Phytoestrogen Exposure Is Associated with Circulating Sex Hormone Levels in Postmenopausal Women and Interact with *ESR1* and *NR1I2* Gene Variants

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Abstract

In this large cross-sectional study, we investigated the relationship between phytoestrogen exposure and circulating sex hormones and sex hormone-binding globulin (SHBG) levels in 1988 healthy postmenopausal women and their interactions with polymorphisms in genes involved in estrogen signaling. Plasma estradiol, testosterone, androstenedione, estrone, and SHBG were measured. Urinary levels of five isoflavones (daidzein, genistein, glycitein, *O*-desmethylangolensin, and equol) and two lignans (enterodiol and enterolactone) were measured and used as biomarkers for dietary intakes. Eighteen polymorphisms in *ESR1*, *ESR2*, and *NR1I2* genes were genotyped. Results showed that lignans were positively associated with plasma SHBG levels (η_p^2 = 1.2%; $P < 0.001$) and negatively associated with plasma testosterone (η_p^2 = 0.2%; $P = 0.042$). Equol was negatively associated with plasma estradiol levels (η_p^2 = 0.3%; $P = 0.028$), whereas *O*-desmethylangolensin was positively associated with plasma estradiol level (η_p^2 = 0.3%; $P = 0.010$). There were significant phytoestrogen interactions with polymorphisms in *ESR1* and *NR1I2* genes in affecting estrone levels. We conclude that phytoestrogens modulate sex hormone and SHBG levels in postmenopausal women and interact with gene variants involved in estrogen signaling. Such phytoestrogen-gene interactions may explain the conflicting literature on the hormonal effects of phytoestrogens. (Cancer Epidemiol Biomarkers Prev 2007;16(5):1009–16)

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Introduction

Phytoestrogens are diphenolic compounds in plants that subsequently form part of the human diet. Dietary phytoestrogens can be classified principally into isoflavones and lignans. Isoflavones are found mainly in legumes, with exceptionally high amounts in soy. Lignans are widespread in many grains, fruit, and vegetables with flaxseed being the richest source.

Phytoestrogens are structurally similar to 17β-estradiol and have been postulated to possess hormonal properties that might protect against breast cancer (1, 2). Phytoestrogens may directly modulate concentrations of circulating estradiol and sex hormone-binding globulin (SHBG) by inhibiting enzymes involved in estrogen biosynthesis and metabolism (3) and stimulating SHBG production (4-6). In addition, phytoestrogens may exert its hormonal effects through direct signaling. Phytoestrogens have been shown to bind to both estrogen receptor α and estrogen receptor β (7), which are encoded by *ESR1* and *ESR2* genes, respectively. In 1998, a new receptor known as the pregnane X receptor (PXR; also known as the steroid and xenobiotic receptor) has been discovered. PXR is a broad-specificity, orphan nuclear receptor (8). Its ligands include a wide variety of structurally diverse, low-affinity exogenous and endogenous chemicals. PXR is encoded by the *NR1I2* (*nuclear receptor subfamily 1, group I, member 2*) gene. On activation through ligand binding, PXR binds to the response element and induces the expression of *CYP3A4*, which plays a major role in the hydroxylation of both estrone and estradiol. PXR is mainly expressed in the liver and gut, the main sites of phytoestrogen exposure and metabolism. Phytoestrogens are able to bind to PXR *in vitro* (9-11). Hence, it is plausible that phytoestrogens may modulate estrogen levels through signaling via PXR binding.

We have recently investigated diet-gene interactions between phytoestrogen exposure and sex hormone metabolism genes (*CYP19*, *HSD17B1*, and *SHBG*) and found significant interaction between isoflavones and *SHBG D356N* polymorphism in affecting circulating SHBG levels in postmenopausal women (12). Lesser is known about interactions between phytoestrogens and estrogen signaling genes. In a previous study on 125 postmenopausal women, we found that isoflavone exposure correlated positively with plasma estradiol and observed evidence of interaction with *ESR1* PvuII polymorphism (13). However, the study was small and only a few polymorphisms were investigated. The interaction with PvuII polymorphism was hard to interpret as the PvuII polymorphism lies in an intron and has no known function. It may be in linkage disequilibrium with another unknown polymorphism that may be functional. With the use of haplotype-tagging single nucleotide polymorphisms (SNP), it would now be possible to examine the whole gene to identify all SNPs that may be truly functional.

Hence, this large cross-sectional study has two aims: (*a*) to investigate the relationship between phytoestrogen exposure and circulating sex hormone levels in healthy postmenopausal women and (*b*) to investigate if such relationships are modified by polymorphisms in genes involved in estrogen signaling. Phytoestrogen exposure was assessed using urinary phytoestrogens as biomarkers. These were studied in association with

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circulating levels of estradiol, estrone, testosterone, androstenedione, and SHBG and 18 polymorphisms in *ESR1*, *ESR2*, and *NR1I2* genes.

Patients and Methods

Study Subjects. The European Prospective Investigation of Cancer and Nutrition (EPIC) is a large multicenter prospective study initiated in 1993 with the aim of investigating the relationship between diet and cancer (14). EPIC-Norfolk is part of the United Kingdom arm of this Europe-wide program (15). Men and women ages 45 to 75 years resident in Norfolk, United Kingdom were recruited in 1993 to 1997 using general practice age sex registers. Participants (30,452) completed a health questionnaire and gave written informed consent. Permission for the study was obtained from The Norfolk and Norwich Hospital Ethics Committee. Individuals (25,630) attended a medical examination and gave blood and an untimed spot urine sample. All subjects were healthy at the time of recruitment. Details on the study design and sample collection had been reported previously (15). Participants were invited to attend a follow-up health check in 1998 to 2000 where updated health and dietary information was collected and further blood and urine samples were taken. Subjects also filled in a Physical Activity questionnaire and the information was used to derive a self-reported physical activity index for each subject (16).

For this study, we randomly selected 2003 healthy women from a subset of women >55 years old who had reported no menstruation for at least 1 year and who had not taken hormone replacement therapy for at least 3 months before attending the follow-up health check. Spot urine samples were stored at −20°C until analyzed for creatinine and phytoestrogens. Plasma samples were stored at −70°C until analyzed for hormones. Whole blood was collected and stored at −30°C before DNA extraction.

Of the 2003 women selected, 15 women were subsequently found to have sex hormone levels inconsistent with postmenopausal status (estradiol, >150 pmol/L) and were excluded, leaving a final total of 1,988 eligible subjects in this study.

Urinary Analysis. All spot urine samples were analyzed for three isoflavones (daidzein, genistein, and glycitein), two metabolites of daidzein [*O*-desmethylangolensin (O-DMA) and equol], and two lignans (enterodiol and enterolactone). Urinary phytoestrogens were analyzed using liquid chromatography/tandem mass spectrometry. We used the method developed by Grace et al. (17), except that the liquid chromatography conditions for separating phytoestrogens on an XTerra C18 column (2.1 × 150 mm) were modified to separate an interference peak present on the enterodiol chromatogram. This was achieved using a gradient of 40% to 60% methanol (in 0.1% ammonium acetate buffer) at 35°C with a flow rate of 250 μL/min. Limits of detection were 0.01 ng/mL for all analytes except for equol where the detection limit was 0.1 ng/mL. The coefficient of variation (CV) for assays was determined using nonblinded quality controls prepared from human urine. The average within-batch CVs were 2.3% for daidzein, 5.4% for genistein, 2.2% for glycitein, 2.5% for O-DMA, 4.0% for equol, 2.5% for enterodiol, and 1.1% for enterolactone. The between-batch CVs were 3.0% for daidzein, 4.0% for genistein, 3.1% for glycitein, 4.9% for O-DMA, 4.2% for equol, 2.8% for enterodiol, and 3.4% for enterolactone.

Urinary creatinine concentrations were measured based on a kinetic modification of the Jaffe reaction using the Roche reagent for creatinine on a Roche Cobas Mira Plus chemistry analyzer (Roche Products Ltd.).

Plasma Hormones and SHBG Analyses. Plasma samples were analyzed for sex hormones and SHBG in the following order: estradiol, testosterone, SHBG, androstenedione, and estrone. More than 98% of the subjects had sufficient plasma samples to complete the measurements of estradiol, testosterone, and SHBG. Seventy-one percent of the subjects had sufficient plasma samples to complete androstenedione measurements, whereas 55% of subjects had sufficient plasma samples for estrone measurements.

Estradiol was measured from plasma by RIA after ether extraction (18). Testosterone was measured using a solid-phase RIA kit (Diagnostic Products). SHBG was measured using a liquid-phase immunoradiometric kit (Orion Diagnostica). Androstenedione was measured using a solid-phase RIA kit (Diagnostic Systems Laboratories). Estrone was extracted with ether and liquid column chromatography on a Lipidex 5000 (Perkin-Elmer) with elution using chloroform/hexane/methanol (50:50:1) and then measured using a RIA kit. CVs for assays were determined using nonblinded quality control samples. The within-batch CVs ranged from 2.1% (SHBG) to 14% (estrone) and the between-batch CVs ranged from 7.4% (SHBG) to 22% (estrone). Details on detection limits and quality control have been described elsewhere (19).

Selection of SNPs for Genotyping. The ESR1 PvuII SNP and SNPs in *ESR2* gene were selected based on available information on known SNPs in these genes at the time of genotyping. Additional SNPs were chosen in *ESR1* gene as haplotype-tagging SNPs to tag the linkage disequilibrium block in *ESR1* gene where the PvuII SNP is located. SNPs in the *NR1I2* gene were chosen to efficiently tag the known SNPs in the gene.

SNPs in *ESR1* gene were identified from the National Institute of Environmental Health Sciences study.[5] There were 113 SNPs with minor allele frequencies >8% in the set of 90 individuals from United States of America in Panel 1. The Graphical Overview of Linkage Disequilibrium package[6] was used to create a summary of pair-wise linkage disequilibrium patterns on the National Institute of Environmental Health Sciences individual genotyping data for these 113 SNPs. Five linkage disequilibrium blocks were identified. The PvuII SNP was found in block 1, which consisted of a total of 19 SNPs. We used the tagSNPs program (20) to identify a set of tagging SNPs[7] for block 1. Seven tagging SNPs were identified, which achieved $R^2_h > 0.8$. One of the SNPs (rs867239) was subsequently found to be very rare in our population set, with <0.5% heterozygotes and no rare homozygotes. This SNP was therefore excluded.

SNPs in *NR1I2* gene were identified from the HapMap study.[8] There were 15 SNPs with minor allele frequencies >5% in the Centre d'Étude du Polymorphisme Humain (Utah residents with ancestry from northern and western Europe) set of individuals. Three linkage disequilibrium blocks were identified. We used the tagSNPs program (20) to identify a set of tagging SNPs[5] for each block based on the full set of 15 SNPs. For block 1, two tagging SNPs were chosen, yielding $R^2_S = 1.00$. For block 2, three tagging SNPs were chosen, yielding $R^2_S = 0.96$. For block 3, three tagging SNPs were chosen, yielding $R^2_S = 0.90$.

Genotype Analyses. All genotyping was carried out using end-point Taqman assays (Applied Biosystems) in 384-well arrays, which included blank wells as negative controls. Assays were run on MJ Tetrad thermal cyclers (Genetics Research Instrumentation) and genotypes were subsequently read on a 7900 Sequence Detector (Applied Biosystems)

[5] http://egp.gs.washington.edu
[6] http://www.sph.umich.edu/csg/abecasis/GOLD/
[7] http://www-rcf.usc.edu/~stram/
[8] http://www.hapmap.org

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Table 1. Frequency of polymorphisms

SNP	Reference SNP ID	Major/Minor allele	No. subjects (% of total)		
			Common homozygote	Heterozygote	Rare homozygote
ESR1-1	rs2077647	t/c	379 (26.2)	753 (52.1)	312 (21.6)
ESR1-2	rs1709182	t/c	587 (41.4)	637 (44.9)	195 (13.7)
ESR1-3	rs9340821	a/g	1,147 (79.2)	286 (19.8)	15 (1.0)
ESR1-4	rs9340835	g/a	648 (44.8)	641 (44.4)	156 (10.8)
ESR1-5 (PvuII)	rs2234693	t/c	533 (30.0)	879 (49.5)	363 (20.5)
ESR1-6 (Xba)	rs9340799	a/g	672 (43.5)	681 (44.1)	192 (12.4)
ESR2-1	rs1256049	g/a	1,280 (93.6)	87 (6.4)	0 (0)
ESR2-2 (a1730g)		a/g	550 (39.9)	627 (45.5)	201 (14.6)
ESR2-3	rs1255998	c/g	1,135 (82.5)	232 (16.9)	9 (0.7)
ESR2-4	rs1256030	t/c	414 (30.0)	680 (49.2)	288 (20.8)
NR1I2-1	rs3814055	c/t	570 (39.8)	663 (46.3)	198 (13.8)
NR1I2-2	rs2472671	t/c	949 (67.1)	416 (29.4)	49 (3.5)
NR1I2-3	rs1403526	a/g	664 (45.9)	648 (44.8)	135 (9.3)
NR1I2-4	rs13059232	c/t	614 (37.9)	795 (49.0)	213 (13.1)
NR1I2-5	rs7643645	a/g	546 (38.2)	676 (47.3)	208 (14.5)
NR1I2-6	rs3732357	a/g	734 (52.2)	563 (40.0)	109 (7.8)
NR1I2-7	rs3732359	a/g	882 (61.4)	481 (33.5)	74 (5.1)
NR1I2-8	rs1054190	c/t	1,108 (76.9)	310 (21.5)	23 (1.6)

according to the manufacturer's instructions. An automated robotic high-throughput system in a low-volume 384-well format was used. Each assay was tested on a specific test set of 96 DNA samples (80 unique, 14 duplicates, and 2 no template controls), before use in the main study, and all gave clear clustering and showed >95% concordance in the duplicates.

Genotype data were obtained on 1,775 women for ESR1 PvuII SNP. A subset of ~1,486 to 1,674 of these women served as healthy controls for a separate breast cancer case control study (21) and were more extensively genotyped. These women were genotyped for the five other SNPs in *ESR1* gene, four SNPs in *ESR2* gene, and eight SNPs in *NR1I2* gene. χ^2 tests were used to test that the genotype distributions for all the polymorphisms analyzed conformed to those expected under Hardy-Weinberg equilibrium.

Data Analysis. The statistical analyses were done using SPSS software version 12.0 (SPSS UK Ltd.). All statistical tests were two sided, and $P < 0.05$ was considered statistically significant. The spot nature of urinary concentrations was corrected using urinary creatinine concentration. Urinary excretion of phytoestrogens was expressed as micrograms per millimole of urinary creatinine. Urinary isoflavones were computed as the sum of the five individual isoflavones (daidzein, genistein, glycitein, O-DMA, and equol). Urinary lignans were computed as the sum of enterdiol and enterolactone. All urinary phytoestrogen and plasma sex hormones and SHBG data were analyzed as continuous variables. All urinary phytoestrogen and plasma sex hormones and SHBG data were skewed. Therefore, data were logarithmically transformed, except for urinary equol that was inversely transformed to obtain approximately normal frequency distributions. All subsequent statistical testings were done on transformed data.

ANCOVA was used to assess the association between phytoestrogen exposure, genetic polymorphisms, and sex hormone and SHBG levels. Analyses were adjusted for the following variables, which are or are potentially associated with sex hormone levels: age (continuous), body mass index (BMI; continuous), physical activity index (continuous), time of day at venipuncture (as quintiles, categorical), and time interval between last meal and venipuncture (continuous). Physical activity index was logarithmically transformed to obtain approximately normal distribution for the purpose of statistical testing. All genetic polymorphisms were initially tested based on an additive mode of action. Where P value is <0.10, the polymorphism was further tested for dominant and recessive modes of action to find the most appropriate genetic model. Phytoestrogen-gene interactions were formally assessed by including interaction terms in the statistical model. To reduce the number of statistical tests conducted, the effects

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Table 2. Urinary levels of phytoestrogens and plasma levels of sex hormones and SHBG

	n	Geometric mean (95% CI)	Percentile		
			25th	50th	75th
Urinary excretion (μg/mmol Cr)	1,985				
Isoflavones		23.3 (21.8, 24.8)	9.7	27.2	58.9
Daidzein		15.2 (14.3, 16.2)	6.0	16.5	37.9
Genistein		5.8 (5.4, 6.2)	1.9	5.9	14.4
Glycitein		1.2 (1.1, 1.3)	0.0	0.4	2.2
O-DMA		1.1 (1.0, 1.2)	0.0	0.2	1.6
Equol		0.6 (0.6, 0.6)	0.3	0.5	1.0
Lignans		123.9 (118.0, 130.1)	73.2	136.7	247.8
Enterodiol		7.0 (6.6, 7.4)	3.2	6.8	14.3
Enterolactone		109.7 (104.1, 115.7)	61.9	125.0	231.0
Plasma levels					
Estradiol (pmol/L)	1,968	16.0 (15.6, 16.4)	11.0	16.0	23.0
Estrone (pmol/L)	1,107	75.3 (73.2, 77.5)	55.0	76.0	106.0
Testosterone (nmol/L)	1,865	0.7 (0.7, 0.7)	0.5	0.7	1.1
Androstenedione (nmol/L)	1,415	3.2 (3.1, 3.3)	2.3	3.2	4.5
SHBG (nmol/L)	1,975	42.0 (41.1, 42.8)	31.0	42.0	58.9

Abbreviation: 95% CI, 95% confidence interval.

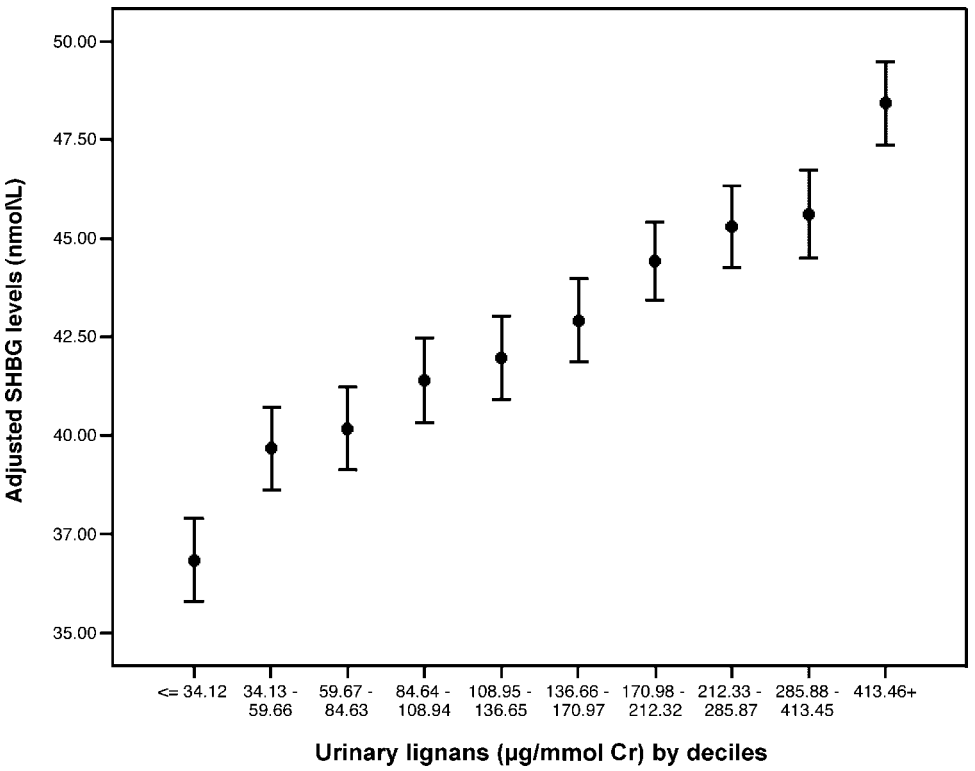

Figure 1. Error bar plot of plasma SHBG levels against urinary lignans by deciles. Plasma SHBG levels were adjusted for age, BMI, physical activity, time of venipuncture, and time interval between last meal and venipuncture.

of phytoestrogens were tested only using equol, O-DMA, and two summary measures of phytoestrogens (isoflavones and lignans) instead of testing each of the seven phytoestrogens individually. For polymorphisms in *ESR1, ESR2*, and *NR1I2* genes, phytoestrogen-gene interactions were assessed for estradiol and estrone levels, the two main estrogens in postmenopausal women. The Bonferroni correction to adjust for multiple testing was not used as it would be too conservative because the different phytoestrogens tested are not independent, as are the different polymorphisms tested. Where a phytoestrogen-gene interaction term was significant ($P < 0.05$), the analysis on the association between phytoestrogens and the hormone was repeated, stratified according to different genotypes. If the phytoestrogen-hormone associations were not significant in the different subgroups, the interaction was not considered to be biologically significant and is not discussed.

Results

Descriptives. Average age of subjects was 67.0 years (SD, 6.7). Subjects had a mean BMI of 26.7 kg/m^2 (SD, 4.3) and were 18.6 years postmenopause on average (SD, 9.0). The genotype frequencies for all the polymorphisms analyzed conformed to that expected under the Hardy Weinberg equilibrium (Table 1).

Urinary isoflavone excretion consisted predominantly of daidzein with equol being the most minor component. One hundred eighteen (5.9%) women did not have detectable levels of equol (≥0.1 ng/mL) in their urine. Eight hundred eleven (40.8%) women did not have detectable levels of O-DMA (≥0.01ng/mL) in their urine. Enterolactone was the predominant lignan. Plasma sex hormone and SHBG levels were compatible with postmenopausal status of these women (Table 2).

Associations between Phytoestrogens and Circulating Sex Hormones and SHBG. Table 3 shows the associations between urinary phytoestrogens and plasma sex hormones and SHBG levels. There was a highly significant positive association between urinary lignans and plasma SHBG levels ($\eta^2_P = 1.2\%$; $P < 0.001$). Urinary lignans explain 1.2% of variance in plasma SHBG level after accounting for potential confounders. This positive association is shown in the error bar plot in Fig. 1 where plasma SHBG levels show a dose-response increase across increasing deciles of urinary lignan levels. Urinary lignans were also negatively associated with plasma testosterone ($\eta^2_P = 0.2\%$; $P = 0.042$), testosterone/SHBG ratio ($\eta^2_P = 1.0\%$; $P < 0.001$), and estradiol/SHBG ratio ($\eta^2_P = 0.5\%$; $P = 0.003$; Table 3).

Urinary isoflavones as a whole showed no significant association with any of the sex hormones. However, two specific metabolites of daidzein (equol and O-DMA) showed significant associations with estradiol. Urinary equol was negatively associated with plasma estradiol level ($\eta^2_P = 0.3\%$; $P = 0.028$) and estradiol/SHBG ratio ($\eta^2_P = 0.2\%$; $P = 0.032$). In contrast, urinary O-DMA was positively associated with plasma estradiol level ($\eta^2_P = 0.3\%$; $P = 0.010$; Table 3). Women with no detectable urinary O-DMA had an adjusted mean plasma estradiol of 16.1 pmol/L (SE, 0.16) compared with 17.0 pmol/L (SE, 0.14) in women with detectable O-DMA ($P < 0.001$).

Phytoestrogen Interactions with *ESR1*, *ESR2*, and *NR1I2* Gene Variants. There were no significant associations between any of the polymorphisms in *ESR1* and *ESR2* genes and circulating estradiol and estrone levels. There was also no significant interaction between phytoestrogens and polymorphisms in *ESR1* and *ESR2* genes in affecting estradiol or estrone levels except for ESR1-4 polymorphism. A significant interaction between ESR1-4 polymorphism and lignans on estrone levels was observed ($P = 0.029$). There was a negative association between lignans and estrone levels in women with gg or ga genotypes ($\eta^2_P = 0.8\%$; $P = 0.018$) but not in women with aa genotype ($\eta^2_P = 0.03\%$; $P = 0.134$; Fig. 2).

There were several significant associations observed between polymorphisms in *NR1I2* gene and circulating estradiol and estrone levels. NR1I2-4 polymorphism was associated

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Table 3. Relationship between phytoestrogens and plasma sex hormones and SHBG levels

	Isoflavones			Equol*		
	β (95% CI)	η^2_P (%)	P	β (95% CI)	η^2_P (%)	P
Estradiol	0.010 (−0.008, 0.028)	0.1	0.267	0.050 (0.005, 0.094)	0.3	0.028
Estrone	0.003 (−0.019, 0.025)	0.0	0.777	0.037 (−0.016, 0.090)	0.2	0.167
Testosterone	0.004 (−0.016, 0.025)	0.0	0.674	0.032 (−0.019, 0.082)	0.1	0.219
Androstenedione	−0.006 (0.027, 0.016)	0.0	0.601	0.028 (−0.022, 0.079)	0.1	0.271
SHBG	0.011 (−0.004, 0.026)	0.1	0.149	−0.016 (−0.053, 0.020)	0.0	0.379
Estradiol/SHBG	0.000 (−0.023, 0.023)	0.0	0.991	0.062 (0.005, 0.119)	0.2	0.032
Testosterone/SHBG	−0.006 (−0.031, 0.018)	0.0	0.614	0.047 (−0.013, 0.108)	0.1	0.124

NOTE: ANCOVA adjusted for age, BMI, physical activity, time of day at venipuncture, and time interval between last meal and venipuncture. η^2_P is partial η^2, which represents the proportion of variance explained by the particular variable after accounting for potential confounders.
*Results shown are for the inverse transformation of equol. Hence, where a β coefficient is positive, it indicates a negative association between urinary equol and the particular sex hormone and vice versa.

with differences in estradiol levels. The NR1I2-4 t allele was significantly associated with estradiol levels (adjusted means: tt, 17.2 pmol/L; ct, 15.2 pmol/L; cc, 16.0 pmol/L; η^2_P = 0.6%; $P_{heterogeneity}$ = 0.009; P_{trend} = 0.455) and is consistent with either a recessive or additive effect. NR1I2-1 and NR1I2-3 polymorphisms were significantly associated with differences in estrone levels. The NR1I2-1 c allele was associated with higher estrone levels and was most consistent with a dominant mode of action (adjusted means: cc, 79.4 pmol/L; ct or tt, 74.0 pmol/L; η^2_P = 0.5%; P = 0.038). The NR1I2-3 g allele was associated with lower estrone levels and was most consistent with a dominant mode of action (adjusted means: ga or gg, 72.9 pmol/L; aa, 80.0 pmol/L; η^2_P = 0.9%; P = 0.006).

There was evidence of isoflavone interaction with NR1I2-6 polymorphism in affecting circulating estrogen levels. A positive association between isoflavones and estradiol was observed in women with gg genotype for NR1I2-6 polymorphism (η^2_P = 0.6%; P = 0.015) but not in women with ga or aa genotypes (η^2_P = 0.0%; P = 0.680). But the isoflavone×NR1I2-6 interaction term did not reach statistical significance (P = 0.061). However, the interaction between isoflavones and NR1I2-6 polymorphism was statistically significant for estrone levels (P = 0.018). There was a positive association between isoflavones and estrone levels in women with aa genotype (η^2_P = 1.5%; P = 0.014) but not in women with ga or gg genotypes (η^2_P = 0.3%; P = 0.267). This interaction is shown in Fig. 3 where the relationship between isoflavone exposure and plasma estrone levels was significantly different in women with different genotype for the NR1I2-6 polymorphism.

Discussion

Human intervention and cross-sectional studies that have investigated the effect of phytoestrogens or soy intake on hormone levels in postmenopausal women have been small and produced inconsistent results (4, 22-38). This study is the largest study ever conducted to investigate the relationship between phytoestrogen exposure and circulating sex hormone levels in postmenopausal women. Using urinary biomarkers, we found a clear positive association between lignans and SHBG levels (Fig. 1). We have shown previously that phytoestrogen concentrations in spot urine correlated with that in serum (r = 0.81-0.94; P < 0.001) and can be used as suitable biomarkers of phytoestrogen intake in Western population with low habitual soy consumption (39). Lignans have been found to stimulate SHBG levels *in vitro* (4) and this study provides evidence that such stimulation may also occur *in vivo*.

Urinary lignans were also negatively associated with testosterone levels (Table 3). This is supported by *in vitro* evidence that lignans can inhibit 17β-hydroxysteroid dehydrogenase type I, which catalyzes the conversion of androstenedione to testosterone (40). Testosterone competes with estradiol for binding to SHBG. Lignans seem to stimulate SHBG levels while lowering testosterone levels. This is likely to promote greater binding of estradiol to SHBG, thus reducing the amount of free estradiol. This would be expected to translate to a lower breast cancer risk. However, the observed hormonal modulatory effects of lignans could also be a manifestation of their estrogenic properties, in which case their effects on breast cancer risk will be harder to predict.

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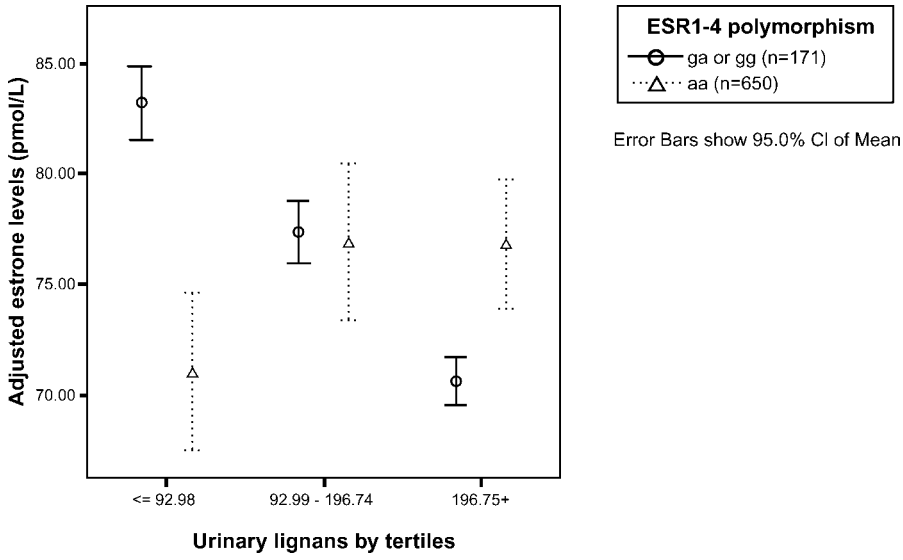

Figure 2. Error bar plot of plasma estrone levels against urinary lignans by tertiles, stratified by ESR1-4 polymorphism. Plasma estrone levels were adjusted for age, BMI, physical activity, time of venipuncture, and time interval between last meal and venipuncture.

Table 3. Relationship between phytoestrogens and plasma sex hormones and SHBG levels (Cont'd)

O-DMA			Lignans		
β (95% CI)	η^2_P (%)	*P*	β (95% CI)	η^2_P (%)	*P*
0.035 (0.008, 0.061)	0.3	0.010	0.001 (−0.021, 0.023)	0.0	0.946
0.002 (−0.031, 0.035)	0.0	0.906	−0.021 (−0.047, 0.005)	0.2	0.107
−0.002 (−0.032, 0.028)	0.0	0.912	−0.026 (−0.052, −0.001)	0.2	0.042
−0.001 (−0.032, 0.031)	0.0	0.961	0.003 (−0.022, 0.028)	0.0	0.815
0.004 (−0.018, 0.026)	0.0	0.707	0.045 (0.027, 0.063)	1.2	<0.001
0.032 (−0.002, 0.066)	0.2	0.066	−0.043 (−0.072, −0.015)	0.5	0.003
−0.004 (−0.040, 0.033)	0.0	0.848	−0.064 (−0.094, −0.034)	1.0	<0.001

Equol and O-DMA are two phytoestrogen metabolites, which are formed by the intestinal bacterial metabolism of daidzein. Approximately 30% to 50% of the human population produce equol, whereas ~80% to 90% produce O-DMA following soy challenge (41). In this study, ~94% of women had detectable urinary equol, whereas 59% had detectable O-DMA. The high proportion of women with small amounts of detectable urinary equol is likely due to preformed equol from the diet and reflects the high sensitivity of the liquid chromatography/tandem mass spectrometry analysis used. Equol has been found in cow's milk (42-44) and is also likely to be present in meat and meat products because animals, including cows, pigs, and sheep, are capable of producing equol (45, 46). In contrast, limited studies have assessed O-DMA production in animals but O-DMA seems to be produced in smaller amounts than equol (47).

In vitro and animal studies have suggested that equol and O-DMA are more biologically active than their precursor daidzein (41). In this study, equol was negatively associated with estradiol level and estradiol/SHBG ratio, suggesting an antiestrogenic effect. This is in contrast to the positive association between O-DMA and estradiol levels (Table 3). Such apparent opposing effects of equol and O-DMA are intriguing but have also manifested in a recent study by Frankenfeld et al. (48). In their study in 55 postmenopausal women, mammographic density was 39% lower in equol producers compared with nonproducers, whereas O-DMA producers had 69% greater mammographic density compared with nonproducers. However, in a similar study in 87 postmenopausal women, Frankenfeld et al. (49) did not observe any significant differences in serum estrogens, androgens, and SHBG between equol producers versus nonproducers and O-DMA producers versus nonproducers. Frankenfeld et al. had used a ''bacteriotyping'' approach, comparing individuals harboring bacteria that are capable or incapable of equol and O-DMA production, but their approach does not take into account dietary intake. On the other hand, our study has assessed equol and O-DMA excretion, which is dependent on dietary intake. The different results obtained using the two approaches suggest that the hormonal effects of equol and O-DMA may be more likely due to their direct actions on sex hormone metabolism pathways rather than due to the actions of bacteria responsible for equol/O-DMA production.

In this study, the magnitude of effects of phytoestrogens on circulating sex hormones and SHBG levels seems to be small, with phytoestrogens explaining not >2% of the variance in hormone levels after accounting for potential confounders. But small endocrine modifications may reach physiologic significance over a long period. In this study, we used a cross-sectional analysis of phytoestrogen biomarkers and circulating hormones to estimate chronic exposure and such estimates will incur random errors. It is likely that the estimates of effect size obtained are attenuated due to these random errors. In addition, this study has been done in subjects with low habitual soy consumption. It is not known whether in people who consume soy regularly, greater effects would be observed.

The possibility of phytoestrogen-gene interactions in affecting circulating sex hormone levels in postmenopausal women was first explored in our preliminary study in 125 postmenopausal women in EPIC-Norfolk (13) in which only estradiol was studied. In this study, we found significant interaction between lignans and ESR1-4 polymorphism in affecting estrone levels (Fig. 2). ESR1-4 polymorphism lies in an intron and has no known function. It tags three of the five most common haplotypes in our subjects. It may lie in a regulatory

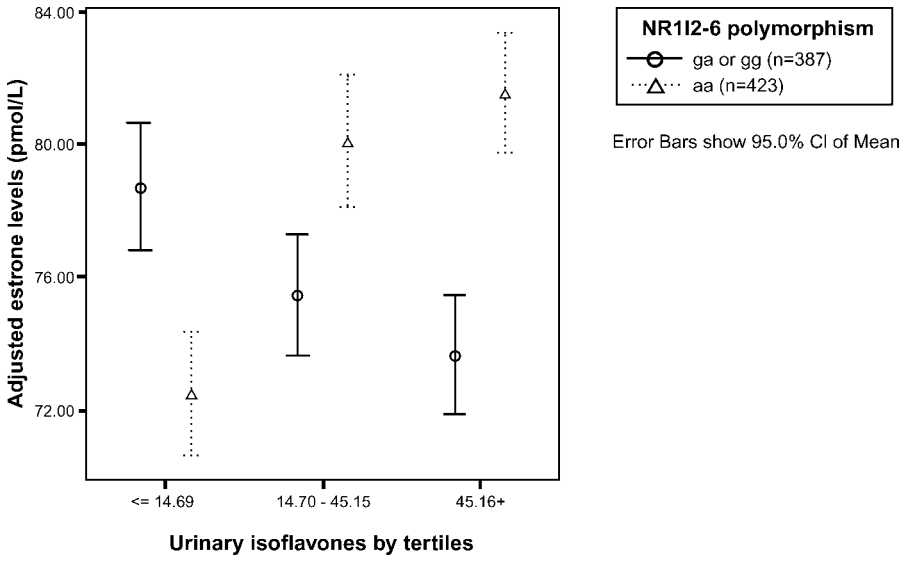

Figure 3. Error bar plot of plasma estrone levels against urinary isoflavones by tertiles, stratified by NR1I2-6 polymorphism. Plasma estrone levels were adjusted for age, BMI, physical activity, time of venipuncture, and time interval between last meal and venipuncture.

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sequence and affect levels of ESR1 expression or is a silent variant in linkage disequilibrium with another as yet unknown variant that may be functional. But the observed interaction provided some indirect evidence for phytoestrogens interacting with estrogen receptor α to exert feedback control on circulating estrogen levels.

Compared with estrogen receptor α and estrogen receptor β, far fewer studies have been conducted on PXR. Phytoestrogens have been shown to bind and activate PXR (9, 10, 50). The binding of phytoestrogens to PXR can result in the transcriptional activation of its principal target gene, *CYP3A4. CYP3A4* is responsible for the oxidation of a variety of structurally unrelated compounds, including steroids and xenobiotics (51). Pertinent to this study, *CYP3A4* catalyzes the hydroxylation of estrone and estradiol, with higher affinity for estrone (52-54). Levels of *CYP3A4* expression exhibit considerable interindividual variation and much of the variation is attributed to genetic factors (55). However, polymorphisms in *CYP3A4* gene do not seem to explain the apparent differences in *CYP3A4* expression levels. This led to increasing attention on PXR polymorphisms as possible explanatory factors (56). In this study, three polymorphisms (NR1I2-1, NR1I2-3, and NR1I2-4) in *NR1I2* gene were associated with differences in circulating estrone or estradiol levels. All three polymorphisms are located in the 5′-untranslated region and have unknown function. In addition, there was suggestive evidence of isoflavones interacting with NR1I2-6 polymorphism to exert differential effects on circulating estrone and estradiol level. NR1I2-6 is a silent polymorphism that lies in an intron. Thus, the mechanism of interaction is unknown. But in a separate study by Zhang et al. (57), several silent PXR polymorphisms that do not result in amino acid changes have been found to correlate with differences in *CYP3A4* activity *in vitro* and *in vivo*. These silent PXR polymorphisms could lie in a regulatory sequence and affect levels of PXR expression. But this remains speculative until further studies are done to investigate the functional effects of these otherwise silent polymorphisms. It is noteworthy that *CYP3A4* is also the main enzyme that is involved in the metabolism of >60% of all therapeutic drugs (58), including the antiestrogen tamoxifen, which is widely used in breast cancer treatment (59, 60), and several statins (simvastatin, lovastatin, cerivastatin, and atorvastatin; ref. 61). Therefore, if phytoestrogens do interact with PXR receptor to affect activation of *CYP3A4,* this could present potential diet-drug interaction problems and deserves further investigation.

In this large cross-sectional study on phytoestrogen-gene associations with circulating sex hormone levels in postmenopausal women, we found significant associations between phytoestrogen exposure and circulating sex hormone and SHBG levels. This is despite of the low habitual soy consumption of these women. Furthermore, several phytoestrogen-gene interactions were evident, resulting in differential effects of phytoestrogens in women with different gene variants. Such phytoestrogen-gene interactions imply that the health effects of phytoestrogen consumption may differ between individuals with different genotype for the relevant genes. Knowledge of such interactions will help to clarify the conflicting literature commonly reported on the hormonal effects of phytoestrogens.

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References

1. Adlercreutz H. Phytoestrogens and breast cancer. J Steroid Biochem Mol Bio 2003;83:113–8.
2. Magee PJ, Rowland IR. Phyto-oestrogens, their mechanism of action: current evidence for a role in breast and prostate cancer. Br J Nutr 2004;91:513–31.
3. Kirk CJ, Harris RM, Wood DM, Waring RH, Hughes PJ. Do dietary phytoestrogens influence susceptibility to hormone-dependent cancer by disrupting the metabolism of endogenous oestrogens? Biochem Soc Trans 2001;29:209–16.
4. Adlercreutz H, Mousavi Y, Clark J, et al. Dietary phytoestrogens and cancer: *in vitro* and *in vivo* studies. J Steroid Biochem Mol Biol 1992;41:331–7.
5. Mousavi Y, Adlercreutz H. Genistein is an effective stimulator of sex hormone-binding globulin production in hepatocarcinoma human liver cancer cells and suppresses proliferation of these cells in culture. Steroids 1993;58:301–4.
6. Loukovaara M, Carson M, Palotie A, Adlercreutz H. Regulation of sex hormone-binding globulin production by isoflavonoids and patterns of isoflavonoid conjugation in HepG2 cell cultures. Steroids 1995;60:656–61.
7. Committee on Toxicity of Chemicals in Food/Consumer Products and the Environment. COT Report—Phytoestrogens and Health. London: Food Standards Agency, 2003.
8. Kliewer SA, Moore JT, Wade L, et al. An orphan nuclear receptor activated by pregnanes defines a novel steroid signaling pathway. Cell 1998;92:73–82.
9. Blumberg B, Sabbagh W, Jr., Juguilon H, et al. SXR, a novel steroid and xenobiotic-sensing nuclear receptor. Genes Dev 1998;12:3195–205.
10. Ricketts ML, Moore DD, Banz WJ, Mezei O, Shay NF. Molecular mechanisms of action of the soy isoflavones includes activation of promiscuous nuclear receptors. A review. J Nutr Biochem 2005;16:321–30.
11. Jacobs MN, Nolan GT, Hood SR. Lignans, bacteriocides and organochlorine compounds activate the human pregnane X receptor (PXR). Toxicol Appl Pharmacol 2005;209:123–33.
12. Low YL, Dunning AM, Dowsett M, et al. Implications of gene-environment interaction in studies of gene variants in breast cancer: an example of dietary isoflavones and the D356N polymorphism in the sex hormone-binding globulin gene. Cancer Res 2006;66:8980–3.
13. Low YL, Taylor JI, Grace PB, et al. Phytoestrogen exposure correlation with plasma estradiol in postmenopausal women in European Prospective Investigation of Cancer and Nutrition-Norfolk may involve diet-gene interactions. Cancer Epidemiol Biomarkers Prev 2005;14:213–20.
14. Riboli E. Nutrition and cancer: Background and rationale of the European Prospective Investigation into Cancer and Nutrition (EPIC). Ann Oncol 1992; 3:783–91.
15. Day NE, Oakes S, Luben R, et al. EPIC-Norfolk: study design and characteristics of the cohort. Br J Cancer 1999;80:95–103.
16. Wareham NJ, Jakes RW, Rennie KL, et al. Validity and repeatability of the EPIC-Norfolk physical activity questionnaire. Int J Epidemiol 2002;31: 168–74.
17. Grace PB, Taylor JI, Botting NP, et al. Quantification of isoflavones and lignans in serum using isotope dilution liquid chromatography/tandem mass spectrometry. Rapid Commun Mass Spectrom 2003;17:1350–7.
18. Dowsett M, Goss PE, Powles TJ, et al. Use of the aromatase inhibitor 4-hydroxyandrostenedione in postmenopausal breast cancer: optimization of therapeutic dose and route. Cancer Res 1987;47:1957–61.
19. Dunning AM, Dowsett M, Healey CS, et al. Polymorphisms associated with circulating sex hormone levels in postmenopausal women. J Natl Cancer Inst 2004;96:936–45.
20. Stram DO, Haiman CA, Hirschhorn JN, et al. Choosing haplotype-tagging SNPS based on unphased genotype data using a preliminary sample of unrelated subjects with an example from the Multiethnic Cohort Study. Hum Hered 2003;55:27–36.
21. Al-Zahrani A, Sandhu MS, Luben R, et al. IGF1 and IGFBP3 tagging polymorphisms are associated with circulating levels of IGF1, IGFBP3 and risk of breast cancer. Hum Mol Genet 2006;15:1–10.
22. Wu AH, Stanczyk FZ, Martinez C, et al. A controlled 2-mo dietary fat reduction and soy food supplementation study in postmenopausal women. Am J Clin Nutr 2005;81:1133–41.
23. Brooks JD, Ward WE, Lewis JE, et al. Supplementation with flaxseed alters estrogen metabolism in postmenopausal women to a greater extent than does supplementation with an equal amount of soy. Am J Clin Nutr 2004;79: 318–25.
24. Teede HJ, Dalais FS, McGrath BP. Dietary soy containing phytoestrogens does not have detectable estrogenic effects on hepatic protein synthesis in postmenopausal women. Am J Clin Nutr 2004;79:396–401.
25. Foth D, Nawroth F. Effect of soy supplementation on endogenous hormones in postmenopausal women. Gynecol Obstet Invest 2003;55:135–8.
26. Persky VW, Turyk ME, Wang L, et al. Effect of soy protein on endogenous hormones in postmenopausal women. Am J Clin Nutr 2002;75:145–53.
27. Nicholls J, Lasley B, Nakajima S, Setchell KDR, Schneeman B. Effects of soy consumption on gonadotropin secretion and acute pituitary responses to gonadotropin-releasing hormone in women. J Nutr 2002;132:708–14.
28. Baird D, Umbach D, Lansdell L, et al. Dietary intervention study to assess estrogenicity of dietary soy among postmenopausal women. J Clin Endocrinol Metab 1995;80:1685–90.
29. Hutchins A, Martini MC, Olson B, Thomas W, Slavin J. Flaxseed consumption influences endogenous hormone concentrations in postmenopausal women. Nutr Cancer 2001;39:58–65.
30. Duncan AM, Underhill K, Xu X, et al. Modest hormonal effects of soy isoflavones in postmenopausal women. J Clin Endocrinol Metab 1999;84: 3479–84.

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31. Oh HY, Kim SS, Chung HY, Yoon S. Isoflavone supplements exert hormonal and antioxidant effects in postmenopausal Korean women with diabetic retinopathy. J Med Food 2005;8:1–7.

32. Goldin BR, Brauner E, Adlercreutz H, Ausman LM, Lichtenstein AH. Hormonal response to diets high in soy or animal protein without and with isoflavones in moderately hypercholesterolemic subjects. Nutr Cancer 2005; 51:1–6.

33. Petri Nahas E, Nahas Neto J, De Luca L, et al. Benefits of soy germ isoflavones in postmenopausal women with contraindication for conventional hormone replacement therapy. Maturitas 2004;48:372–80.

34. Adlercreutz H, Höckerstedt K, Bannwart C, et al. Effect of dietary components, including lignans and phytoestrogens, on enterohepatic circulation and liver metabolism of estrogens and on sex hormone binding globulin (SHBG). J Steroid Biochem Mol Biol 1987;27:1135–44.

35. Shoff SM, Newcomb PA, Mares-Perlman JA, et al. Usual consumption of plant foods containing phytoestrogens and sex hormone levels in postmenopausal women in Wisconsin. Nutr Cancer 1998;30:207–12.

36. Thomas HV, Davey GK, Key TJ. Oestradiol and sex hormone-binding globulin in premenopausal and post-menopausal meat-eaters, vegetarians and vegans. Br J Cancer 1999;80:1470–5.

37. Verkasalo P, Appleby P, Davey G, Key T. Soy milk intake and plasma sex hormones: a cross-sectional study in pre- and postmenopausal women (EPIC-Oxford). Nutr Cancer 2001;40:79–86.

38. Wu AH, Stanczyk FZ, Seow A, Lee H-P, Yu MC. Soy intake and other lifestyle determinants of serum estrogen levels among postmenopausal Chinese women in Singapore. Cancer Epidemiol Biomarkers Prev 2002;11: 844–51.

39. Grace PB, Taylor JI, Low Y-L, 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 2004;13: 698–708.

40. Brooks JD, Thompson LU. Mammalian lignans and genistein decrease the activities of aromatase and 17β-hydroxysteroid dehydrogenase in MCF-7 cells. J Steroid Biochem Mol Biol 2005;94:461–7.

41. Atkinson C, Frankenfeld CL, Lampe JW. Gut bacterial metabolism of the soy isoflavone daidzein: exploring the relevance to human health. Exp Biol Med (Maywood) 2005;230:155–70.

42. Bannwart C, Adlercreutz H, Wahala K, et al. Identification of the phytoestrogen 3′,7-dihydroxyisoflavan, an isomer of equol, in human urine and cow's milk. Biomed Environ Mass Spectrom 1988;17:1–6.

43. Antignac JP, Cariou R, Le Bizec B, Cravedi JP, Andre F. Identification of phytoestrogens in bovine milk using liquid chromatography/electrospray tandem mass spectrometry. Rapid Commun Mass Spectrom 2003;17: 1256–64.

44. King R, Mano M, Head R. Assessment of isoflavonoid concentrations in Australian bovine milk samples. J Dairy Res 1998;65:479–89.

45. Lundh TJ, Pettersson H, Kiessling KH. Liquid chromatographic determination of the estrogens daidzein, formononetin, coumestrol, and equol in bovine blood plasma and urine. J Assoc Off Anal Chem 1988;71:938–41.

46. Lundh T. Metabolism of estrogenic isoflavones in domestic animals. Proc Soc Exp Biol Med 1995;208:33–9.

47. Blair RM, Appt SE, Franke AA, Clarkson TB. Treatment with antibiotics reduces plasma equol concentration in cynomolgus monkeys (*Macaca fascicularis*). J Nutr 2003;133:2262–7.

48. Frankenfeld CL, McTiernan A, Aiello EJ, et al. Mammographic density in relation to daidzein-metabolizing phenotypes in overweight, postmenopausal women. Cancer Epidemiol Biomarkers Prev 2004;13:1156–62.

49. Frankenfeld CL, McTiernan A, Tworoger SS, et al. Serum steroid hormones, sex hormone-binding globulin concentrations, and urinary hydroxylated estrogen metabolites in post-menopausal women in relation to daidzein-metabolizing phenotypes. J Steroid Biochem Mol Biol 2004;88:399–408.

50. Jacobs MN, Nolan GT, Hood SR. Lignans, bacteriocides, and organochlorine compounds activate the human pregnane X receptor (PXR). Toxicol Appl Pharmacol 2005;209:123–33.

51. Keshava C, McCanlies EC, Weston A. CYP3A4 polymorphisms—potential risk factors for breast and prostate cancer: a HuGE review. Am J Epidemiol 2004;160:825–41.

52. Shou M, Korzekwa KR, Brooks EN, et al. Role of human hepatic cytochrome *P*450 1A2 and 3A4 in the metabolic activation of estrone. Carcinogenesis 1997;18:207–14.

53. Lee AJ, Cai MX, Thomas PE, Conney AH, Zhu BT. Characterization of the oxidative metabolites of 17β-estradiol and estrone formed by 15 selectively expressed human cytochrome *p*450 isoforms. Endocrinology 2003;144: 3382–98.

54. Yamazaki H, Shaw PM, Guengerich FP, Shimada T. Roles of cytochromes *P*450 1A2 and 3A4 in the oxidation of estradiol and estrone in human liver microsomes. Chem Res Toxicol 1998;11:659–65.

55. Ozdemir V, Kalowa W, Tang BK, et al. Evaluation of the genetic component of variability in CYP3A4 activity: a repeated drug administration method. Pharmacogenetics 2000;10:373–88.

56. Kliewer SA, Goodwin B, Willson TM. The nuclear pregnane X receptor: a key regulator of xenobiotic metabolism. Endocr Rev 2002; 23:687–702.

57. Zhang J, Kuehl P, Green ED, et al. The human pregnane X receptor: genomic structure and identification and functional characterization of natural allelic variants. Pharmacogenetics 2001;11:555–72.

58. Goodwin B, Redinbo MR, Kliewer SA. Regulation of CYP3A gene transcription by the pregnane X receptor. Annu Rev Pharmacol Toxicol 2002;42:1–23.

59. Parte P, Kupfer D. Oxidation of tamoxifen by human flavin-containing monooxygenase (FMO) 1 and FMO3 to tamoxifen-N-oxide and its novel reduction back to tamoxifen by human cytochromes *P*450 and hemoglobin. Drug Metab Dispos 2005;33:1446–52.

60. Desai PB, Nallani SC, Sane RS, et al. Induction of cytochrome *P*450 3A4 in primary human hepatocytes and activation of the human pregnane X receptor by tamoxifen and 4-hydroxytamoxifen. Drug Metab Dispos 2002;30: 608–12.

61. Paoletti R, Corsini A, Bellosta S. Pharmacological interactions of statins. Atheroscler Suppl 2002;3:35–40.

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