scientific reports

Check for updates

OPEN

Inverted U-shape association between urine equol levels and cancer: a national population-based cross-sectional study

Jufa Li[1,4], Jing Lv[1,4], Yuwei Zhang[2,3,4], Zhihao Zhou[1], Haochen Geng[1], Yuhua Zhou[1], Chun Yang[1,3]✉ & Ninghan Feng[1,2,3]✉

Equol, a naturally occurring phytoestrogen derived from the fermentation of soy and soy-based products by gut bacteria, is recognized for its diverse health benefits. While there is speculation about its association with cancer prevention, the scientific community has yet to reach a consensus due to the variability in research findings. Our study aims to shed light on this topic by examining the correlation between urine equol concentrations and the cancer risk among the American population. The National Health and Nutrition Examination Survey (NHANES) is a national survey of U.S. civilians in which cancer participants are enrolled in a database by a sample questionnaire. This study included 2797 Americans aged 40 years and older in the NHANES database (2005–2010). The relationship between urine equol concentration and cancer was analysed using weighted logistic regression models, stratified analysis, smoothed curve fitting and threshold effect analysis were also performed. Among the 2797 participants in our study, 390 individuals received a cancer diagnosis. Our findings indicate a positive correlation between urine equol levels and the risk of cancer. Notably, individuals in the highest quartile of equol excretion exhibited a significantly elevated risk of cancer, with a 25.4% increase compared to those in the lowest quartile (POR = 1.254, 95% CI: 1.252, 1.256), after fully adjusting for confounders. Similar results were observed in other adjusted models. A non-linear relationship in the shape of an inverted U-shape can be observed by smoothed curve fitting, and the inflection point is 25.5. Urinary equol concentrations below 25.5 ng/ml were positively associated with cancer risk, while equol concentrations above 25.5 ng/ml showed a slight negative trend in cancer risk. However, further prospective studies are needed to provide more robust evidence and confirmed in large clinical trials.

Keywords Cancer risk, Equol, Gut metabolites, Soy, Urine, NHANES

Abbreviations

BMI	body mass index
NHANES	National Health and Nutrition Examination Survey
NCHS	National Center for Health Statistics
CDC	Centers for Disease Control and Prevention
POR	prevalence odds ratio
CIs	confidence intervals
SCFAs	short-chain fatty acids
TMAO	trimethylamine N-oxide
Ref	reference
PIR	poverty income ratio
TME	tumor microenvironment
TAMs	tumor-associated macrophages
HPLC-APPI-MS/MS	high performance liquid chromatography-atmospheric pressure photoionization-tandem mass spectrometry

[1]Wuxi School of Medicine, Jiangnan University, Wuxi, China. [2]Medical School of Nantong University, Nantong, China. [3]Department of Urology, Jiangnan University Medical Center, Wuxi, China. [4]Jufa Li, Jing Lv and Yuwei Zhang contributed equally. ✉email: yang-chun@126.com; n.feng@jiangnan.edu.cn

Cancer is a major global public health problem, threatening the lives of millions of people and imposing a severe social and economic burden. A recent study has revealed that in 2022, there were approximately 20 million new cases of cancer and 9.7 million deaths related to the disease[1]. The report further indicates that by 2050, the number of new cancer cases is projected to surpass 35 million per year[1]. Despite the diversity of cancers, many epidemiological factors have been identified as being associated with cancer, and by intervening in these factors, the incidence of certain types of cancer has been significantly reduced. For example, controlling smoking reduces the incidence of lung, liver and bladder cancers[2–4]. The control of cancer-related risk factors is becoming increasingly important due to their potential role in disease prevention.

In recent years, the significance of the gut microbiome has been thrust into the spotlight, recognized for its crucial role in combating the production of harmful substances and facilitating the absorption of essential nutrients that the body cannot directly assimilate[5]. The gut microbiota plays a crucial role in various physiological, pathological and metabolic processes in the human body, such as inflammation, tumours, immunomodulation and nervous system development[6–8]. Gut bacterial metabolites are produced by gut bacteria metabolising food, can be detected in human blood and urine and are important for human life and health. For example, short-chain fatty acids (SCFAs) are metabolites produced by gut bacteria that play a crucial role in maintaining intestinal and systemic homeostasis, and have the ability to prevent tumour cell proliferation and invasion, induce apoptosis, and arrest the cell cycle[9]. The metabolite trimethylamine N-oxide (TMAO) produced by gut bacteria is involved in the regulation of a variety of disease processes, including cancer, diabetes, obesity, atherosclerosis, inflammation and other diseases[10].

Some studies have demonstrated that the intake of soybeans and soy products is of great significance to human health[11,12]. Soybeans are rich in various bioactive compounds, such as soy isoflavones, soy lecithin, and soy peptides, which have been shown to confer significant health benefits[13]. Research has found that increased intake of soy isoflavones is closely associated with the prevention of multiple diseases, including reduced risk of cardiovascular disease[14], decreased incidence of hormone-dependent cancers (such as breast and prostate cancer)[15,16], and prevention of osteoporosis[17]. The chemical structure of soy isoflavones and some of their metabolites is similar to that of the endogenous estrogen 17β-estradiol, and they can exert estrogen-like biological activities, which is why they are referred to as "phytoestrogens"[18]. Soy isoflavones in the human body mainly originate from the consumption of soybeans and soy-derived foods, with the primary components being genistein, daidzein, and glycitein[19].

Equol is a downstream metabolite derived from soy isoflavones, primarily generated through microbial transformation in the small intestine and colon[20,21]. Equol was first identified in the urine of pregnant mares in 1932[22]. equol exists as both R- and S-isomers, but the human gut microbiota only produces S-equol[23]. Equol boasts superior stability and absorption compared to its precursor, daidzein, coupled with a slower clearance rate[24]. Moreover, it exhibits a more pronounced estrogenic activity than other isoflavones or their metabolites[25]. Equol has a high affinity for estrogen receptors, and acts as an estrogen modulator, playing a beneficial role in various hormone-dependent diseases[26,27]. These benefits include relief of menopausal symptoms, prevention of osteoporosis, and reduced risk of developing cancer[28–31]. In addition, equol is the isoflavone-derived compound with the strongest antioxidant activity[32], which inhibits oxidative stress damage, promotes the expression of antioxidant genes in cells, and enhances the activity of antioxidant enzymes[33,34]. Antioxidants are believed to play an important role in the onset and development of different chronic diseases, including cancer[35]. In conclusion, equol plays an extremely important role in promoting human health, especially in tumour suppression[36].

However, the role of equol on tumours is controversial, with some studies finding a positive association between equol and tumours. In a European prospective investigation, equol was found to increase the risk of breast cancer, with a doubling of the concentration increasing the chance of developing breast cancer by 20–45%[37]. Other studies have found a positive correlation between urine equol levels and breast cancer, endometrial cancer, ovarian cancer and cancer markers[38–40]. It's important to note that the relationship between equol and cancer is not yet fully understood and may be influenced by a variety of factors, including individual differences in metabolism, overall diet, and the presence of specific gut bacteria that can produce equol from soy isoflavones.

As a metabolite derived from soya and its products, equol is produced by certain gut bacteria and can be detected in urine, suggesting its potential as a biomarker for cancer risk. While some studies have suggested a positive association between equol and specific types of cancer, a comprehensive link with overall cancer risk has not been definitively established. In an effort to explore this further, we used the National Health and Nutrition Examination Survey (NHANES) data from 2005 to 2010 to evaluate the relationship between urine equol levels and the prevalence of overall cancer. This analysis aims to provide a more comprehensive understanding of equol's role in cancer risk.

Materials and methods

Study design and participants

In this cross-sectional study, all data were obtained from the National Health and Nutrition Examination Survey (NHANES) database. NHANES is a nationally representative survey of the U.S. civilian population conducted by the National Center for Health Statistics (NCHS), a division of the Centers for Disease Control and Prevention (CDC).The protocol for NHANES was approved by the NCHS Research Ethics Review Board, and informed written consent was obtained from all participants. The datasets generated and analysed in the current study are available on the NHANES website (https://www.cdc.gov/nchs/nhanes/index.htm). We downloaded 3 cycles of NHANES from 2005 to 2010 (2005–2006, 2007–2008, 2009–2010). The data for each cycle consisted of five sections: demographics data, dietary data, examination data, laboratory data, and questionnaire data. In this study, we selected individuals aged 40 and above as our study population. According to the literature, cancer patients under the age of 40 account for only 7.8%, while those aged 40 and above represent a substantial

92.2%[41]. Moreover, the incidence and mortality rates of cancer increase significantly among individuals over 40 years old[42],. Therefore, setting the lower age limit for our study sample at 40 years not only ensures the representativeness of the study population but also focuses on the age group with the highest cancer incidence. This selection will help enhance the practicality and relevance of the study results, providing more valuable references for early cancer screening and intervention.

In order to maintain the integrity and reliability of the results, participants who met one of the following criteria were excluded: (1) age < 40 years ($N = 19818$); (2) participants with missing data from the cancer questionnaire ($N = 18$); (3) participants with urine equol concentrations below the lower limit of measurement and missing ($N = 7874$); and (4) participants who were missing information on cancer-related covariates ($N = 527$). Ultimately, 2797 participants were enrolled in this study (Fig. 1).

Determination of cancer outcomes

In the NHANES survey, participants were asked, "'Have you ever been told by a doctor or other health professional that you had cancer or a malignancy of any kind?" Those who responded "Yes" were classified as having cancer.

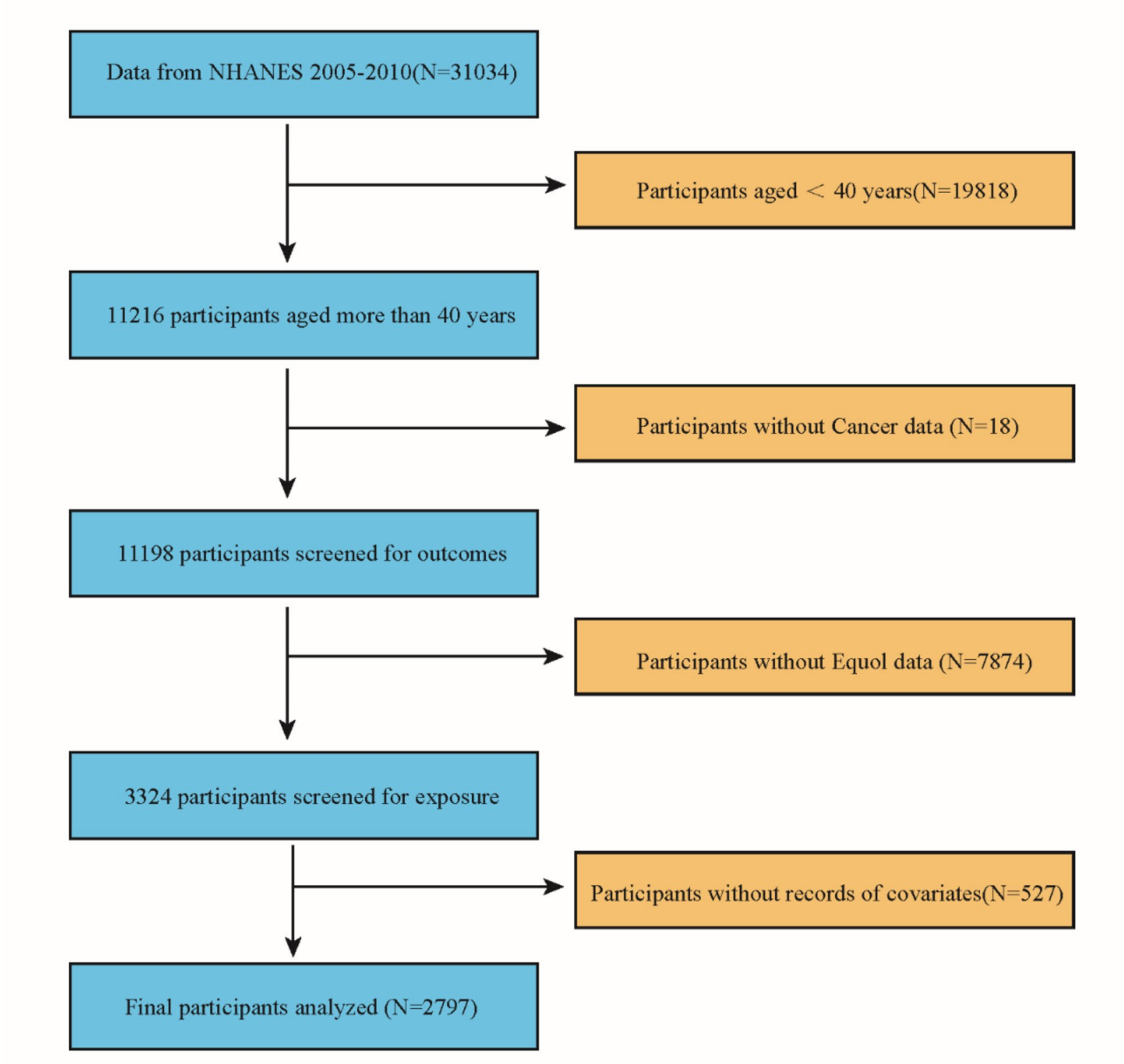

Fig. 1. Flowchart of the participants' selection from NHANES 2005–2010.

Measurement of urine equol concentrations

In the NHANES survey, urine specimens were collected at a mobile testing centre and stored at −20 °C prior to analysis. Urinary equol concentrations in the NHANES database were quantified using high performance liquid chromatography-atmospheric pressure photoionisation-tandem mass spectrometry (HPLC-APPI-MS/MS). Its lower limit of detection is 0.04 ng/ml. Samples below the lower limit in our study will be excluded.

Assessment of covariates

We extracted several participant characteristics from the NHANES database, including gender, age, race, education level, family Poverty Income Ratio (PIR), body mass index (BMI), smoking, drinking, hypertension, diabetes, heart attack, and stroke. Specifically, participants were included if they were aged ≥ 40 years. Race was categorized into Mexican American and non-Mexican American. Education level was classified into three groups: less than high school, high school or equivalent, and college or above. BMI was categorized as < 25 (normal weight), ≥ 25 and < 30 (overweight), and ≥ 30 (obese). Smoking was based on whether one had 'Smoked at least 100 cigarettes in life?'(SMQ020). Drinking was based on whether one had 'Had at least 12 alcohol drinks/1yr?'(ALQ101). Diagnoses of hypertension (BPD020), diabetes (DIQ010), heart attack (MCQ160E) and stroke (MCQ160F) were based on self-reported questionnaire data.

Statistical analyses

Due to the complex sampling methodology of the NHANES database, we used weighted analyses with variables for urine metabolite measurements. The weighting of equol concentrations in the 2005–2006 and 2007–2008 cycles is WTSB2YR, and the weighting of sample equol concentrations in 2009–2010 is WTSA2YR. The aim of this study was to investigate whether urine equol concentrations are associated with cancer. The whole statistical analysis process was divided into four steps. First, we analysed the characteristics of the participants according to the following principles: (1) cancer was used as a dichotomous variable, classified as cancer and non-cancer. continuous variables were expressed as mean ± standard deviation (normal distribution) or median (quartiles) (skewed distribution), and categorical variables were expressed as frequency (per cent); (2) one-way analysis of variance (normal distribution) was used, Kruskal-Wallis H (skewed distribution) test and chi-square test (categorical variables) to determine whether there were significant differences between the means and proportions of the groups. Second, we analysed the relationship between urine equol concentration and cancer using weighted logistic regression. Three statistical models were also analysed: Crude Model, unadjusted for covariates; Model I, adjusted for covariates such as age, gender and race; and Model II, adjusted for age, race, gender, BMI, education level, Family PIR, hypertension, diabetes, heart attack, stroke, smoking, drinking. Third, we used a stratified analysis model stratified by age (40–60 or ≥ 60 years), education level, BMI (< 25; ≥25 and < 30; ≥30), smoking, alcohol consumption, hypertension and diabetes. Stratified analysis interaction p-values were calculated by performing a chi-square test on the interaction terms. Finally, through smoothed curve fitting and threshold effect analysis, we visualised the relationship and inflection points between urine equol concentration and cancer. All analyses were performed using IBM SPSS Statistics 26 and EmpowerStats 4.0. $P < 0.05$ was considered as statistically significant difference.

Results

Characteristics of study participants

We show the general characteristics of the 2797 participants (Table 1). The mean age was 59.63 years. All participants were divided into non-cancer (2407) and cancer (390). Supplementary Table 1 shows cancer types. Significant differences were found between the cancer and non-cancer groups in terms of age, race, education level, family PIR, BMI, hypertension, heart attack, stroke, and smoking ($p < 0.05$). In addition, the proportion of cancer patients was higher in the higher quartiles of urine equol concentrations.

Relationships of urine equol concentration with cancer

The correlation between urine equol concentration and cancer was analysed by weighted logistic regression (Table 2). Urine equol concentration was turned into a categorical variable by quartiles. Urine equol concentration had the strongest positive correlation with cancer in the unadjusted model, and in the model I, adjusted age, gender, and race. In the model II, adjusted all covariates. The PORs of these two adjusted models trended in the same direction as those of the unadjusted model, and all $P < 0.05$, indicating that urine equol concentration was significantly positively associated with cancer.

Stratified analyses

To evaluate the differences in the association between urinary equol concentration and cancer risk across various subgroups, we conducted stratified analyses based on several variables, including age; race; gender; BMI; education level; family PIR; hypertension; diabetes; heart attack; stroke; smoking and drinking (Table 3). The results showed that the relationship between urinary equol concentration and cancer risk was consistent across all subgroups. However, the interaction tests were not significant ($P > 0.05$), indicating that none of the examined subgroups significantly altered the association between urinary equol concentration and cancer risk.

Smoothed curve fitting and threshold effect analysis

We plotted a smoothed curve fitting for the association between urine equol concentration and cancer. Adjusted: age; race; gender; BMI; education level; family PIR; hypertension; diabetes; heart attack; stroke; smoking; drinking. To address the skew in urinary equol concentrations, we applied the Trimming method to exclude extreme values exceeding the 95th percentile. We found a non-linear relationship between urine

Characteristics	Total ($N=2,797$)	Non-cancer ($N=2,407$)	Cancer ($N=390$)	P-value
Age (years), Mean ± SD	59.63 ± 12.46	58.33 ± 12.09	67.64 ± 11.68	< 0.001
Gender, N (%)				0.802
Female	1382 (49.41)	1187 (49.31)	195 (50.00)	
Male	1415 (50.59)	1220 (50.69)	195 (50.00)	
Race, N (%)				< 0.001
Mexican American	464 (16.59)	434 (18.03)	30 (7.69)	
Other Race	2333 (83.41)	1973 (81.97)	360 (92.31)	
Equol (ng/ml), Median (Q1-Q3)	6.21 (2.79–13.30)	6.05 (2.65–13.10)	8.39 (3.56–15.47)	< 0.001
Equol quartile (ng/ml), N (%)				0.003
Q1 1.40 (0.04–2.78)	698 (24.96)	627 (26.05)	71 (18.21)	
Q2 4.29 (2.79–6.19)	697 (24.92)	604 (25.09)	93 (23.85)	
Q3 9.25 (6.20–13.20)	700 (25.03)	588 (24.43)	112 (28.72)	
Q4 24.15 (13.21-max)	702 (25.10)	588 (24.43)	114 (29.23)	
BMI (kg/m^2), N (%)				0.204
< 25	706 (25.24)	601 (24.97)	105 (26.92)	
≥ 25 and < 30	994 (35.54)	846 (35.15)	148 (37.95)	
≥ 30	1097 (39.22)	960 (39.88)	137 (35.13)	
Education level, N (%)				0.029
Below high school	814 (29.10)	721 (29.95)	93 (23.85)	
High school	690 (24.67)	594 (24.68)	96 (24.62)	
Above high school	1293 (46.23)	1092 (45.37)	201 (51.54)	
Family PIR, Mean ± SD	2.73 ± 1.62	2.70 ± 1.62	2.97 ± 1.59	0.002
Hypertension, N (%)				< 0.001
No	1474 (52.70)	1317 (54.72)	157 (40.26)	
Yes	1323 (47.30)	1090 (45.28)	233 (59.74)	
Diabetes, N (%)				0.074
No	2352 (84.09)	2036 (84.59)	316 (81.03)	
Yes	445 (15.91)	371 (15.41)	74 (18.97)	
Heart attack, N (%)				< 0.001
No	2635 (94.21)	2283 (94.85)	352 (90.26)	
Yes	162 (5.79)	124 (5.15)	38 (9.74)	
Stroke, N (%)				< 0.001
No	2656 (94.96)	2300 (95.55)	356 (91.28)	
YES	141 (5.04)	107 (4.45)	34 (8.72)	
Smoking, N (%)				0.023
< 100 cigarettes in life	1369 (48.95)	1199 (49.81)	170 (43.59)	
≥ 100 cigarettes in life	1428 (51.05)	1208 (50.19)	220 (56.41)	
Drinking, N (%)				0.957
< 12 drinks/year	828 (29.60)	713 (29.62)	115 (29.49)	
≥ 12 drinks/year	1969 (70.40)	1694 (70.38)	275 (70.51)	

Table 1. The characteristics of participants.

equol and cancer, showing an inverted U-shape (Fig. 2). Threshold effect analysis revealed an inflection point of 25.5(Table 4), which indicated that urine equol concentrations ≤ 25.5 ng/ml were positively associated with cancer risk (POR = 1.02, 95% CI: 1.00, 1.04), $p = 0.0125$, and > 25.5 ng/ml were negatively associated with cancer risk (POR = 0.96, 95% CI: 0.91, 1.01), $p = 0.0901$.

Discussion

We examined whether there is an association between urine equol and cancer. In this study, which included 2797 Americans, we assessed the relationship between urine equol and cancer risk using several statistical models. In the weighted logistic regression model, we discovered that higher urine equol was associated with a higher risk of cancer. The results of the stratified analyses and interaction testing indicated that this connection was similar across populations. In the analysis of smooth curve fitting, we found an inverse U-shaped relationship between urinary equol concentration and cancer risk, with an inflection point at 25.5 ng/mL. Specifically, when the urinary equol concentration is below 25.5 ng/mL, the risk of cancer increases (POR = 1.02, 95% CI = 1.00–1.04). Conversely, when the equol concentration exceeds 25.5 ng/mL, a slight negative correlation trend with cancer

Exposure	Crude Model (POR, 95%CI)	Model I (POR, 95%CI)	Model II (POR, 95%CI)
Equol quartile (ng/ml)			
Q1 1.40 (0.04–2.78)	Ref	Ref	Ref
Q2 4.29 (2.79–6.19)	1.267(1.265, 1.270)	1.184(1.182, 1.186)	1.174(1.172, 1.176)
Q3 9.25 (6.20–13.20)	1.210(1.372, 1.377)	1.210(1.208, 1.213)	1.171(1.169, 1.173)
Q4 24.15 (13.21-max)	1.261(1.368, 1.372)	1.261(1.259, 1.263)	1.254(1.252, 1.256)

Table 2. Relationship between urine equol and cancer in different models. Crude Model adjust for: None, Model I adjust for: Age; Race; Gender, Model II adjust for: Age; Race; Gender; BMI; Education level; Family PIR; Hypertension; Diabetes; Heart attack; Stroke; Smoking; Drinking. 95% CI, 95% Confidence Interval; POR, Prevalence Odds Ratio; Ref: reference.

Characteristics	Q1 POR(95%CI)	Q2 POR(95%CI)	Q3 POR(95%CI)	Q4 POR(95%CI)	*P*-interaction
Age (years)					0.49
≥ 40 and < 60	Ref	0.97 (0.41, 2.33)	1.01 (0.47, 2.15)	1.27 (0.63, 2.59)	
≥ 60	Ref	1.49 (0.89, 2.52)	1.57 (0.98, 2.52)	1.35 (0.86, 2.14)	
Gender					0.26
Female	Ref	1.77 (1.07, 2.91)	1.50 (0.96, 2.35)	1.90 (1.09, 3.29)	
Male	Ref	0.78 (0.36, 1.70)	1.25 (0.63, 2.47)	0.95 (0.47, 1.93)	
Race					0.97
Mexican American	Ref	1.29 (0.42, 3.93)	1.21 (0.34, 4.33)	1.00 (0.27, 3.70)	
Other Race	Ref	1.25 (0.81, 1.92)	1.34 (0.89, 2.01)	1.32 (0.84, 2.08)	
BMI (kg/m)					0.72
<25	Ref	1.36 (0.57, 3.24)	1.24 (0.61, 2.49)	0.91 (0.39, 2.12)	
≥ 25 and < 30	Ref	1.46 (0.75, 2.86)	1.35 (0.61, 3.00)	1.60 (0.85, 3.03)	
≥ 30	Ref	1.03 (0.49, 2.19)	1.60 (0.85, 3.03)	1.62 (0.76, 3.46)	
Education level					0.12
Below high school	Ref	2.26 (1.10, 4.64)	0.99 (0.40, 2.46)	2.35 (0.97, 5.72)	
High school	Ref	0.71 (0.29, 1.75)	1.03 (0.47, 2.29)	1.02 (0.46, 2.26)	
Above high school	Ref	1.34 (0.71, 2.51)	1.68 (1.00, 2.82)	1.34 (0.78, 2.30)	
Family PIR					0.52
<1	Ref	1.92 (0.65, 5.70)	1.86 (0.59, 5.81)	3.21 (0.87, 11.82)	
≥ 1	Ref	1.21 (0.78, 1.86)	1.31 (0.88, 1.95)	1.25 (0.80, 1.95)	
Hypertension					0.85
No	Ref	1.08 (0.54, 2.13)	1.11 (0.64, 1.93)	1.26 (0.70, 2.27)	
Yes	Ref	1.39 (0.79, 2.43)	1.55 (0.90, 2.67)	1.52 (0.91, 2.56)	
Diabetes					0.13
No	Ref	1.26 (0.78, 2.01)	1.22 (0.82, 1.83)	1.27 (0.81, 2.02)	
Yes	Ref	1.37 (0.69, 2.72)	2.37 (1.06, 5.31)	2.36 (1.18, 4.74)	
Heart attack					0.66
No	Ref	1.28 (0.80, 2.04)	1.33 (0.87, 2.03)	1.41 (0.92, 2.17)	
Yes	Ref	1.03 (0.27, 3.88)	1.67 (0.39, 7.19)	0.86 (0.18, 4.06)	
Stroke					0.95
No	Ref	1.25 (0.81, 1.92)	1.38 (0.93, 2.05)	1.37 (0.87, 2.14)	
Yes	Ref	1.38 (0.22, 8.55)	1.12 (0.21, 6.07)	1.39 (0.30, 6.33)	
Smoking					0.07
<100 cigarettes in life	Ref	1.61 (0.86, 3.01)	1.21 (0.61, 2.40)	1.06 (0.56, 2.00)	
≥ 100 cigarettes in life	Ref	0.99 (0.54, 1.81)	1.56 (0.97, 2.49)	1.73 (1.03, 2.91)	
Drinking					0.83
<12 drinks/year	Ref	1.75 (0.77, 3.94)	1.51 (0.70, 3.23)	1.52 (0.75, 3.08)	
≥ 12 drinks/year	Ref	1.12 (0.68, 1.83)	1.33 (0.83, 2.14)	1.32 (0.78, 2.25)	

Table 3. Relationship between urine equol and cancer in different subgroups.

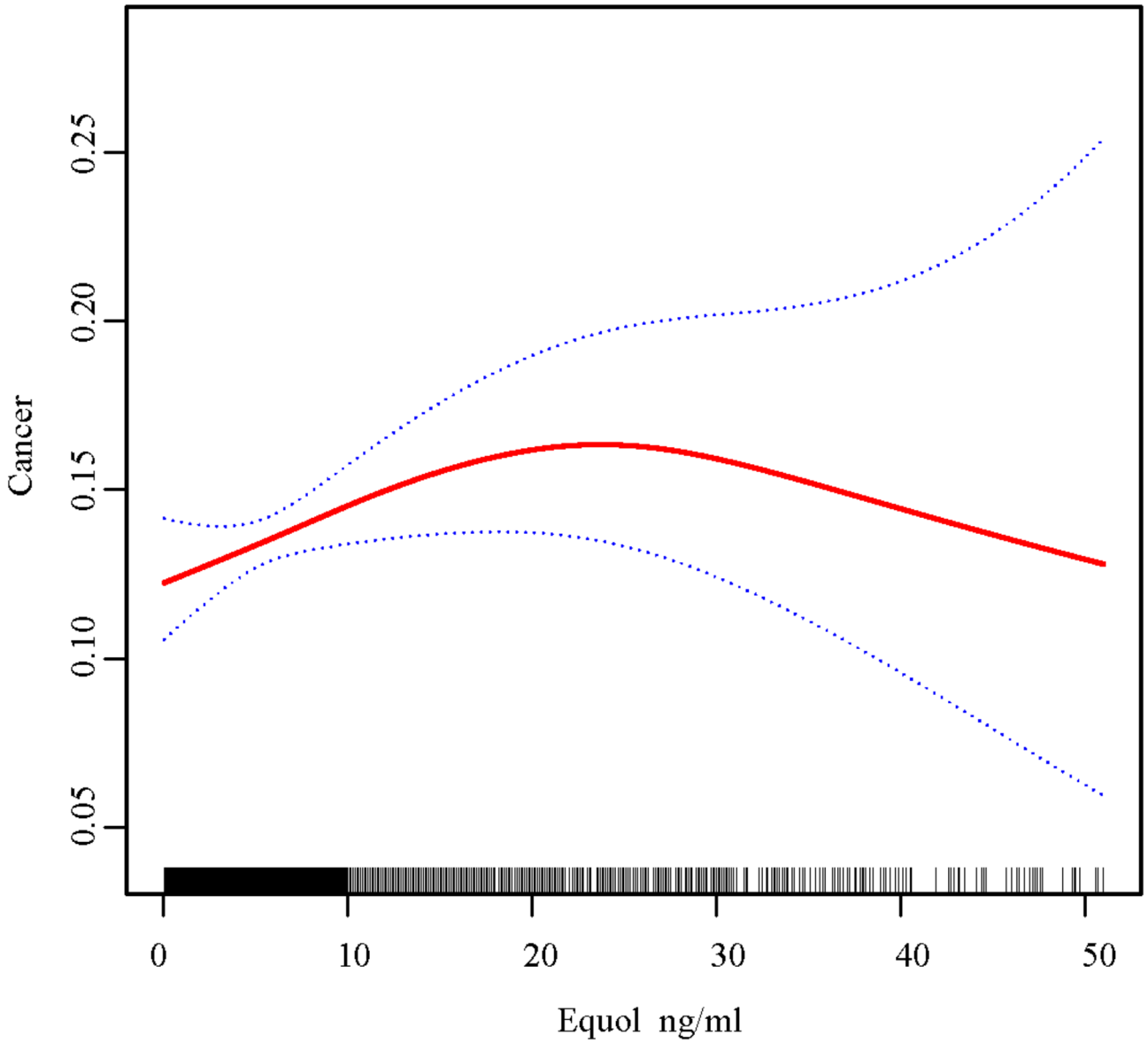

Fig. 2. Association between urine equol concentration and cancer. Adjusted for: Age; Race; Gender; BMI; Education level; Family PIR; Hypertension; Diabetes; Heart attack; Stroke; Smoking; Drinking. The solid and dotted lines represent the estimated values and their corresponding 95% CIs, respectively.

	Adjusted POR (95% CI), *P* Value
Inflection point	25.5
equol ≤ 25.5 ng/ml	1.02 (1.00, 1.04) 0.0125
equol > 25.5 ng/ml	0.96 (0.91, 1.01) 0.0901
P for log-likelihood ratio	0.025

Table 4. Threshold effect analysis of urine equol on cancer using a linear regression **model.** Adjusted for: Age; Race; Gender; BMI; Education level; Family PIR; Hypertension; Diabetes; Heart attack; Stroke; Smoking; Drinking. 95% CI, 95% Confidence Interval; POR, Prevalence Odds Ratio.

risk is observed (POR = 0.96, 95% CI = 0.91–1.01). However, this negative correlation has not reached statistical significance, and thus, further validation with more data or additional studies is needed.

Some studies have indicated that elevated levels of equol in urine are associated with an increased risk of breast cancer, endometrial cancer, and ovarian cancer[38,39]. A prospective cohort study conducted in Europe also found that higher serum equol levels were linked to a greater risk of breast cancer[37]. Relatedly, Liu et al.

observed a positive correlation between urinary equol concentrations and the breast cancer biomarker CA15-3[40]. However, this positive correlation has been reported only in breast, endometrial, and ovarian cancers, and its relevance to other types of cancer requires further investigation.

Equol is a phytoestrogen produced by the metabolism of soy and other plant-based foods by specific gut microbiota[36]. Epidemiological studies have shown that the intake of soy products rich in phytoestrogens is associated with a reduced risk of prostate cancer[16,43]. Moreover, multiple studies have found a significant inverse correlation between plasma equol concentrations and the incidence of prostate cancer[44,45]. Most in vitro studies have indicated that equol exerts inhibitory effects on the progression of various cancers, including breast, prostate, liver, and colon cancers[46–49]. Experimental results further confirm that the estrogenic activity of equol plays a key role in its anticancer properties[46,47]. Specifically, equol has been shown to bind to estrogen receptors ERα and ERβ, thereby inhibiting the proliferation of breast cancer cells[46]. Eun et al. reported that equol effectively inhibits tumor cell proliferation by triggering apoptosis through the activation of caspase cascades in breast cancer cells[30]. Similarly, Johanna et al. discovered that equol enhances the transcriptional activity of estrogen-related receptors, subsequently inhibiting the growth of PC-3 prostate cancer cells[47]. At higher concentrations, equol has been observed to inhibit the growth of prostate cancer cells by inducing DNA damage[50]. Furthermore, equol has been found to suppress the invasiveness of DU145 cells by downregulating the expression of matrix metalloproteinase-2 and matrix metalloproteinase-9[51]. Liang et al. noted that equol induces cell cycle arrest in human hepatocellular carcinoma cells and promotes apoptosis through the activation of caspase-12 and caspase-8, as well as the upregulation of endoplasmic reticulum stress-related molecules such as Chop and Bip, thereby exerting a tumor-suppressive effect[48]. Yang et al. also found that equol may impede the proliferation of human gastric cancer MGC-803 cells by inducing G1/G0 arrest and apoptosis through the modulation of the AKT pathway[52] .

Equol and cancer risk have opposite trends in in vivo and in vitro studies, and the following are possible explanations for this difference[37,39,46–48,52]. Firstly, by smoothed curve fitting, the association may be concentration-dependent, with low concentrations of equol potentially promoting cancer development while higher concentrations may exert inhibitory effects. The study by Hatono et al. demonstrated this phenomenon, finding that low concentrations of equol promoted tumor cell growth in breast cancer cell lines, while high concentrations of equol exerted anti-tumor effects[53]. This is reminiscent of the dose-dependent function of estrogens, where low doses can stimulate breast cancer growth, but high doses may have therapeutic benefits[54]. Similarly, low doses of estradiol have been shown to increase prostate tumor growth, while higher doses have a significant inhibitory effect[55]. As a phytoestrogen, equol's impact on cancer may also follow a dose-dependent pattern. Secondly, the physiological mean concentration of equol was determined to be 0.15 ng/ml[56]. The concentrations of equol used in in vitro experiments exceeded physiological concentrations by a factor of 1000[30,51,52], which could lead to nonspecific inhibitory effects. This difference in equol concentration between the in vivo and in vitro environments is a key factor to be considered when interpreting the results of experimental studies and highlights the possibility of different biological responses at different concentrations. Thirdly, there are inherent differences between in vivo and in vitro conditions, as cellular environments differ markedly. In the complex in vivo setting, tumors are embedded within a tumor microenvironment (TME) that includes a variety of non-cancerous cells. These cells can both support and inhibit tumor growth. For instance, tumor-associated macrophages (TAMs) are a critical component of the TME and can influence tumor progression and drug resistance by establishing an immunosuppressive milieu[57].

Our analysis revealed a divergence between the outcomes of our weighted logistic regression, which assessed urine equol concentration in quartiles, and the smoothed curve fitting model, which examined the continuous relationship of urine equol concentration to cancer risk. Specifically, the logistic regression pointed to a direct correlation between higher equol levels and increased cancer risk, while the curve fitting model suggested a more complex, inverted U-shaped relationship. This apparent contradiction might stem from the skewed distribution of equol concentrations in urine, as depicted in supplementary Fig. 1. Given that over 88% of our study's participants (2475 individuals) had urinary equol levels at or below 25.5 ng/ml, the findings from both models are, in fact, complementary rather than contradictory. They collectively highlight the nuanced relationship between urinary equol and cancer risk, underscoring the importance of considering the full spectrum of equol concentrations in epidemiological studies. Our study has revealed a link between the concentration of equol in urine and the likelihood of cancer; however, the precise molecular mechanisms driving this association are not yet fully understood. It is essential to delve deeper into the biological processes that may explain this connection, as uncovering these mechanisms could have significant implications for cancer prevention and early detection strategies.

In this study, we employed multiple statistical models to thoroughly investigate the potential correlation between urinary equol levels and cancer risk. However, our research is not without its limitations. The NHANES survey, which our study is based on, has a cross-sectional design, which means that equol exposure was assessed years after the cancer diagnosis was established. This timing could introduce measurement bias, as changes in equol levels following diagnosis might not reflect the exposure levels prior to or at the time of cancer onset. Moreover, the cross-sectional nature of the NHANES survey limits our ability to draw conclusions about the causality between urinary equol and cancer. The study's focus on an American population also narrows the generalizability of our findings, given the known East-West differences in equol production rates, with approximately 30% of Western populations and 60% of Asian populations being equol producers[58]. Future research should aim to include a more diverse range of populations to better understand the global implications of equol in cancer risk. Additionally, due to the constraints imposed by the sample size of the NHANES database, our analysis was limited to overall cancer prevalence. It is plausible that the relationship between urinary equol and specific cancer types could vary and may require separate analyses in future studies. These potential

variations warrant further investigation to provide a more nuanced understanding of equol's role in different types of cancer.

Conclusions

In conclusion, our study points to a correlation that's worth noting: for folks over 40, higher urinary equol levels, those under 25.5 ng/ml, are linked to a bump in cancer risk. But here's the twist: once equol levels in the urine hit above 25.5 ng/ml, they actually seem to lower the risk of cancer. The potential of urinary equol as a simple, non-invasive biomarker holds promise for its utility in cancer diagnostics and therapeutics. Nonetheless, further extensive and long-term studies are essential to confirm these preliminary findings and to explore their full implications.

Data availability

All data are available at NHANES website https://www.cdc.gov/ nchs/nhanes/index.htm (accessed on 20 July 2024).

Received: 29 October 2024; Accepted: 24 February 2025
Published online: 28 February 2025

References

1. Bray, F. et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J. Clin.* **74**, 229–263. https://doi.org/10.3322/caac.21834 (2024).
2. Jemal, A., Ma, J., Rosenberg, P. S., Siegel, R. & Anderson, W. F. Increasing lung cancer death rates among young women in Southern and Midwestern States. *J. Clin. Oncol.* **30**, 2739–2744. https://doi.org/10.1200/JCO.2012.42.6098 (2012).
3. Islami, F. et al. Proportion and number of cancer cases and deaths attributable to potentially modifiable risk factors in the united States. *CA Cancer J. Clin.* **68**, 31–54. https://doi.org/10.3322/caac.21440 (2018).
4. Cumberbatch, M. G. K. et al. Epidemiology of bladder cancer: A systematic review and contemporary update of risk factors in 2018. *Eur. Urol.* **74**, 784–795. https://doi.org/10.1016/j.eururo.2018.09.001 (2018).
5. Kuziel, G. A. & Rakoff-Nahoum, S. The gut Microbiome. *Curr. Biol.* **32**, R257–R264. https://doi.org/10.1016/j.cub.2022.02.023 (2022).
6. Kamada, N., Seo, S. U., Chen, G. Y. & Nunez, G. Role of the gut microbiota in immunity and inflammatory disease. *Nat. Rev. Immunol.* **13**, 321–335. https://doi.org/10.1038/nri3430 (2013).
7. Park, E. M. et al. Targeting the gut and tumor microbiota in cancer. *Nat. Med.* **28**, 690–703. https://doi.org/10.1038/s41591-022-01779-2 (2022).
8. Wang, Q., Yang, Q. & Liu, X. The microbiota-gut-brain axis and neurodevelopmental disorders. *Protein Cell.* **14**, 762–775. https://doi.org/10.1093/procel/pwad026 (2023).
9. Li, Y. et al. The roles and applications of short-chain fatty acids derived from microbial fermentation of dietary fibers in human cancer. *Front. Nutr.* **10**, 1243390. https://doi.org/10.3389/fnut.2023.1243390 (2023).
10. Zhou, Y. et al. The gut microbiota derived metabolite trimethylamine N-oxide: its important role in cancer and other diseases. *Biomed. Pharmacother.* **177**, 117031. https://doi.org/10.1016/j.biopha.2024.117031 (2024).
11. Belobrajdic, D. P., James-Martin, G., Jones, D. & Tran, C. D. *Soy Gastrointest. Health: Rev. Nutrients* **15**, doi:https://doi.org/10.3390/nu15081959 (2023).
12. Kim, I. S., Kim, C. H. & Yang, W. S. Physiologically active molecules and functional properties of soybeans in human Health-A current perspective. *Int. J. Mol. Sci.* **22** https://doi.org/10.3390/ijms22084054 (2021).
13. Nakai, S., Fujita, M. & Kamei, Y. Health promotion effects of soy isoflavones. *J. Nutr. Sci. Vitaminol (Tokyo).* **66**, 502–507. https://doi.org/10.3177/jnsv.66.502 (2020).
14. Zhang, X. et al. Potential protective effects of equol (Soy isoflavone Metabolite) on coronary heart Diseases-From molecular mechanisms to studies in humans. *Nutrients* **13** https://doi.org/10.3390/nu13113739 (2021).
15. Yamamoto, S. et al. Soy, isoflavones, and breast cancer risk in Japan. *J. Natl. Cancer Inst.* **95**, 906–913. https://doi.org/10.1093/jnci/95.12.906 (2003).
16. Cicero, A. F. G. et al. Nutraceutical treatment and prevention of benign prostatic hyperplasia and prostate cancer. *Arch. Ital. Urol. Androl.* **91** https://doi.org/10.4081/aiua.2019.3.139 (2019).
17. Akhlaghi, M., Ghasemi Nasab, M., Riasatian, M. & Sadeghi, F. Soy isoflavones prevent bone resorption and loss, a systematic review and meta-analysis of randomized controlled trials. *Crit. Rev. Food Sci. Nutr.* **60**, 2327–2341. https://doi.org/10.1080/10408398.2019.1635078 (2020).
18. Canivenc-Lavier, M. C. & Bennetau-Pelissero, C. *Phytoestrogens Health Eff. Nutrients* **15**, doi:https://doi.org/10.3390/nu15020317 (2023).
19. Krizova, L., Dadakova, K., Kasparovska, J. & Kasparovsky, T. *Isoflavones Molecules* **24**, doi:https://doi.org/10.3390/molecules24061076 (2019).
20. Setchell, K. D. & Clerici, C. Equol: history, chemistry, and formation. *J. Nutr.* **140**, 1355S–1362S. https://doi.org/10.3945/jn.109.119776 (2010).
21. Schwen, R. J., Nguyen, L. & Jackson, R. L. Elucidation of the metabolic pathway of S-equol in rat, monkey and man. *Food Chem. Toxicol.* **50**, 2074–2083. https://doi.org/10.1016/j.fct.2012.03.048 (2012).
22. Marrian, G. F. & Haslewood, G. A. Equol, a new inactive phenol isolated from the Ketohydroxyoestrin fraction of mares' urine. *Biochem. J.* **26**, 1227–1232. https://doi.org/10.1042/bj0261227 (1932).
23. Setchell, K. D. et al. S-equol, a potent ligand for Estrogen receptor beta, is the exclusive enantiomeric form of the soy isoflavone metabolite produced by human intestinal bacterial flora. *Am. J. Clin. Nutr.* **81**, 1072–1079. https://doi.org/10.1093/ajcn/81.5.1072 (2005).
24. Setchell, K. D. et al. Comparing the pharmacokinetics of Daidzein and genistein with the use of 13 C-labeled tracers in premenopausal women. *Am. J. Clin. Nutr.* **77**, 411–419. https://doi.org/10.1093/ajcn/77.2.411 (2003).
25. Jackson, R. L., Greiwe, J. S. & Schwen, R. J. Emerging evidence of the health benefits of S-equol, an Estrogen receptor beta agonist. *Nutr. Rev.* **69**, 432–448. https://doi.org/10.1111/j.1753-4887.2011.00400.x (2011).
26. Fatima, A., Khan, M. S. & Ahmad, M. W. Therapeutic potential of equol: A comprehensive review. *Curr. Pharm. Des.* **26**, 5837–5843. https://doi.org/10.2174/1381612826999201117122915 (2020).
27. Mayo, B., Vazquez, L., Florez, A. B. & Equol A Bacterial Metabolite from The Daidzein Isoflavone and Its Presumed Beneficial Health Effects. *Nutrients* 11, (2019). https://doi.org/10.3390/nu11092231
28. Chen, L. R. & Chen, K. H. Utilization of isoflavones in soybeans for women with menopausal syndrome: an overview. *Int. J. Mol. Sci.* **22** https://doi.org/10.3390/ijms22063212 (2021).

29. Ni, X. et al. Equol exerts a protective effect on postmenopausal osteoporosis by upregulating OPG/RANKL pathway. *Phytomedicine* **108**, 154509. https://doi.org/10.1016/j.phymed.2022.154509 (2023).
30. Choi, E. J., Ahn, W. S. & Bae, S. M. Equol induces apoptosis through cytochrome c-mediated caspases cascade in human breast cancer MDA-MB-453 cells. *Chem. Biol. Interact.* **177**, 7–11. https://doi.org/10.1016/j.cbi.2008.09.031 (2009).
31. Lu, Z. et al. S-equol, a secondary metabolite of natural anticancer isoflavone Daidzein, inhibits prostate Cancer growth in vitro and in vivo, though activating the Akt/FOXO3a pathway. *Curr. Cancer Drug Targets*. **16**, 455–465. https://doi.org/10.2174/1568009616666151207105720 (2016).
32. Choi, E. J. & Kim, G. H. The antioxidant activity of Daidzein metabolites, O–desmethylangolensin and Equol, in HepG2 cells. *Mol. Med. Rep.* **9**, 328–332. https://doi.org/10.3892/mmr.2013.1752 (2014).
33. Choi, E. J. Evaluation of equol function on anti- or prooxidant status in vivo. *J. Food Sci.* **74**, H65–71. https://doi.org/10.1111/j.1750-3841.2008.01039.x (2009).
34. Watanabe, A., Muraki, K., Tamaoki, J. & Kobayashi, M. Soy-Derived equol induces antioxidant activity in zebrafish in an Nrf2-Independent manner. *Int. J. Mol. Sci.* **23** https://doi.org/10.3390/ijms23095243 (2022).
35. Harris, I. S. & DeNicola, G. M. The complex interplay between antioxidants and ROS in Cancer. *Trends Cell. Biol.* **30**, 440–451. https://doi.org/10.1016/j.tcb.2020.03.002 (2020).
36. Lv, J. et al. Equol: a metabolite of gut microbiota with potential antitumor effects. *Gut Pathog.* **16**, 35. https://doi.org/10.1186/s13099-024-00625-9 (2024).
37. Grace, P. B. et al. Phytoestrogen concentrations in serum and spot urine as biomarkers for dietary phytoestrogen intake and their relation to breast cancer risk in European prospective investigation of cancer and nutrition-norfolk. *Cancer Epidemiol. Biomarkers Prev.* **13**, 698–708 (2004).
38. Ward, H. et al. Breast cancer risk in relation to urinary and serum biomarkers of phytoestrogen exposure in the European prospective into Cancer-Norfolk cohort study. *Breast Cancer Res.* **10**, R32. https://doi.org/10.1186/bcr1995 (2008).
39. Lee, A. W., Poynor, V. & McEligot, A. J. Urinary phytoestrogen levels are associated with female hormonal cancers: an analysis of NHANES data from 1999 to 2010. *Nutr. Cancer*. **74**, 2748–2756. https://doi.org/10.1080/01635581.2021.2020304 (2022).
40. Liu, F. et al. Association of urinary phytoestrogens with hormone-related cancers and cancer biomarkers: NHANES 1999–2010. *Br. J. Nutr.* **130**, 750–764. https://doi.org/10.1017/S0007114522003877 (2023).
41. Ribelles, N. et al. Increasing annual Cancer incidence in patients age 20–49 years: A Real-Data study. *JCO Glob Oncol.* **10**, e2300363. https://doi.org/10.1200/GO.23.00363 (2024).
42. Han, B. et al. Cancer incidence and mortality in China, 2022. *J. Natl. Cancer Cent.* **4**, 47–53. https://doi.org/10.1016/j.jncc.2024.01.006 (2024).
43. Applegate, C. C., Rowles, J. L., Ranard, K. M., Jeon, S. & Erdman, J. W. Soy Consumption and the Risk of Prostate Cancer: An Updated Systematic Review and Meta-Analysis. *Nutrients* 10, (2018). https://doi.org/10.3390/nu10010040
44. Van der Eecken, H., Joniau, S., Berghen, C., Rans, K. & De Meerleer, G. The use of soy isoflavones in the treatment of prostate cancer: A focus on the cellular effects. *Nutrients* **15** https://doi.org/10.3390/nu15234856 (2023).
45. Zhang, Q. et al. Phytoestrogens and risk of prostate cancer: an updated meta-analysis of epidemiologic studies. *Int. J. Food Sci. Nutr.* **68**, 28–42. https://doi.org/10.1080/09637486.2016.1216525 (2017).
46. Hod, R., Maniam, S. & Mohd Nor, N. H. A systematic review of the effects of equol (Soy Metabolite) on breast Cancer. *Molecules* **26** https://doi.org/10.3390/molecules26041105 (2021).
47. Hirvonen, J. et al. Transcriptional activity of estrogen-related receptor gamma (ERRgamma) is stimulated by the phytoestrogen equol. *J. Steroid Biochem. Mol. Biol.* **123**, 46–57. https://doi.org/10.1016/j.jsbmb.2010.11.001 (2011).
48. Liang, X. L., Li, M., Li, J. & Wang, X. L. Equol induces apoptosis in human hepatocellular carcinoma SMMC-7721 cells through the intrinsic pathway and the Endoplasmic reticulum stress pathway. *Anticancer Drugs*. **25**, 633–640. https://doi.org/10.1097/CAD.0000000000000085 (2014).
49. Cai, Y. F., Zhang, H. M., Niu, W. Y., Zou, Y. Q. & Ma, D. F. [Effects of equol on colon cancer cell proliferation]. *Beijing Da Xue Xue Bao Yi Xue Ban*. **49**, 383–387 (2017).
50. Mitchell, J. H., Duthie, S. J. & Collins, A. R. Effects of phytoestrogens on growth and DNA integrity in human prostate tumor cell lines: PC-3 and LNCaP. *Nutr. Cancer*. **38**, 223–228. https://doi.org/10.1207/S15327914NC382_12 (2000).
51. Zheng, W. et al. +/-)Equol inhibits invasion in prostate cancer DU145 cells possibly via down-regulation of matrix metalloproteinase-9, matrix metalloproteinase-2 and urokinase-type plasminogen activator by antioxidant activity. *J. Clin. Biochem. Nutr.* **51**, 61–67. https://doi.org/10.3164/jcbn.11-54 (2012).
52. Yang, Z. P. et al. Equol inhibits proliferation of human gastric carcinoma cells via modulating Akt pathway. *World J. Gastroenterol.* **21**, 10385–10399. https://doi.org/10.3748/wjg.v21.i36.10385 (2015).
53. Hatono, M. et al. Effect of isoflavones on breast cancer cell development and their impact on breast cancer treatments. *Breast Cancer Res. Treat.* **185**, 307–316. https://doi.org/10.1007/s10549-020-05957-z (2021).
54. Ingle, J. N. Estrogen as therapy for breast cancer. *Breast Cancer Res.* **4**, 133–136. https://doi.org/10.1186/bcr436 (2002).
55. Nakajima, Y. et al. Estrogen exhibits a biphasic effect on prostate tumor growth through the Estrogen receptor beta-KLF5 pathway. *Mol. Cell. Biol.* **36**, 144–156. https://doi.org/10.1128/MCB.00625-15 (2016).
56. Peeters, P. H. et al. Variations in plasma phytoestrogen concentrations in European adults. *J. Nutr.* **137**, 1294–1300. https://doi.org/10.1093/jn/137.5.1294 (2007).
57. Li, M. et al. Metabolism, metabolites, and macrophages in cancer. *J. Hematol. Oncol.* **16** https://doi.org/10.1186/s13045-023-01478-6 (2023).
58. Rafii, F. The role of colonic bacteria in the metabolism of the natural isoflavone Daidzin to equol. *Metabolites* **5**, 56–73. https://doi.org/10.3390/metabo5010056 (2015).

Acknowledgements

We thank the participants of the NHANES databases.

Author contributions

J. Li. and J. Lv. conceived the study design and are responsible for the overall content. J. Li. and Y. Zhang. analyzed and interpreted the data. J. Li., Z. Z, and H.G prepared the manuscript. J. Lv., Y. Zhang. and Y. Zhou. edited the manuscript. N. F., Y. Zhang. and C.Y. provided financial and technical support. All authors approved the submitted and final versions.

Funding

This study was supported by the National Natural Science Foundation of China (Item No:82370777), Postgraduate Research & Practice Innovation Program of Jiangsu Province (Item No: KYCX24_3596).

Declarations

Ethics approval and consent to participate
The study was conducted in accordance with the guidelines set out in the Declaration of Helsinki. The protocols of NHANES were approved by the institutional review board of the National Center for Health Statistics, CDC (https://www.cdc.gov/nchs/nhanes/irba98.htm). NHANES has obtained written informed consent from all participants.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Authors' information
1 Wuxi School of Medicine, Jiangnan University, Wuxi, China.
2 Medical School of Nantong University, Nantong, China.
3 Department of Urology, Jiangnan University Medical Center, Wuxi, China.

Additional information
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-91846-8.

Correspondence and requests for materials should be addressed to C.Y. or N.F.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.