Urinary Phytoestrogen Metabolites Positively Correlate with Serum 25(OH)D Level Based on National Health and Nutrition Examination Survey 2009–2010

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Summary Studies showed that vitamin D (25-hydroxyvitamin D) level in the human blood circulation could be affected by exogenous estrogen exposure. This study aims to explore the relationships between urinary phytoestrogens metabolites and serum total 25(OH)D in general population, urinary phytoestrogens metabolites (daidzein, enterodiol, enterolactone, equol, genistein and o-desmethylangolensin). Totally 2,609 adults ≥6 y old from the 2009–2010 National Health and Nutrition Examination Surveys (NHANES) were recruited into the cross-sectional analyses and information including demographic, socioeconomic, examinations and laboratory test were collected. All analyses were performed using Stata13.0, one-way analysis of variance and multivariable regression were utilised according to data characteristics, respectively. It showed that age, race, education level, body mass index (BMI), and sampling season had significant effects on serum 25(OH)D level (all p<0.001). In the whole population, urinary enterodiol and equol were significantly positively associated with serum total 25(OH)D level (β=0.86, 95%CI=0.08–1.65, p<0.05; β=1.68, 95%CI=0.91–2.45, p<0.001). Equol was also found significantly positively correlated with total 25(OH)D in both female and male separately (β=1.69, 95%CI=0.51–2.87, p<0.05; β=1.66, 95%CI=0.63–2.69, p<0.05). Phytoestrogen concentrations in the urinary and 25(OH)D levels in the serum had proved a positive correlation in our study, which provide theoretical basis and reference for the dietary nutrient intake in the population.

Key Words NHANES, urinary phytoestrogen, 25-hydroxyvitamin D, enterodiol, equol

Vitamin D in the human body comes from endogenous production and dietary uptake. It is generated mainly through the photolytic reaction of 7-dehydrocholesterol. After the hydroxylation by 25-hydroxylase in hepatomicrosome and then 1α-hydroxylation in renal cell mitochondria, vitamin D can be transformed into 25(OH)D, which is bioactive and can bind specifically to intracellular vitamin D receptors (1–4). Thus serum 25 concentration had been shown to be the best indicator of human vitamin D level (5). The most important physiological function of vitamin D is to regulate calcium and phosphorus metabolism in the skeletal system. Currently researchers found that vitamin D might be a protective factor against viruses, such as COVID-19 (6, 7).

The deficiency of vitamin D in the human body is a ubiquitous public health problem around the world and leads to multiple systemic dysfunctions (8–10). Serum vitamin D concentration is affected by not only individual varieties, but also exposure of environmental factors especially chemicals with estrogenic activities (11, 12). Johns et al. found that adults exposed to phthalates and bisphenol A had higher serum levels of 25(OH)D (12).

Phytoestrogen, which is one big group of EDCs, contains natural non-steroidal compounds with estrogenic activities and has been used in hormone replacement therapy (13). Phytoestrogens were found in a wide variety of drugs and our daily diet (14, 15), and their analogues could increase the expression of 1α-hydroxylase and further promote 1,25-dihydroxyvitamin D synthesis (16). Previous studies suggested that serum 25(OH)D concentration was elevated after phytoestrogen treatment in women (5, 17–20). We would like to further explore the relationship between the phytoestrogen levels and blood circulating vitamin D in human body. Accordingly, the aim of this study is to analyze the associations between the levels of urinary phytoestrogen metabolites and the serum 25(OH)D level in the general population by dealing with the data from the National Health and Nutrition Examination Surveys (NHANES) 2009–2010.

MATERIALS AND METHODS

The information and data were obtained from NHANES, 2009–2010 (https://wwwn.cdc.gov). NHANES
is a program of studies designed to evaluate the health and nutritional status of adults and children in the United States. This cross-sectional investigation containing demographic, interview, examinations, questionnaire, and laboratory data, which are available through an extensive series of scientific publications and articles. The data used for this study were publicly available. Prior to public release, the study protocol (continuation of Protocol No. 2005–06) was approved by the NCHS Research Ethics Review Board. In our study, a total of 10,537 participants were recruited from NHANES 2009–2010, but totally 2,609 people (1,299 males and 1,310 females, aged at 6 y or older), whose data of urinary phytoestrogens, urinary creatinine, and serum 25(OH)D were complete, were included in our primary analyses and totally 2,587 participants (22 participants without data on body mass index (BMI)) were included in final regression analyses by covariates (Fig. 1).

The urinary phytoestrogen metabolites (daidzein, enterodiol, enterolactone, equol, genistein and o-desmethylangolensin) and serum vitamin D (25(OH)D$_2$ and 25(OH)D$_3$) of the participants from NHANES 2001–2010 were detected by the Division of Laboratory Sciences, the National Centre for Environmental Health in Centre for Disease Control and Prevention using high performance liquid chromatography-atmospheric pressure photoionization-tandem mass spectrometry (HPLC-APPI-MS/MS). The limits of detection (LODs) of daidzein, enterodiol, enterolactone, equol, genistein and o-desmethylangolensin were 0.40, 0.04, 0.10, 0.06, 0.20 and 0.20 ng/mL respectively (21, 22).

Information on race/ethnicity, 6-mo sampling period, education level, family income to poverty ratio (PIR), BMI, age, and 30-d vitamin D supplementary was collected from demographic questionnaire and each was considered as potential confounding variables. The whole people had been divided into five race groups (Non-Hispanic White, Non-Hispanic Black, Mexican American, Other Hispanic, and Other Race), two types of 6-mo sampling period (summer months: May 1st–October 31st; winter months: November 1st–April 30th), three education level group (Less than high school, High school/GED, and More than high school), and two PIR group (PIR<1 or PIR≥1). Also, there are four groups of BMI (Underweight: <18.5, Normal weight: 18.5–25, Overweight: 25–30, and Obesity: ≥30) (23), and five groups of age (6 to <12, 12 to <20, 20 to <40, 40 to <60, and 60 to <80). Moreover, 30-d vitamin D supplementary were obtained from dietary analysis, and two categories on 30-d vitamin D supplement use were defined as: No: without vitamin D supplement; Yes: with vitamin D supplement.

STATA Version 13 (Stata Corp. College Station, Texas, USA) was used to do the analysis. Urinary phytoestrogen concentration was standardised with creatinine and then normal distribution was checked. Data had been natural logarithm (ln)-transformed when the right-skewed distribution was found.

One-way ANOVA was used to analyze the difference of serum vitamin D levels in subgroups of race, education, age and BMI, and then Bonferroni was applied to make multiple comparisons among groups. Multivariable regression models by gender were used to exclude its effect in overall population. In the final regression model, urinary phytoestrogen was adjusted by Race (categorical), Sampling season (categorical), Education level (categorical), PIR (continuous), BMI (continuous), Age (continuous), and 30-d Vitamin D supplement (categorical). To increase the accuracy of analysis, analysis weights were applied throughout the analysis process. The associations between each urinary phytoestrogens and serum vitamin D were examined through linear regression analysis.

Because isoflavones and lignans are the most common phytoestrogens in human diet (24, 25), we also examined the associations of total serum 25(OH)D with the sum of isoflavones (equol, daidzein, genistein, and o-desmethylangolensin), the sum of lignans (enterolactone, and enterodiol), and the sum of all phytoestrogens, respectively. Both appropriate weighted and unweighted analyses were utilised. It was considered statistically significant when \( p<0.05 \).

**RESULTS**

Demographic population characteristics of 2,609 participants were recruited from NHANES 2009–2010.
people recruited in NHANES 2009–2010 were listed in Table 1. In weighted analysis, it was shown that 30.64% participants were 40–60 y old and 64.99% were Non-Hispanic White. Nearly half participants’ educational level was more than high school. Overweighed participants occupied 28.35% and 30.82% reached obesity. Participants with PIR≥1 occupied 77.26% and sampling season of 58.58% was summer months. In unweighted analysis, 24.91% participants were 20–40 y old and 42.78% participant were Non-Hispanic. The participants whose education level was less than high school account for 45.69%. Moreover, 27.83% participants were over weighted, and 30.19% reached obesity.

### Table 1. Demographic and laboratory characteristics in NHANES 2009–2010 population.

| Population characteristics | Unweighted (%) | Weighted (%) | Total 25(OH)D levels, weighted mean (SD) |
|----------------------------|----------------|--------------|------------------------------------------|
| Age***                     |                |              |                                          |
| 6 to <12                   | 300            | 11.50        | 7.02                                     | 70.83 (20.78)1 |
| 12 to <20                  | 409            | 15.68        | 11.71                                    | 64.99 (23.30)2 |
| 20 to <40                  | 650            | 24.91        | 30.50                                    | 62.72 (25.33)3 |
| 40 to <60                  | 617            | 23.65        | 30.64                                    | 69.38 (26.50)4 |
| 60 to <80                  | 633            | 24.26        | 20.13                                    | 73.69 (28.65)  |
| Race***                    |                |              |                                          |
| Mexican American           | 570            | 21.85        | 10.16                                    | 54.39 (16.82)5 |
| Other Hispanic             | 268            | 10.27        | 5.09                                     | 59.39 (19.63)6 |
| Non-Hispanic White*        | 1,116          | 42.78        | 64.99                                    | 75.63 (25.36)7 |
| Non-Hispanic Black         | 491            | 18.82        | 11.82                                    | 48.47 (21.78)8 |
| Other Race                 | 164            | 6.29         | 7.94                                     | 55.10 (24.00)  |
| Education level***         |                |              |                                          |
| Less than highschool       | 1,192          | 45.69        | 32.10                                    | 64.54 (23.55)  |
| High school/GED            | 475            | 18.21        | 20.80                                    | 66.78 (27.30)  |
| More than highschool       | 942            | 36.11        | 47.10                                    | 70.49 (27.14)9 |
| BMI, kg/m²***              |                |              |                                          |
| Underweight (<18.5)        | 258            | 9.97         | 7.49                                     | 71.23 (22.38)  |
| Normal weight (18.5–25)    | 828            | 32.01        | 33.34                                    | 71.03 (27.39)  |
| Overweight (25–30)         | 720            | 27.83        | 28.35                                    | 69.20 (25.33)  |
| Obesity (≥30)              | 781            | 30.19        | 30.82                                    | 62.44 (25.80)10 |
| 30-d vitamin D supplement***|              |              |                                          |
| Yes                        | 779            | 29.86        | 36.17                                    | 79.60 (26.74)  |
| No                         | 1,830          | 70.14        | 63.83                                    | 61.12 (23.39)  |
| PIR category***            |                |              |                                          |
| <1                         | 844            | 32.35        | 22.74                                    | 61.17 (24.05)  |
| ≥1                         | 1,765          | 67.65        | 77.26                                    | 69.76 (26.49)  |
| Sampling season***         |                |              |                                          |
| Winter months              | 1,239          | 47.49        | 41.42                                    | 60.76 (24.06)  |
| Summer months              | 1,370          | 52.51        | 58.58                                    | 72.79 (26.51)  |

*p<0.05.

1 The group of age 6 to 12 had significant higher 25(OH)D level comparing to 12 to 20 group and 20 to 40 group (p<0.05, p<0.001, respectively).

2 The group of age 12 to 20 had significantly lower 25(OH)D level comparing to 60 to 80 group (p<0.001).

3 The group of age 20 to 40 had significantly lower 25(OH)D level comparing to 60 to 80 group and 60 to 80 group (p<0.001, p<0.001, respectively).

4 The group of 40 to 60 had significantly lower 25(OH)D level comparing to 60 to 80 group (p<0.05).

5 Mexican American had significantly lower 25(OH)D level comparing to Other Hispanic, Non-Hispanic White (both p<0.05) and they had significantly higher 25(OH)D level compared to Non-Hispanic Black (p<0.001).

6 Other Hispanic had significantly lower 25(OH)D level comparing to Non-Hispanic White (p<0.001), and significantly higher 25(OH)D level comparing to Non-Hispanic Black (p<0.05).

7 Non-Hispanic White had significantly higher 25(OH)D level comparing to Non-Hispanic Black and Other Race (both p<0.001).

8 Non-Hispanic Black had significant lower 25(OH)D level comparing to Other Race (p<0.05).

9 “More than highschool” group had significantly higher 25(OH)D level compared to “Less than highschool” group (p<0.001).

10 Obesity group had significantly lower 25(OH)D level compared to underweight group, normal weight group, and overweight group (all p<0.001).
compared to the groups aged 12 to 20 and 20 to 40, the group aged 6 to 12 had significantly higher 25(OH)D level compared to Non-Hispanic Black and 0.05). Non-Hispanic White had significantly higher 25(OH)D level compared to Non-Hispanic Black (p<0.01), and significantly higher 25(OH)D level compared to Other Hispanic (p<0.001). Other Hispanic had significantly higher 25(OH)D level compared to Non-Hispanic Black (p<0.001). In the crude model, lignans, equol and sum of phytoestrogens were significantly positively associated with total 25(OH)D in gender-stratified model and overall population (all p<0.05). These results were also observed in weighted and unweighted analyses (Table 3). In the unweighted crude analysis, isoflavones were significantly positively associated with total 25(OH)D in both females (β=0.96, 95%CI=0.05–1.86, p<0.05) and overall population (β=0.72, 95%CI=0.13–1.31, p<0.05).

In unweighted analysis, after having been adjusted with age, race, education, BMI, 30-d vitamin D supplement use, PIR, sampling season in male and female models, the association between equol and 25(OH)D in the whole population, in males (β=2.02, 95%CI=1.40–2.63, p<0.001) and females (β=1.84, 95%CI=1.03–2.65, p<0.001). In unweighted analysis, after having been adjusted with gender variable, the results showed that equol and 25(OH)D had significant association in the whole population (β=2.02, 95%CI=1.40–2.63, p<0.001) (Table 4). In both the whole population and female population, the associations were significant between 25(OH)D and enterodiol (β=0.80, 95%CI=0.25–1.36, p<0.05; β=1.27, 95%CI=0.47–2.07, p<0.05, respectively). In weighted analysis, significantly positive associations between enterodiol and 25(OH)D in both the whole population and in female population had been detected (β=0.86, 95%CI=0.08–1.65, p<0.05; β=1.10, 95%CI=0.01–2.20, p<0.05, respectively) (Table 4).

In this study, urinary phytoestrogen and serum 25(OH)D levels of 2,609 participants from NHANES 2009–2010 were analyzed and results showed that all the demographic factors (age, race, education, BMI, 30-d vitamin D supplement use, PIR, sampling season)
### Table 3. Urinary phytoestrogen associations with total 25(OH)D (unadjusted).

| Urinary analyte            | Overall population, $\beta$ (95%CI) Unweighted | Weighted | Females, $\beta$ (95%CI) Unweighted | Weighted | Males, $\beta$ (95%CI) Unweighted | Weighted |
|----------------------------|------------------------------------------------|----------|------------------------------------|----------|-----------------------------------|----------|
| Lignans                    | 1.89 (1.26, 2.51)**                           | 2.06 (1.44, 2.98)** | 2.41 (1.46, 3.27)**                | 2.21 (1.23, 3.19)** | 1.33 (0.51, 2.14)**                | 1.65 (0.86, 2.45)** |
| Enterodiol                 | 1.90 (1.32, 2.48)**                           | 2.16 (1.58, 2.73)** | 2.49 (1.63, 3.34)**                | 2.61 (1.73, 3.49)** | 1.19 (0.40, 1.98)**                | 1.41 (0.65, 2.18)** |
| Enterolactone              | 1.34 (0.78, 1.89)**                           | 1.45 (0.89, 2.01)** | 1.59 (0.75, 2.43)**                | 1.33 (0.46, 2.20)** | 1.05 (0.32, 1.78)**                | 1.37 (0.66, 2.09)** |
| Isoflavones                | 0.72 (0.13, 1.31)*                            | 0.50 (−0.12, 1.11) | 0.96 (0.05, 1.86)*                 | 0.39 (−0.54, 1.32) | 0.47 (−0.29, 1.23)                 | 0.38 (−0.41, 1.17) |
| Daidzein                   | 0.33 (−0.22, 0.88)                            | 0.18 (−0.40, 0.75) | 0.44 (−0.42, 1.29)                 | 0.03 (−0.85, 0.91) | 0.21 (−0.50, 0.92)                 | 0.14 (−0.61, 0.88) |
| Genistein                  | 0.39 (−0.18, 0.97)                            | 0.21 (−0.38, 0.80) | 0.45 (−0.43, 1.33)                 | −0.02 (−0.91, 0.86) | 0.32 (−0.41, 1.06)                 | 0.31 (−0.45, 1.08) |
| o-Desmethylangolensin      | 0.40 (−0.01, 0.82)                            | 0.27 (−0.16, 0.69) | 0.49 (−0.14, 1.12)                 | 0.05 (−0.60, 0.69) | 0.30 (−0.25, 0.84)                 | 0.34 (−0.22, 0.89) |
| Equol                      | 3.15 (2.49, 3.80)**                           | 3.03 (2.34, 3.72)** | 3.95 (2.92, 4.98)**                | 3.65 (2.54, 4.75)** | 2.43 (1.61, 3.25)**                | 2.34 (1.50, 3.18)** |
| Sum of phytoestrogens      | 2.01 (1.26, 2.76)**                           | 2.20 (1.45, 2.95)** | 2.94 (1.79, 4.10)**                | 2.69 (1.50, 3.87)** | 1.10 (0.14, 2.05)*                 | 1.37 (0.42, 2.31)** |

* $p<0.05$, ** $p<0.01$, *** $p<0.001$.

### Table 4. Associations between urinary phytoestrogen and total 25(OH)D (adjusted).

| Urinary analyte            | Overall population, $\beta$ (95%CI) Unweighted | Weighted | Females, $\beta$ (95%CI) Unweighted | Weighted | Males, $\beta$ (95%CI) Unweighted | Weighted |
|----------------------------|------------------------------------------------|----------|------------------------------------|----------|-----------------------------------|----------|
| Lignans                    | 0.47 (−0.14, 1.08)                           | 0.46 (−0.33, 1.24) | 0.64 (−0.28, 1.55)                 | 0.21 (−0.91, 1.33) | 0.32 (−0.47, 1.11)                | 0.58 (−0.49, 1.65) |
| Enterodiol                 | 0.80 (0.25, 1.36)**                           | 0.86 (0.08, 1.65) | 1.27 (0.47, 2.07)**                | 1.10 (0.01, 2.20)** | 0.29 (−0.46, 1.05)                 | 0.48 (−0.65, 1.62) |
| Enterolactone              | 0.23 (−0.30, 0.77)                            | 0.17 (−0.50, 0.85) | 0.25 (−0.55, 1.06)                 | −0.18 (−1.16, 0.79) | 0.24 (−0.45, 0.93)                 | 0.49 (−0.43, 1.40) |
| Isoflavones                | 0.33 (−0.21, 0.87)                            | −0.00 (−0.76, 0.76) | 0.33 (−0.48, 1.14)                 | −0.26 (−1.38, 0.85) | 0.33 (−0.38, 1.03)                 | 0.22 (−0.77, 1.20) |
| Daidzein                   | 0.13 (−0.37, 0.64)                            | −0.14 (−0.86, 0.59) | 0.21 (−0.56, 0.97)                 | −0.24 (−1.30, 0.82) | 0.09 (−0.56, 0.74)                 | −0.04 (−0.98, 0.89) |
| Genistein                  | 0.09 (−0.43, 0.61)                            | −0.15 (−0.88, 0.59) | −0.02 (−0.79, 0.75)                | −0.48 (−1.53, 0.57) | 0.19 (−0.49, 0.88)                 | 0.20 (−0.78, 1.19) |
| o-Desmethylangolensin      | 0.13 (−0.26, 0.52)                            | −0.14 (−0.68, 0.39) | −0.01 (−0.59, 0.57)                | −0.49 (−1.28, 0.30) | 0.25 (−0.26, 0.77)                 | 0.17 (−0.48, 0.82) |
| Equol                      | 2.02 (1.40, 2.63)**                           | 1.68 (0.91, 2.45)** | 2.20 (1.27, 3.14)**                | 1.69 (0.51, 2.87)** | 1.84 (1.03, 2.65)**                | 1.66 (0.63, 3.26)** |
| Sum of phytoestrogens      | 0.52 (−0.17, 1.21)                            | 0.52 (−0.41, 1.45) | 0.90 (−0.17, 1.97)                 | 0.50 (−0.88, 1.88) | 0.15 (−0.73, 1.03)                 | 0.38 (−0.86, 1.63) |

All linear regression models were unweighted for complex survey design and adjusted for age, race, education, BMI, 30-d vitamin D supplement use, PIR, sampling season category. Overall population models had been additionally adjusted for gender.

* $p<0.05$, ** $p<0.01$, *** $p<0.001$. 
had significant effects on serum 25(OH)D concentration.

Compared to peoples aged 12–40, children and old people were paid more attention and had more possibilities to develop vitamin D deficiency. They might prefer to take dietary supplements, which contained vitamin D, to prevent disease (26–28). That might be the reason why this age group had more serum vitamin D than peoples aged 12–40. Different dietary cultures and vitamin D metabolism ability in different race could affected serum vitamin D level (29, 30). As a result, serum vitamin D level was species-specific. With the improvement of education level, the medical theories especially nutrition conceptions would be more likely to be accepted by the population and further affected their daily-care behaviors. People with good education tended to maintain a healthy diet (31). High-income families were more inclined to use dietary supplements and fortified foods. They might have more ways to reach vitamin D supplement (32, 33). Thus, people whose family PIR was bigger than 1 had higher serum 25(OH)D concentration than people whose family PIR was less than 1. Moreover, serum vitamin D levels could also increase after sunlight and ultraviolet light exposure (34, 35). Therefore, peoples sampled in summer months, exposed to more sunlight and ultraviolet light, had more vitamin D in serum compared to peoples in winter months.

Our study clearly showed that urinary phytoestrogens or their metabolites in human were significantly positively correlated with serum 25(OH)D concentration. The levels of urinary phytoestrogen metabolites might be markers to indicate serum 25(OH)D level, which could provide a non-invasive inspection means of vitamin D.

Although the significant relationship between phytoestrogens and serum 25(OH)D concentration had been found, whether there was a direct causal link between these two still need to be explored. Since phytoestrogen acts in the body as an exogenous estrogen, it could act as a cause. Previous studies suggested estrogenic chemicals intake could lead the increase of circulating 1,25-(OH)2D3 in females in both human and animals. As early as 1989, studies had shown that circulating 1,25-(OH)2D3 in postmenopausal women increased after exogenous estrogen uptake (36, 37). In a survey of 1,662 African American women aged 23–34, the concentration of serum 25(OH)D increased following the recent use of estrogen-containing contraceptives (20). Combined treatment of isoflavones and calcium had been found to prevent vitamin D loss and the blood 25(OH)D level was partially restored after cowpea isoflavones supplementation in rat (38). 1,25-(OH)2D3 receptor (VDR) might play a key role in the process of elevated 25(OH)D by phytoestrogen. Previous studies had shown that phytoestrogens could up-regulate VDR protein expression, thereby enhancing cellular sensitivity to 1,25-(OH)2D3. An early study demonstrated that 17β-estradiol could increase the expression of VDR in human osteoblast-like cells and regulate the biological effects of 1,25-(OH)2D3 (39). Duque and his colleagues found that the expression of VDR in skeletal muscle decreased with age and estrogen attenuation in mice, but it could be restored after enterodiol supplementation by enhancing the bioactivity of VDR (40). Although increased VDR might also elevated CYP24A1 transcription, which catabolizes 25D3 and 1,25D3, estrogen or phytoestrogen had been reported to inhibit CYP24A1 (41, 42). Thus estrogen or phytoestrogen could help increase VDR sensitivity and decrease 25(OH)D catabolism at the same time.

Our results showed among the phytoestrogen metabolites, enterodiol and equol were more related with serum 25(OH)D level in both weighted models and unweighted models, with/without covariates being taken into the regression model in the whole population. Specific metabolism might be involved. Enterodiol and equol could be metabolised from polyphenols and daidzein by intestinal flora and had more estrogenic and antioxidant activity than their precursors (43–46). Equol could affected VDR expression by activating extracellular signal-regulated kinase signaling pathway and regulating VDR gene Sp-1 site (47). On the other hand, VDR deficiency in the intestine has been reported to affect the intestinal flora and thus local equol production is disrupted (48). Since enterobacteriaceae is largely involved in the metabolism of phytoestrogen, internal phytoestrogen metabolite levels may vary due to individual intestinal flora structure difference (49). This might partly explain why urinary phytoestrogen concentrations varied greatly in our results. It could be inferred that antibiotic application could also affect phytoestrogen metabolism. However, antibiotics-taken information was not provided by NHANES. It would be helpful to include antibiotics questions in the survey in the future. Enterodiol is one type of lignans and mainly ingested from beverages, vegetables, nuts and seeds, bread, and fruits (50, 51). Equol, one type of isoflavones, is mainly ingested from legumes such as soy, beans (52, 53). Thus, taking these foods, contain enterodiol and equol, may affect the levels of serum 25(OH)D level, which provides a new way to increase the levels of serum 25(OH)D level. The intake of these foods may be closely related with urinary phytoestrogen metabolite concentrations. The detailed information on intake and status of phytoestrogen-contained foods could not be obtained from NHANES. Well designed surveys focusing on food intake and internal phytoestrogen concentrations would be launced to analyzed their association further.

Sex-stratified analysis showed that the correlations urinary phytoestrogen metabolite concentrations and serum 25(OH)D level were stronger in women than in men. Phytoestrogens mainly acted by binding to the estrogen receptor (ER) which was expressed more in females than in males (13, 54, 55). Some behaviors such as taking oral contraceptives and wearing sunscreen, which were observed more in women than in men also contributed to the sex-specific difference in the relationship between phytoestrogens and the vitamin D level (56, 57).
There is no reported mechanism clearly explaining the correlation between urinary phytoestrogens and vitamin D. Even if phytoestrogen metabolites could activate the transcription mechanism via VDR, the mechanism that promotes the expression and activity of CYP27A1, which metabolizes vitamin D to 25-OH-D, is still unknown. More details need to be explored. The correlation we found was based on the observation in the population. Scientific hypotheses could be made and tested to reveal the mechanism underlying.

Our study results indicated a significant correlation between urinary phytoestrogen concentrations and serum 25(OH)D level. Specific phytoestrogen metabolites enterodiol and equol were closely related to the level of vitamin D in human body. More human investigations and animal experiments would verify the urinary phytoestrogen metabolites as the new biomarker of vitamin D and the possibility of supplying vitamin D by increasing phytoestrogen-containing food intake.

Authorship
Methodology, investigation, data curation, writing-original draft, validation: NC; conceptualization, methodology, investigation, formal analysis, writing-review & editing: NL; methodology, investigation, data curation: JJ; methodology, investigation: XY; project administration, supervision, writing-review & editing: DW.
NC and NL contributed equally to this work.

Disclosure of state of COI
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data accessibility statement
The data were from NHANES, 2009–2010. NHANES is an ongoing cross-sectional study containing demographic, interview, examinations, questionnaire, and laboratory data, designed to assess the health and nutrition status of general US population.

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