14/879	1·00 (Ref)	
Q2	3·74–7·87	22/784	1·59 (0·41–6·08)	
Q3	7·88–15·94	23/635	5·79 (1·67–20·08)	
Q4	15·95–19392·16	16/647	3·06 (0·85–11·01)	
Total lignan				0·077
Q1	0·12–181·84	24/827	1·00 (Ref)	
Q2	181·85–472·69	12/749	1·51 (0·57–3·97)	
Q3	472·70–1087·40	18/737	1·09 (0·44–2·68)	
Q4	1087·41–142797·70	21/632	2·64 (1·03–6·78)	
Enterodiol				0·098
Q1	0·01–19·25	24/842	1·00 (Ref)	
Q2	19·26–51·93	13/799	1·53 (0·52–4·54)	
Q3	51·94–122·49	18/696	1·82 (0·64–5·19)	
Q4	122·50–17400·00	20/608	2·21 (0·83–5·86)	
Enterolactone				0·107
Q1	0·09–109·37	25/776	1·00 (Ref)	
Q2	109·38–386·22	14/783	2·00 (0·73–5·50)	
Q3	386·23–944·78	14/738	0·63 (0·20–2·00)	
Q4	944·79–130888·95	22/648	3·09 (1·20–8·00)	

Urinary phytoestrogens	Range, μg/g creatinine	N (HRC/non-HRC)	OR (95% CI)	*P* for trend
>60 y (n=1,392)				
Total phytoestrogen				0·799
Q1	2·93–559·37	36/373	1·00 (Ref)	
Q2	559·38–1089·75	38/324	1·08 (0·47–2·48)	
Q3	1089·76–2140·55	32/282	1·23 (0·54–2·80)	
Q4	2140·56–65885·47	40/267	1·07 (0·46–2·49)	
Total isoflavone				0·219
Q1	2·48–57·78	33/372	1·00 (Ref)	
Q2	57·79–140·75	34/299	1·40 (0·67–2·93)	
Q3	140·76–466·52	41/301	1·91 (0·81–4·49)	
Q4	466·53–64712·84	38/274	1·62 (0·67–3·95)	
Daidzein				0·133
Q1	0·27–24·05	27/360	1·00 (Ref)	
Q2	24·06–68·62	42/300	2·40 (1·02–5·64)	
Q3	68·63–241·13	35/306	1·42 (0·76–2·66)	
Q4	241·14–58947·37	42/280	2·24 (0·99–5·05)	
Genistein				0·152
Q1	0·12–11·82	29/347	1·00 (Ref)	
Q2	11·83–33·27	42/321	1·40 (0·62–3·20)	
Q3	33·28–122·12	36/298	2·22 (0·98–5·05)	
Q4	122·13–11183·44	39/280	1·63 (0·69–3·87)	
O-DMA				0·569
Q1	0·05–1·06	28/251	1·00 (Ref)	
Q2	1·07–5·49	35/302	1·18 (0·51–2·72)	
Q3	5·50–31·67	40/364	0·87 (0·39–1·92)	
Q4	31·68–34166·67	43/329	0·94 (0·43–2·03)	
Equol				0·285
Q1	0·14–4·64	47/368	1·00 (Ref)	
Q2	4·65–9·32	28/340	0·56 (0·26–1·18)	
Q3	9·33–18·14	35/264	1·19 (0·65–2·16)	
Q4	18·15–11117·65	36/274	1·17 (0·61–2·24)	
Total lignan				0·405
Q1	0·39–308·15	36/347	1·00 (Ref)	
Q2	308·16–771·15	43/343	1·14 (0·57–2·30)	
Q3	771·16–1432·13	29/283	0·82 (0·35–1·91)	
Q4	1432·14–47545·45	38/273	0·76 (0·32–1·80)	
Enterodiol				0·002
Q1	0·02–26·89	25/382	1·00 (Ref)	
Q2	26·90–65·79	32/350	0·80 (0·34–1·89)	
Q3	65·80–147·51	48/264	2·20 (0·89–5·46)	
Q4	147·52–40909·09	41/250	2·79 (1·26–6·20)	
Enterolactone				0·234
Q1	0·33–202·15	35/336	1·00 (Ref)	
Q2	202·16–652·84	41/351	0·95 (0·46–1·95)	
Q3	652·85–1293·54	34/283	0·78 (0·35–1·74)	
Q4	1293·55–26491·23	36/276	0·61 (0·25–1·48)	

Fig. 3. The stratified analysis between urinary phytoestrogens and HRC among females from NHANES 1999–2010. The analysis was stratified by (a) race/ethnicity (White and non-White) and (b) age group (≤ 60 years and > 60 years). Adjusted for age (continuous, not for age-stratified analysis), race/ethnicity (non-Hispanic White, non-Hispanic Black, Mexican American or other, not for race/ethnicity-stratified analysis), education level (below high school, high school or college or above), marital status (married/living with partner, divorced/separated/widowed or never married), poverty income ratio (0–1·30, 1·31–3·50, or 3·51–), BMI (< 25, 25–30 or > 30 kg/m^2), physical activity (vigorous, moderate or inactive), smoking status (non-smoker, current smoking or former smokers), alcohol intake (no drinking, moderate drinking or heavy drinking), hypertension (yes or no), diabetes (yes or no), dyslipidaemia (yes or no), total energy intake (continuous) and fat intake (continuous). HRC, hormone-related cancer; NHANES, National Health and Nutrition Examination Survey; NH, non-Hispanic; O-DMA, O-desmethylangolensin.

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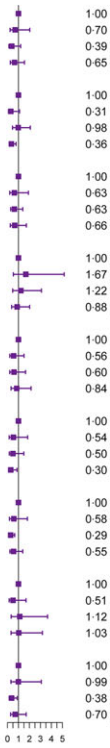
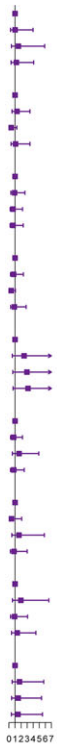
(a)

Urinary phytoestrogens	Range, μg/g creatinine	N (HRC/non-HRC)	OR (95% CI)	*P* for trend
NH white (n=2,132)				
Total phytoestrogen				0·469
Q1	5·49–267·82	8/467	1·00 (Ref)	
Q2	267·83–627·00	15/515	0·70 (0·24–2·03)	
Q3	627·01–1302·01	26/533	0·39 (0·13–1·20)	
Q4	1302·02–54004·41	40/528	0·65 (0·26–1·55)	
Total isoflavone				0·189
Q1	1·66–41·98	18/480	1·00 (Ref)	
Q2	41·97–90·86	13/506	0·31 (0·09–1·10)	
Q3	90·87–271·01	34/526	0·98 (0·46–2·08)	
Q4	271·02–33908·97	24/531	0·36 (0·16–0·78)	
Daidzein				0·425
Q1	0·23–16·88	18/501	1·00 (Ref)	
Q2	16·89–44·74	19/486	0·63 (0·21–1·91)	
Q3	44·75–142·70	28/522	0·63 (0·29–1·39)	
Q4	142·71–22961·26	24/534	0·66 (0·26–1·72)	
Genistein				0·499
Q1	0·09–8·30	12/479	1·00 (Ref)	
Q2	8·31–21·39	22/504	1·67 (0·54–5·16)	
Q3	21·40–65·99	28/512	1·22 (0·48–3·09)	
Q4	66·00–7059·03	27/548	0·88 (0·38–2·02)	
O-DMA				0·862
Q1	0·03–0·66	15/513	1·00 (Ref)	
Q2	0·67–2·92	18/512	0·56 (0·21–1·51)	
Q3	2·93–14·81	24/250	0·60 (0·21–1·65)	
Q4	14·82–3939·53	32/498	0·84 (0·33–2·15)	
Equol				0·037
Q1	0·05–3·52	17/467	1·00 (Ref)	
Q2	3·53–6·97	20/509	0·54 (0·16–1·84)	
Q3	6·98–13·88	31/505	0·50 (0·17–1·49)	
Q4	13·89–11635·77	21/562	0·30 (0·10–0·87)	
Total lignan				0·354
Q1	0·25–124·10	11/465	1·00 (Ref)	
Q2	124·11–391·74	13/524	0·58 (0·18–1·82)	
Q3	391·75–893·67	20/525	0·29 (0·13–0·66)	
Q4	893·68–52892·53	45/529	0·55 (0·22–1·39)	
Enterodiol				0·627
Q1	0·02–15·95	11/522	1·00 (Ref)	
Q2	15·96–38·41	19/525	0·51 (0·16–1·69)	
Q3	38·42–89·85	26/523	1·12 (0·34–3·65)	
Q4	89·86–11689·61	33/473	1·03 (0·33–3·18)	
Enterolactone				0·366
Q1	0·08–80·47	9/473	1·00 (Ref)	
Q2	80·48–316·62	16/506	0·99 (0·32–3·08)	
Q3	316·63–797·99	21/519	0·38 (0·16–0·89)	
Q4	798·00–43386·86	43/545	0·70 (0·29–1·70)	

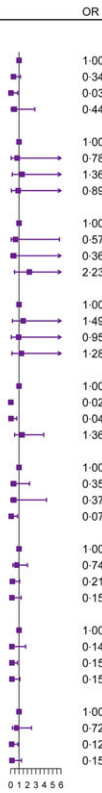


Urinary phytoestrogens	Range, μg/g creatinine	N (HRC/non-HRC)	OR (95% CI)	*P* for trend
Non-NH white (n=2,300)				
Total phytoestrogen				0·655
Q1	1·03–229·23	10/558	1·00 (Ref)	
Q2	229·24–517·16	13/557	1·01 (0·26–3·97)	
Q3	517·17–1084·74	19/579	1·55 (0·41–5·90)	
Q4	1084·75–31443·60	21/543	1·22 (0·36–4·08)	
Total isoflavone				0·807
Q1	0·77–32·15	15/601	1·00 (Ref)	
Q2	32·16–81·74	19/542	1·33 (0·51–3·46)	
Q3	81·75–262·58	8/572	0·37 (0·11–1·30)	
Q4	262·59–31399·00	21/522	1·09 (0·35–3·46)	
Daidzein				0·469
Q1	0·08–14·66	17/606	1·00 (Ref)	
Q2	14·67–44·56	16/548	0·92 (0·32–2·63)	
Q3	44·57–155·11	13/561	0·67 (0·20–2·24)	
Q4	155·12–23200·00	17/522	0·66 (0·19–2·35)	
Genistein				0·766
Q1	0·06–7·72	16/577	1·00 (Ref)	
Q2	7·73–19·75	15/549	0·77 (0·25–2·38)	
Q3	19·76–68·67	12/591	0·37 (0·13–1·06)	
Q4	68·68–11514·39	20/520	0·89 (0·28–2·83)	
O-DMA				0·151
Q1	0·02–0·44	9/604	1·00 (Ref)	
Q2	0·45–2·09	16/537	2·50 (0·55–11·30)	
Q3	2·10–12·66	18/556	2·96 (0·63–13·79)	
Q4	12·67–9557·52	20/540	3·14 (0·68–14·64)	
Equol				0·936
Q1	0·06–1·98	20/559	1·00 (Ref)	
Q2	1·99–4·06	12/535	0·77 (0·26–2·23)	
Q3	4·07–8·09	15/564	1·67 (0·57–4·92)	
Q4	8·10–7660·55	16/579	0·79 (0·25–2·52)	
Total lignan				0·665
Q1	0·15–100·12	12/533	1·00 (Ref)	
Q2	100·13–279·20	7/547	0·42 (0·08–2·10)	
Q3	279·21–674·08	19/579	1·65 (0·47–5·84)	
Q4	674·09–22473·21	25/578	0·84 (0·24–3·01)	
Enterodiol				0·932
Q1	0·02–10·36	12/593	1·00 (Ref)	
Q2	10·37–26·78	19/554	1·96 (0·58–6·58)	
Q3	26·79–64·63	12/578	0·92 (0·27–3·08)	
Q4	64·64–4578·30	20/512	1·37 (0·42–4·44)	
Enterolactone				0·707
Q1	0·09–68·72	11/526	1·00 (Ref)	
Q2	68·73–233·32	10/547	1·74 (0·53–5·74)	
Q3	233·33–593·40	17/576	1·53 (0·44–5·37)	
Q4	593·41–20267·86	25/588	1·49 (0·40–5·52)	



(b)

Urinary phytoestrogens	Range, μg/g creatinine	N (HRC/non-HRC)	OR (95% CI)	*P* for trend
<=60 y (n=3,106)				
Total phytoestrogen				0·132
Q1	1·03–228·86	4/800	1·00 (Ref)	
Q2	228·87–535·53	2/803	0·34 (0·10–1·16)	
Q3	535·54–1139·12	2/777	0·03 (0·00–0·85)	
Q4	1139·13–54004·41	5/713	0·44 (0·07–2·88)	
Total isoflavone				0·777
Q1	0·77–36·45	2/841	1·00 (Ref)	
Q2	36·45–83·95	4/737	0·78 (0·06–9·60)	
Q3	83·96–259·16	5/767	1·36 (0·19–9·51)	
Q4	259·17–33908·97	2/748	0·89 (0·09–8·76)	
Daidzein				0·409
Q1	0·09–16·03	2/856	1·00 (Ref)	
Q2	16·04–43·41	4/728	0·57 (0·06–5·81)	
Q3	43·42–142·67	4/745	0·36 (0·02–6·18)	
Q4	142·68–23200·00	3/764	2·23 (0·46–10·71)	
Genistein				0·964
Q1	0·06–7·71	3/785	1·00 (Ref)	
Q2	7·72–19·71	3/782	1·49 (0·21–10·44)	
Q3	19·72–62·90	4/761	0·95 (0·08–11·75)	
Q4	62·91–11514·39	3/765	1·28 (0·15–10·76)	
O-DMA				0·957
Q1	0·02–0·55	3/856	1·00 (Ref)	
Q2	0·56–2·49	1/743	0·02 (0·00–0·29)	
Q3	2·50–13·51	3/759	0·04 (0·00–0·73)	
Q4	13·52–9557·52	6/735	1·36 (0·47–3·93)	
Equol				0·051
Q1	0·05–2·74	4/852	1·00 (Ref)	
Q2	2·75–5·80	2/780	0·35 (0·05–2·25)	
Q3	5·81–11·93	6/772	0·37 (0·03–4·27)	
Q4	11·94–11635·77	1/689	0·07 (0·01–0·82)	
Total lignan				0·046
Q1	0·21–98·72	4/768	1·00 (Ref)	
Q2	98·73–311·15	1/830	0·74 (0·27–2·00)	
Q3	311·16–727·02	3/774	0·21 (0·04–1·06)	
Q4	727·03–52892·53	5/721	0·15 (0·02–1·25)	
Enterodiol				0·036
Q1	0·02–12·67	4/878	1·00 (Ref)	
Q2	12·68–31·97	2/785	0·14 (0·01–1·79)	
Q3	31·98–76·91	1/774	0·15 (0·03–0·82)	
Q4	76·92–11689·61	6/656	0·15 (0·02–1·11)	
Enterolactone				0·044
Q1	0·08–63·78	4/750	1·00 (Ref)	
Q2	63·79–257·24	2/834	0·72 (0·21–2·48)	
Q3	257·25–643·96	2/788	0·12 (0·02–0·88)	
Q4	643·97–43386·86	5/721	0·15 (0·02–1·28)	



Urinary phytoestrogens	Range, μg/g creatinine	N (HRC/non-HRC)	OR (95% CI)	*P* for trend
>60 y (n=1,326)				
Total phytoestrogen				0·254
Q1	1·27–314·58	34/353	1·00 (Ref)	
Q2	461·73–711·17	29/284	0·83 (0·38–1·80)	
Q3	853·82–1520·08	40/293	1·36 (0·69–2·68)	
Q4	1696·72–27355·07	36/257	1·28 (0·65–2·53)	
Total isoflavone				0·529
Q1	0·94–43·89	39/325	1·00 (Ref)	
Q2	46·49–103·27	28/280	0·49 (0·19–1·25)	
Q3	112·00–334·21	35/298	0·93 (0·41–2·09)	
Q4	305·28–24420·12	37/284	0·66 (0·35–1·27)	
Daidzein				0·387
Q1	0·08–18·28	38/313	1·00 (Ref)	
Q2	18·90–50·83	28/281	0·65 (0·26–1·63)	
Q3	55·00–176·94	37/304	0·96 (0·46–2·02)	
Q4	173·64–18352·94	36/289	0·64 (0·33–1·26)	
Genistein				0·774
Q1	0·07–8·98	35/306	1·00 (Ref)	
Q2	10·19–23·30	30/287	0·96 (0·46–1·98)	
Q3	25·71–79·61	35/304	0·93 (0·46–1·87)	
Q4	80·54–10020·65	39/290	0·91 (0·48–1·73)	
O-DMA				0·515
Q1	0·03–0·73	28/343	1·00 (Ref)	
Q2	0·85–3·33	38/289	1·72 (0·76–3·89)	
Q3	3·66–19·35	30/271	1·24 (0·51–3·00)	
Q4	15·68–3939·53	43/284	1·44 (0·69–2·97)	
Equol				0·341
Q1	0·06–3·34	46/395	1·00 (Ref)	
Q2	4·22–6·98	31/270	1·12 (0·52–2·41)	
Q3	7·53–14·47	39/264	1·13 (0·55–2·35)	
Q4	14·99–5893·13	23/258	0·63 (0·27–1·46)	
Total lignan				0·168
Q1	0·15–153·06	31/347	1·00 (Ref)	
Q2	261·49–436·13	32/296	1·31 (0·57–3·02)	
Q3	613·88–1001·71	40/289	1·55 (0·76–3·16)	
Q4	1227·41–27259·86	36/255	1·58 (0·75–3·35)	
Enterodiol				0·048
Q1	0·03–16·40	32/363	1·00 (Ref)	
Q2	18·72–43·15	34/321	2·23 (1·23–4·04)	
Q3	46·55–106·67	34/276	1·93 (0·79–4·71)	
Q4	103·03–11077·02	39/227	2·78 (1·29–5·98)	
Enterolactone				0·167
Q1	0·10–94·14	31/331	1·00 (Ref)	
Q2	199·36–351·43	34/310	1·33 (0·62–2·86)	
Q3	530·61–883·05	36/284	1·33 (0·62–2·86)	
Q4	1102·13–26760·56	38/262	1·64 (0·83–3·24)	

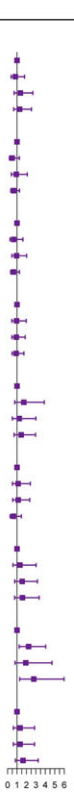


Fig. 4. The stratified analysis between urinary phytoestrogens and HRC among males from NHANES 1999–2010. The analysis was stratified by (a) race/ethnicity (White and non-White) and (b) age group (≤ 60 years and > 60 years). Adjusted for age (continuous, not for age-stratified analysis), race/ethnicity (non-Hispanic White, non-Hispanic Black, Mexican American or other, not for race/ethnicity-stratified analysis), education level (below high school, high school or college or above), marital status (married/living with partner, divorced/separated/widowed or never married), poverty income ratio (0–1·30, 1·31–3·50, or 3·51–), BMI (< 25, 25–30 or > 30 kg/m^2), physical activity (vigorous, moderate or inactive), smoking status (non-smoker, current smoking or former smokers), alcohol intake (no drinking, moderate drinking or heavy drinking), hypertension (yes or no), diabetes (yes or no), dyslipidaemia (yes or no), total energy intake (continuous) and fat intake (continuous). HRC, hormone-related cancer; NHANES, National Health and Nutrition Examination Survey; NH, non-Hispanic; O-DMA, O-desmethylangolensin.

Table 3. Correlation of urinary phytoestrogens with female cancer biomarkers among 678 participants from NHANES 2001–2002

	CA125		CA15·3		HE4	
Urinary phytoestrogens, μg/g creatinine	$r_{partial}$	$P_{partial}$	$r_{partial}$	$P_{partial}$	$r_{partial}$	$P_{partial}$
All participants (*n* 678)						
Total phytoestrogen	–0·023	0·559	0·044	0·258	0·014	0·727
Isoflavone	–0·011	0·780	0·019	0·625	0·016	0·687
Daidzein	–0·005	0·899	0·020	0·607	0·030	0·464
Genistein	0·001	0·993	0·021	0·591	0·015	0·706
O-DMA	–0·035	0·375	–0·005	0·894	–0·002	0·955
Equol	–0·004	0·913	0·002	0·956	–0·009	0·822
Lignan	–0·005	0·898	0·038	0·329	–0·004	0·921
Enterodiol	0·014	0·727	0·015	0·698	0·001	0·972
Enterolactone	–0·009	0·814	0·026	0·495	–0·004	0·913
With HRC group (*n* 24)						
Total phytoestrogen	0·189	0·401	0·145	0·510	0·478	0·098
Isoflavone	–0·012	0·957	0·218	0·319	0·132	0·668
Daidzein	–0·083	0·713	0·304	0·159	0·017	0·957
Genistein	0·001	0·999	–0·080	0·717	0·194	0·525
O-DMA	–0·017	0·941	0·050	0·821	0·177	0·563
Equol	0·097	0·666	0·448	0·032	0·226	0·457
Lignan	0·321	0·145	0·107	0·624	0·488	0·090
Enterodiol	0·293	0·185	0·006	0·978	0·377	0·205
Enterolactone	0·345	0·116	0·093	0·673	0·511	0·074
Normal group (*n* 654)						
Total phytoestrogen	–0·039	0·336	0·046	0·245	–0·005	0·898
Isoflavone	–0·012	0·773	0·015	0·701	0·009	0·830
Daidzein	–0·003	0·950	0·015	0·695	0·025	0·539
Genistein	–0·001	0·993	0·025	0·526	0·007	0·856
O-DMA	–0·036	0·365	–0·005	0·892	–0·008	0·855
Equol	–0·011	0·786	–0·017	0·670	–0·013	0·757
Lignan	–0·028	0·491	0·041	0·291	–0·022	0·591
Enterodiol	–0·003	0·936	0·021	0·590	–0·009	0·831
Enterolactone	–0·031	0·438	0·029	0·455	–0·020	0·630

Abbreviations: CA125, cancer antigen 125; CA15.3, cancer antigen 15.3; HE4, human epididymal secretory protein E4; HRC, hormone-related cancers; NHANES, National Health and Nutrition Examination Survey; O-DMA, O-desmethylangolensin.
Data were expressed as the weighted correlation coefficient (*r*) and adjusted *P*-value.
For CA125, partial correlation analyses adjusted for age (continuous) and hypertension (yes or no). For CA15.3, partial correlation analyses adjusted for marital status (married/living with partner, divorced/separated/widowed or never married). For HE4, partial correlation analyses adjusted for age (continuous), race/ethnicity (non-Hispanic White, non-Hispanic Black, Mexican American, or other), education (below high school, high school or college or above), marital status (married/living with partner, divorced/separated/widowed or never married), smoking status (non-smoker, current smoking or former smokers), hypertension (yes or no), diabetes (yes or no), dyslipidaemia (yes or no), total energy intake (continuous) and fat intake (continuous). A level of $< 0·05$ for two-sided *P* values was considered statistically significant.

postmenopausal majority. Menopausal status as an effect modifier may have an influence on the association between phytoestrogens and female cancer risk. Ward *et al.*[20] stratified by menopausal status and detected a stronger association between total urinary isoflavones and breast cancer risk in premenopausal and perimenopausal women (OR = 1·30), but null findings in postmenopausal women (OR = 1·01). In our study, total urinary isoflavones were positively associated with HRC in both pre- and post-menopausal women, but the OR in premenopausal women was quite larger than that in post-menopausal women. For certain quartiles, the OR of Q2 and Q4 for pre-menopausal and Q3 for post-menopausal were significant. Similarly, the effects of lignans seemed more significant in pre-menopausal women. Since phytoestrogens have dual effects, it is necessary to understand the effects of phytoestrogens on HRC in different menopausal states and hormone levels. Nonetheless, the evidence suggests that there may be circumstances where phytoestrogen exposure increases HRC (e.g. breast cancer) risk, and thus the evidence on the role of phytoestrogens modified by menopausal status deserves further study.

Herein, we found that participants with HRC had higher serum HE4 concentrations and lower PSA ratios than those without HRC, which was in line with our expectations. HE4 is a novel tumour marker for ovarian cancer with higher specificity and sensitivity than CA125[53]. PSA ratio has proven to be an effective tool in prostate cancer screening, especially in the setting of total PSA in the intermediate range of 4–10 ng/ml and a negative digital rectal examination[54]. Knowledge is still limited about the association of phytoestrogens and cancer biomarkers. A case report showed that the serum CA125 concentration of a post-operative patient with ovarian cancer dropped from 756 μg/ml to 311 μg/ml after an intervention of the fermented soybean product for 6 weeks[31]. In our study, we did not find any effect of phytoestrogens on CA125, but a stimulative effect of equol on CA15.3 in twenty-four HRC patients. We further analysed the correlation between CA15.3 and urinary equol in twenty-four HRC patients by cancer type and found that there was a discrepant correlation in the various cancers (for breast cancer: *n* 16, $r = 0·420$, $P = 0·119$; for uterine cancer: *n* 7, $r = 0·843$; $P = 0·035$; data not shown). Studies have suggested that

Table 4. Correlation of urinary phytoestrogens with male cancer biomarkers among 2159 participants from NHANES 2001–2010

	Total PSA		Free PSA		PSA ratio	
Urinary phytoestrogens, μg/g creatinine	$r_{partial}$	$P_{partial}$	$r_{partial}$	$P_{partial}$	$r_{partial}$	$P_{partial}$
All participants (*n* 2159)						
Total phytoestrogen	–0·036	0·108	–0·015	0·498	0·032	0·143
Isoflavone	–0·011	0·610	–0·014	0·539	0·005	0·832
Daidzein	–0·015	0·511	–0·016	0·447	0·008	0·712
Genistein	–0·018	0·412	–0·026	0·247	–0·002	0·940
O-DMA	–0·009	0·673	–0·001	0·982	0·020	0·362
Equol	0·018	0·431	0·016	0·467	–0·013	0·548
Lignan	–0·021	0·358	0·008	0·718	0·032	0·144
Enterodiol	–0·032	0·160	–0·032	0·159	0·018	0·401
Enterolactone	–0·022	0·322	0·012	0·593	0·035	0·107
Prostate cancer group (*n* 13)						
Total phytoestrogen	–0·796	0·204	–0·648	0·551	–0·079	0·818
Isoflavone	0·934	0·067	0·951	0·200	–0·301	0·368
Daidzein	0·900	0·100	0·934	0·232	–0·206	0·544
Genistein	0·884	0·116	0·805	0·404	–0·439	0·177
O-DMA	–0·628	0·372	–0·902	0·284	–0·136	0·691
Equol	–0·201	0·799	0·421	0·723	0·356	0·282
Lignan	–0·987	0·014	–0·993	0·072	0·423	0·194
Enterodiol	–0·905	0·095	–0·871	0·328	0·035	0·918
Enterolactone	–0·979	0·021	–0·986	0·105	0·636	0·036
With HRC group (*n* 18)						
Total phytoestrogen	–0·085	0·873	0·162	0·795	–0·088	0·747
Isoflavone	0·242	0·644	–0·005	0·993	–0·379	0·148
Daidzein	0·382	0·455	0·130	0·834	–0·307	0·247
Genistein	0·223	0·671	–0·020	0·975	–0·442	0·087
O-DMA	0·383	0·454	0·455	0·442	–0·289	0·277
Equol	–0·002	0·997	0·249	0·686	0·298	0·262
Lignan	–0·460	0·359	–0·065	0·917	0·292	0·272
Enterodiol	0·159	0·763	0·539	0·349	0·016	0·953
Enterolactone	–0·553	0·255	–0·177	0·776	0·445	0·084
Normal group (*n* 2141)						
Total phytoestrogen	–0·038	0·095	–0·017	0·446	0·032	0·139
Isoflavone	–0·015	0·502	–0·016	0·473	0·007	0·735
Daidzein	–0·019	0·402	–0·019	0·402	0·010	0·633
Genistein	–0·021	0·354	–0·027	0·238	0·002	0·931
O-DMA	–0·012	0·580	–0·001	0·958	0·220	0·310
Equol	0·015	0·501	0·011	0·615	–0·015	0·489
Lignan	–0·020	0·371	0·005	0·816	0·028	0·189
Enterodiol	–0·033	0·143	–0·035	0·119	0·017	0·419
Enterolactone	–0·022	0·339	0·009	0·694	0·031	0·157

Abbreviations: HRC, hormone-related cancers; NHANES, National Health and Nutrition Examination Survey; O-DMA, O-desmethylangolensin; PSA, prostate-specific antigen.
Data were expressed as the weighted correlation coefficient (*r*) and adjusted *P*-value.
For total PSA, partial correlation analyses were adjusted for age (continuous), BMI (< 25, 25–30, or > 30 kg/m^2), hypertension (yes or no), total energy intake (continuous) and fat intake (continuous). For free PSA, partial correlation analyses adjusted for age (continuous), BMI (< 25, 25–30, or > 30 kg/m^2), smoking status (non-smoker, current smoking or former smokers), hypertension (yes or no), total energy intake (continuous) and fat intake (continuous). For PSA ratio, partial correlation analyses adjusted for race/ethnicity (non-Hispanic White, non-Hispanic Black, Mexican American or other) and marital status (married/living with partner, divorced/separated/widowed or never married).

CA15.3 levels significantly correlated with prognostic factors and were a useful prognostic tool for endometrial (uterine) cancer[55]. However, whether equol impacts the initiation and development of endometrial cancer by affecting the level of CA15.3 needs further research verification. A randomised, double-blind, placebo-controlled study indicated that a daily diet containing four slices of bread rich in heat-treated soy grits favourably influenced the PSA level and the free/total PSA ratio in patients with prostate cancer, which provides some evidence to support epidemiologic studies claiming that male populations who consume high phytoestrogen diets have a decreased risk of the PSA abnormality and even prostate cancer[56]. Additionally, a cross-sectional study examining the relationship between the serum PSA level and urine phytoestrogen concentration from NHANES 2001–2004 revealed that in general healthy US men, 40+ years old without a diagnosis of prostate cancer, urinary isoflavone and lignan concentrations were not associated with serum PSA level[57]. Similar results were found among healthy men without HRC from NHANES 2001–2010 in this study. Of note, among small-scale prostate cancer patients, enterolactone seemed to favourably reduce total PSA levels and increase PSA ratio. The hormone-related activity of lignans may exert anti-hormonal activity and somehow alter physiological hormonal homeostasis in the presence of prostate cancer. Current research is ongoing to better understand the mode of action of phytoestrogens on cancer.

Theoretically, our findings enriched the study of the potential effects of phytoestrogens on HRC and for the first time showed a positive correlation of urinary equol with CA15.3 and a negative and positive correlation of urinary

enterolactone with total PSA and PSA ratio in men, respectively. However, there are some limitations and challenges to be settled in our research. First, the cross-sectional data reduces our capacity for evidence. More prospective studies are warranted to explore the causal association. Second, selection bias and recall bias may exist. For instance, information on the history of cancer was recalled and orally reported by the subjects, not confirmed by cancer registries or medical records. And information on cancer treatment or stage of cancer was unavailable. Third, information on dietary phytoestrogens was not included in our analysis and urinary phytoestrogens reflect short term (24–48 h) rather than long-term dietary intake. Meanwhile, since the phytoestrogen data for most of our cancer patients reflect a point in time after diagnosis, it is possible that the eating habits have changed and cannot reflect the typical dietary behaviours before the cancer experience. Such information, if included, would make our analysis more convincing. Besides, even though the potential covariates of interest had been adjusted in the partial correlation analyses, there may be other unknown variables affecting the relationship between phytoestrogens and cancer biomarkers. Therefore, further research is needed to further clarify this issue.

In conclusion, our findings indicated that higher concentrations of total isoflavones and enterodiol were positively associated with HRC among females and equol was negatively linked to HRC among males, differentiated by race/ethnicity and age. Urinary equol excretion was related to serum CA15.3 among participants with HRC, and enterolactone concentration may affect PSA levels in prostate cancer patients. Nonetheless, further prospective studies and a larger number of subjects are warranted to be verified and provide stronger evidence.

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The National Center for Health Statistics Ethics Review Board at the Centers for Disease Control and Prevention approved collection of the NHANES data and posting of the NHANES public-use files, which were required for this investigation. All NHANES participants provided written informed consent.

Supplementary material

For supplementary material/s referred to in this article, please visit https://doi.org/10.1017/S0007114522003877

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