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Original Article

Correlations of urinary phytoestrogen excretion with life-style factors and dietary intakes among middle-aged and elderly Chinese women

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Abstract: Isoflavones and lignans, two major groups of phytoestrogens, have been postulated to have multiple health benefits, including anti-estrogenic, anti-cancer, pro-cardiovascular health, and ameliorating menopausal symptoms. Urinary excretion of isoflavonoids, including daidzein, genistein, glycitein, O-desmethylangolensin (O-DMA), dihydrodaidzein, dihydrogenistein, and equol, and lignans, including enterodiol and enterolactone, have been used as biomarkers of phytoestrogen exposure in epidemiologic studies. We evaluated the urinary excretion of phytoestrogens and their correlations with lifestyle and dietary factors among 2,165 women who participated in the Shanghai Women's Health Study (SWHS), a population-based prospective cohort study of 74,942 urban Chinese women aged 40-70 years at study enrollment (1996-2000). The medians (in nmol/mg creatinine) were: isoflavonoids, 17.13; daidzein, 5.57; genistein, 2.41; glycitein, 0.94; O-DMA, 1.52; dihydrodaidzein, 0.81; dihydrogenistein, 0.19; equol, 0.11; enterodiol, 0.30; and enterolactone, 1.18. These levels are 2- (enterodiol) to 126- (O-DMA) fold higher than levels among US women similar in age range with the exception of enterolactone, for which a similar level was observed for both populations. Urinary isoflavonoid excretion was higher among older women and women who engaged in regular exercise and significantly associated with soy food intake, but was inversely related to fruit intake. Urinary excretions of dihydrodaidzein, dihydrogenistein, equol, enterodiol, and enterolactone were inversely associated with body mass index (BMI). Urinary excretion of isoflavones correlated with soy food intake and healthy lifestyle but was inversely associated with fruit intake among middle-aged and elderly Chinese women. Our study adds important information to the rapidly growing body of research on the potential health benefits of phytoestrogens.

Keywords: Isoflavonoids, lignans, phytoestrogens, dietary intakes, lifestyle factors

Introduction

Phytoestrogens are natural, plant-derived substances that can bind to estrogen receptors and have previously been inversely associated with risk for breast cancer, colorectal cancer, atherosclerosis, and osteoporosis [1-6]. Two groups of phytoestrogens, which are structurally and functionally similar to the hormone 17β-estrodiol, are commonly found in the human diet. These include isoflavones (daidzein, genistein, and glycitein), which are abundant in soy foods, and lignans (enterodiol and enterolactone), which are abundant in nuts, grains, and other seeds. When metabolized by gut flora, daidzein is converted to dihydrodaidzein, O-desmethylangolensin (O-DMA) and equol, while genistein is metabolized to dihydrogenistein [7]. Both precursors and metabolites can be absorbed into the blood and then excreted, mainly in urine [8]. Substantial interindividual variation in the bioavailability of phytoestrogens after ingestion of isoflavones and lignans has been observed [9, 10]. Urinary levels of phytoestrogen excretions are aggregate measurements of amounts of consumption, metabolism, and absorption of dietary phytoestrogen intake and, thus, have been used as biomarkers in epidemiologic studies [5, 7, 11, 12].

However, at the population level, little information is available on excretion of urinary phytoestrogens and their associations with lifestyle factors and dietary intakes in Asian countries where soy food intake is high. Understanding how well levels of phytoestrogens reflect dietary intake and their associations with lifestyle factors is essential to the implementation and interpretation of epidemiologic research on associations between these biomarkers and health effects. We evaluated urinary excretion of phytoestrogens, isoflavonoids, including daidzein, genistein, glycitein, O-DMA, dihydrodaidzein, dihydrogenistein, and equol, and lignans, including enterodiol and enterolactone, and their correlations with lifestyle factors and dietary intakes by using resources from the Shanghai Women's Health Study (SWHS).

Materials and methods

Study population

The SWHS is an ongoing, population-based, prospective cohort study. From 1996 to 2000, 74,942 Chinese women aged 40 to 70 years residing in seven typical urban communities of Shanghai were recruited for the cohort study (participation rate: 92%) [13]. Participants signed a consent form at study enrollment and completed a detailed in-person survey that collected information on demographic characteristics, physical activity, educational attainment, income, occupation, and other lifestyle factors. A spot urine sample was collected from 65,754 women (87.7% of study participants) by using a sterilized cup containing 125 mg of ascorbic acid. The samples were transported in a portable, insulated bag with ice packs (at approximately 0 to 4°C), processed within 6 hours of collection, and then stored at -80°C. The study was approved by the relevant institutional review boards for human research of all participating institutes. Isoflavone excretion in the urine samples of 2,165 women was measured as part of several nested case-control studies that evaluated the relationship between dietary soy intake and risk of breast cancer risk and coronary heart disease. These women, all of whom were free of cancer and coronary heart disease at the time of the urine sample collection, were included in the current analysis.

Dietary assessment and selected lifestyle factors

Information on usual dietary intake over the 12 months preceding the baseline interview was collected with a validated food frequency questionnaire (FFQ) [14]. A total of 71 food items and food groups were included in the questionnaire, which covered virtually all soy foods consumed in Shanghai, including soy milk, tofu, other soy products, dried soybeans, soybean sprouts, and fresh soybeans. Participants were asked how frequently (daily, weekly, monthly, yearly, or never) they consumed each food or food item, followed by a question on the amount of consumption in liang (50 g/liang, Chinese ounce) per unit of time. The intake level of major nutrients, including protein, fat, soy protein, and soy isoflavones, was estimated using the Chinese Food Composition Tables [15]. Our previous investigation indicated that the SWHS FFQ can reliably and accurately measure usual intake of major nutrients and food groups among women in Shanghai [14]. Anthropometrics, including weight, height, and waist and hip circumferences, were measured according to a standard protocol. Body mass index (BMI, kg/m^2) was calculated from weight and height. Smoking and alcohol consumption were defined according to current status (yes or no). Information on tea consumption habits, and vitamin supplement use including any use of vitamins A, B, C, E, and multi-vitamins, was also collected. Physical activity, assessed by a validated physical activity questionnaire, was measured in metabolic equivalents (MET-h/day/year) based on total daily physical activity and exercise participation [16].

Laboratory methods

Daidzein, genistein, glycitein, O-DMA, dihydrodaidzein, dihydrogenistein, equol, enterodiol, and enterolactone were analyzed using liquid chromatography-mass spectrometry with isotope dilution electrospray ionization (negative mode) tandem mass spectrometry as described previously [1, 17, 18]. Briefly, mixed with triethylamine acetate (pH 7.0), β-glucuronidase, and arylsulfatase, urine was incubated for 1 h at 37°C. After being extracted three times with 2 ml of ethyl ether, the combined organic phases were dried under nitrogen, redissolved in a

Table 1. Age-adjusted characteristics for participants of the current study and all participants of the Shanghai Women's Health Study

Characteristics*	Current study subjects (n = 2,165)	SWHS (n = 74,942)	P value†
Demographic factors			
Age (years) at recruitment, mean (SD)	56.70 (9.39)	52.13 (9.08)	<.0001
Body mass index, mean (SD)	24.03 (0.01)	24.31 (0.07)	0.0001
Waist-to-hip ratio, mean (SD)	0.814 (0.001)	0.811 (0.000)	0.0035
Education elementary or less (%)	22.19	21.87	0.1518
Exercise regularly (%)	34.57	35.65	0.4303
Family income <10,000 per year (%)	17.16	16.27	0.2748
Current smoking (%)	2.57	2.40	0.4184
Current alcohol consumption (%)	2.32	1.94	0.3244
Tea consumption (%)	30.04	29.73	0.7592
Nutritional factors			
Total energy intake (kcal/day), mean (SD)	1697.54 (8.71)	1675.77 (1.48)	0.0138
Total fat intake (g/day), mean (SD)	29.74 (0.29)	29.43 (0.05)	0.2865
Total fruit and vegetable intake (g/day), mean (SD)	568.61 (6.23)	560.72 (1.06)	0.2113
Total soy intake (g/day), mean (SD)	144.32 (2.66)	141.28 (0.45)	0.2617
Soy protein intake (g/day), mean (SD)	9.10 (0.14)	8.92 (0.02)	0.2164
Soy isoflavones intake (mg/day), mean (SD)	31.29 (0.49)	30.52 (0.08)	0.1196

*Adjusted for age at study enrollment; †One-sample t-test was used for continuous variables; chi-square test was used for categorical variables.

methanol/acetate buffer and were either analyzed immediately by injecting 20 μl into the liquid chromatography system or stored at -20°C until analysis. Liquid chromatography photodiode array mass spectrometry analyses were performed by using a HydroBond PS reversed-phase guard and analytical column. Limits of quantitation (LOQ) were 1-5 nM. The range for between-day coefficients of variation was 4-18% for all analytes, while intra-day variation was half or less of that. Urinary concentrations of phytoestrogens were adjusted for urinary creatinine concentration and expressed in nanomoles per milligram creatinine units. Urinary creatinine concentrations were measured with a Roche-Cobas MiraPlus chemistry auto analyzer by using a kit from Randox Laboratories (Crumlin, UK) that is based on a kinetic modification of the Jaffé reaction with a LOQ of < 15 μM and mean inter-assay coefficient of variation of 0.8% at 187 μM (21.1 mg/L).

Statistical analysis

The basic demographic characteristics, selected dietary intakes, and lifestyle factors of participants in the current study were compared with participants of the parent cohort by using the one-sample t-test for continuous variables and the chi-square test for categorical variables after adjustment for age at study enrollment. Symmetrical distributions were obtained after log transformation of the skewed distributions of the original phytoestrogen concentrations. The distributions of urinary phytoestrogen excretions were expressed as selected percentiles including the 1st, 5th, 10th, 25th, 50th, 75th, 90th, 95th, and 100th, geometric means, and 95% confidence intervals (95% CI). Correlations between each type of phytoestrogen and selected lifestyle factors and dietary intakes were estimated by Spearman correlation coefficients. All data were analyzed by using SAS (version 9.2; SAS Institute, Inc., Cary, NC) and two-sided $P < 0.05$ was considered statistically significant.

Results

The 2,165 women who participated in the current study were slightly older and had a lower BMI, higher waist-to-hip ratio (WHR), and higher total energy intake compared with women in the parent cohort, reflecting the age distributions of the original, nested case-control studies of breast cancer and coronary heart disease. There was little difference between the 2,165 women and the entire SWHS cohort in education, income, current smoking, current alcohol consumption, tea consumption, or selected nutritional factors (intakes of fat, fruits and vegetables, soy, soy protein, and soy isoflavones) at baseline (Table 1).

Table 2 presents selected percentiles and geometric means (95% CI) of urinary phytoestrogen excretions in spot urine samples for the participants of the current study and for U.S. women (n=1,081) who were aged 40 to 70 years and participated in the National Health and Nutrition Examination Survey (NHANES 1999-2000, 2001-2002, and 2003-2004). In our population, median levels of urinary phytoestrogen excretions were as follows: total isoflavonoids, 17.13; daidzein, 5.57; genistein, 2.41; glycitein, 0.94; O-DMA, 1.52; dihydrodaidzein, 0.81; dihydrogenistein, 0.19; equol, 0.11; enterodiol, 0.30; and enterolactone, 1.18. The levels for Chinese women in our study were 2 (enterodiol) to 126 (O-DMA) times higher than levels among the U.S. women. However, the level of urinary enterolactone excretion was similar between Chinese and U.S. women.

Urinary isoflavanoid excretion was higher among older women and women who regularly participated in exercise (Table 3). The urinary excretion levels of total isoflavonoids, daidzein, genistein, and glycitein were positively correlated with soy food intake ($0.20 \leq r \leq 0.22$), but weakly and inversely related to fruit intake ($-0.07 \leq r \leq -0.06$); in addition, genistein was inversely associated with total vegetable intake (r = -0.05). Urinary excretion of isoflavone metabolites, including O-DMA, dihydrodaidzein, and dihydrogenistein, were weakly associated with soy food intake ($0.09 \leq r \leq 0.11$), and O-DMA was inversely related to fruit intake (r = -0.05). Urinary enterolactone excretion was weakly related to fat intake (r = 0.07). Urinary isoflavonoid excretion was unrelated to total energy, fat, and protein intakes (Table 4).

Table 5 shows that urinary isoflavanoid excretion was associated with regular exercise ($0.04 \leq r \leq 0.07$). Urinary excretions of total isoflavonoids and daidzein were weakly related to vitamin supplement use (r = 0.04 and r = 0.05), and daidzein was additionally related to smoking (r = 0.04). Urinary excretions of dihydrodaidzein, dihydrogenistein, equol, enterodiol, and enterolactone were inversely related to BMI ($-0.08 \leq r \leq -0.05$). Urinary equol and enterodiol excretions were weakly related to menopausal status (r = -0.05 and r = 0.07), and enterolactone was correlated with tea consumption (r = 0.06).

In addition, urinary excretions of isoflavones, daidzein, genistein, and glycitein were significantly associated with the average dietary intakes of isoflavones, daidzein, genistein, and glycitein from the baseline FFQ and the 1st follow-up FFQ in our study ($0.13 \leq r \leq 0.19$).

Discussion

In this large cross-sectional analysis of 2,165 middle-aged and elderly Chinese women, we found that urinary levels of daidzein, genistein, and glycitein correlated well with soy food intake and several other lifestyle factors. The levels of urinary isoflavones and enterodiol among women in our study were much higher than among U.S. women of comparable age, while the level of enterolactone was similar between Chinese and US women.

Soy and its products are the richest food sources of isoflavones with a ratio of daidzein, genistein, and glycitein of roughly 1:1:0.1 [18]. After being absorbed into the blood, these compounds are mainly excreted in urine. Our results are in agreement with previous studies that have found that high concentrations of isoflavones in urine were positively associated with the consumption of soy products [19-21]. Our data add further support to previous findings that isoflavones measured in a spot urine sample can serve as biomarkers for soy consumption in epidemiologic studies [22].

Of the nine phytoestrogens we measured, the urinary daidzein level was the highest and concentrations of isoflavones were much higher than lignans, which is consistent with the typical Asian diet, in which soy products, rather than whole grains and cereals, contribute the bulk of phytoestrogens. The phytoestrogen levels of Chinese women in our study were higher than the average levels found among all women included in a recent study conducted in several Asian countries, including Vietnam, Cambodia, India, and Japan [23]. However, levels of daidzein, genistein, and equol were 1.5- to 2.2-fold lower than levels reported for Vietnamese (Hanoi) and Japanese women. The isoflavonoid levels of Chinese women in our study were far higher than those of US women, consistent with the fact that soy food consumption is much higher among Chinese women than US women (the average of isoflavone intakes among Chinese women and U.S. women were 47 mg/d and 1- 6 mg/d, respectively) [4, 24]. Our previ-

Table 2. Geometric means and 95% confidence intervals (95% CI) and selected percentiles of urinary phytoestrogen excretion (in nmol/mg creatinine) and comparison with NHANES* participants

Phytoestrogens	Geometric mean (95%CI)	Selected percentiles									Ratios† (SWHS/ NHANES)
		1st	5th	10th	25th	50th	75th	90th	95th	100th	
Total isoflavonoids‡											
SWHS	15.25 (14.42-16.12)	0.34	1.45	2.61	7.04	17.13	38.20	72.07	105.59	669.95	39.84
NHANES	0.52 (0.47-0.57)	0.0239	0.0547	0.0857	0.17	0.43	1.29	3.87	8.58	218.48	
Daidzein											
SWHS	4.99 (4.71-5.30)	0.14	0.46	0.78	2.05	5.57	13.74	27.02	38.99	212.86	23.21
NHANES	0.23 (0.21-0.26)	0.0045	0.0139	0.0274	0.0674	0.24	0.70	2.12	4.23	175.56	
Genistein											
SWHS	2.07 (1.93-2.22)	0.0243	0.13	0.25	0.79	2.41	6.54	14.77	23.07	182.80	26.05
NHANES	0.10 (0.09-0.11)	0.0007	0.0053	0.0126	0.0323	0.0925	0.32	1.01	1.98	41.61	
Glycitein											
SWHS	0.81 (0.76-0.87)	0.0089	0.0516	0.0983	0.32	0.94	2.53	5.35	8.22	91.29	NA
NHANES	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	
O-DMA											
SWHS	0.82 (0.73-0.93)	0.0001	0.0171	0.0496	0.320	1.52	4.4700	9.84	15.17	135.97	126.67
NHANES	0.01 (0.01-0.02)	0.0001	0.0002	0.0005	0.002	0.012	0.0774	0.37	1.20	41.98	
Dihydrodaidzein											
SWHS	0.58 (0.52-0.65)	<0.0001	0.0165	0.0381	0.15	0.81	3.29	7.52	13.18	90.66	NA
NHANES	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	
Dihydrogenistein											
SWHS	0.18 (0.16-0.20)	<0.0001	0.0075	0.0148	0.0546	0.19	0.82	3.43	7.49	162.34	NA
NHANES	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	
Equol											
SWHS	0.06 (0.05-0.08)	<0.0001	<0.0001	0.0028	0.0202	0.11	1.5200	8.56	18.21	216.00	3.94
NHANES	0.03 (0.02-0.03)	<0.0001	0.0036	0.0058	0.0116	0.0279	0.0600	0.12	0.21	35.68	
Enterodiol											
SWHS	0.26 (0.24-0.29)	0.0026	0.0117	0.0345	0.11	0.30	0.78	1.92	3.53	41.30	2.00
NHANES	0.12 (0.11-0.13)	0.0006	0.0065	0.0169	0.0574	0.15	0.34	0.63	1.06	33.91	
Enterolactone											
SWHS	0.34 (0.27-0.42)	<0.0001	0.0000	0.0109	0.17	1.18	4.19	10.87	17.49	88.54	0.98
NHANES	0.90 (0.81-1.00)	0.0042	0.0255	0.0754	0.38	1.21	2.96	5.75	8.97	143.68	

*Derived from female participants of NHANES 1999-2000, 2001-2002, and 2003-2004 aged 40 to 70 years, data downloaded from http://www.cdc.gov/nchs/nhanes/nhanes_questionnaires.htm; †The ratio of median levels between SWHS (*n*=2,165) and NHANES (*n*=1,081); ‡Total isoflavonoids includes daidzein, genistein, glycitein, O-DMA, dihydrodaidzein, dihydrogenistein, and equol for the SWHS and daidzein, genistein, O-DMA, and equol for NHANES.

Table 3. Urinary excretion of phytoestrogens (geometric mean, nmol/mg creatinine) by selected demographic factors

Characteristics	Total isoflavonoids‡	Daidzein	Genistein	Glycitein	O-DMA	Dihydrodaidzein	Dihydrogenistein	Equol	Enterodiol	Enterolactone
Age at study enrollment (years)										
40-49	12.16	3.68	1.53	0.60	0.63	0.51	0.12	0.07	0.28	0.57
50-59	15.75	5.38	2.30	0.82	0.97	0.47	0.21	0.03	0.24	0.20
≥ 60	17.79	6.06	2.47	1.02	0.92	0.72	0.22	0.09	0.27	0.26
P value†	<.0001	<.0001	<.0001	<.0001	0.0004	0.0002	<.0001	0.0001	0.7708	0.0001
Income, whole family (yuan)*										
< 10,000	15.20	4.79	2.08	0.81	0.92	0.66	0.18	0.08	0.27	0.41
10,000 – 19,999	15.58	5.11	2.09	0.84	0.72	0.60	0.06	0.06	0.27	0.35
20,000 – 29,999	14.53	4.91	1.98	0.78	0.87	0.55	0.07	0.07	0.23	0.29
≥ 30,000	15.64	5.11	2.16	0.82	0.89	0.52	0.05	0.05	0.29	0.32
P value†	0.7536	0.7547	0.5745	0.5364	0.8220	0.8122	0.4156	0.8194	0.8768	0.9234
Education*										
≤ Elementary school	16.62	5.34	2.43	0.92	0.93	0.71	0.19	0.10	0.27	0.24
Middle school	14.26	4.67	1.91	0.77	0.72	0.50	0.17	0.07	0.24	0.37
High school	15.65	5.20	2.06	0.80	0.79	0.52	0.19	0.04	0.28	0.36
≥ College	13.84	4.63	1.72	0.69	0.91	0.67	0.17	0.04	0.30	0.41
P value†	0.2100	0.4021	0.0645	0.2319	0.5050	0.2518	0.5182	0.0828	0.4452	0.8921
Exercise participation*										
No	14.24	4.75	1.96	0.77	0.75	0.57	0.17	0.06	0.26	0.37
Yes	16.89	5.39	2.26	0.88	0.94	0.61	0.20	0.07	0.28	0.28
P value†	0.0045	0.0203	0.0389	0.0476	0.0028	0.0351	0.0465	0.8226	0.2024	0.9812

*Adjusted for age at study enrollment; †ANOVA on ranks adjusted for age at study enrollment; ‡Total isoflavonoids including daidzein, genistein, glycitein, O-DMA, dihydrodaidzein, dihydrogenistein, and equol.

Table 4. Spearman correlation coefficients (95% confidence intervals) between urinary phytoestrogen excretion and dietary intakes

Characteristics	Total isoflavonoids*	Daidzein	Genistein	Glycitein	O-DMA	Dihydrodaidzein	Dihydrogenistein	Equol	Enterodiol	Enterolactone
Total energy intake†	-0.04 (-0.08,0.00)	-0.03 (-0.08,0.01)	-0.03 (-0.08,0.01)	-0.03 (-0.07,0.02)	-0.03 (-0.07,0.02)	-0.02 (-0.06,0.02)	-0.02 (-0.06,0.02)	-0.02 (-0.07,0.03)	-0.02 (-0.07,0.03)	-0.03 (-0.08,0.01)
Protein‡	0.03 (-0.01,0.07)	0.03 (-0.01,0.07)	0.01 (-0.03,0.05)	0.03 (-0.01,0.07)	0.01 (-0.03,0.05)	0.01 (-0.03,0.05)	0.00 (-0.04,0.04)	0.01 (-0.03,0.06)	0.00 (-0.05,0.05)	0.04 (-0.01,0.09)
Fats‡	0.01 (-0.03,0.05)	0.02 (-0.02,0.06)	0.00 (-0.05,0.04)	0.00 (-0.04,0.04)	0.00 (-0.04,0.04)	0.01 (-0.03,0.06)	-0.01 (-0.05,0.04)	0.01 (-0.03,0.06)	0.03 (-0.02,0.07)	0.07 (0.02,0.11) §
Vegetables‡	-0.03 (-0.08,0.01)	-0.03 (-0.07,0.01)	-0.05 (-0.09,-0.01)§	-0.04 (-0.08,0.00)	0.00 (-0.04,0.04)	-0.04 (-0.08,0.00)	-0.04 (-0.08,0.01)	-0.03 (-0.07,0.01)	0.01 (-0.03,0.06)	-0.01 (-0.06,0.03)
Fruits‡	-0.07 (-0.11,-0.03)§	-0.06 (-0.11,-0.02)§	-0.06 (-0.11,-0.02)§	-0.06 (-0.10,-0.02)§	-0.05 (-0.09,-0.01)§	-0.03 (-0.07,0.01)	-0.04 (-0.08,0.01)	-0.02 (-0.07,0.02)	0.04 (-0.01,0.08)	0.03 (-0.02,0.08)
Total dietary soy ‡	0.21 (0.17,0.25)§	0.22 (0.18,0.26)§	0.20 (0.16,0.24)§	0.20 (0.16,0.24)§	0.11 (0.07,0.15)§	0.10 (0.06,0.14)§	0.09 (0.05,0.14)§	0.04 (0.00,0.08)	0.01 (-0.04,0.06)	0.01 (-0.04,0.06)
Soy Isoflavone intake‡	0.20 (0.16,0.24)§	0.20 (0.16,0.24)§	0.18 (0.14,0.22)§	0.18 (0.14,0.22)§	0.11 (0.07,0.15)§	0.11 (0.07,0.15)§	0.10 (0.06,0.14) §	0.05 (0.01,0.10)§	0.01 (-0.03,0.06)	0.02 (-0.03,0.07)

*Total isoflavonoids including daidzein, genistein, glycitein, O-DMA, dihydrodaidzein, dihydrogenistein, and equol; values in nmol/mg; †Adjusted for age at study enrollment, BMI, and exercise participation; ‡Adjusted for age at study enrollment, BMI, exercise participation, and total energy intake; §*P* < 0.05.

Table 5. Association (Spearman correlation coefficients [95% confidence intervals]) of urinary phytoestrogen excretion and lifestyle factors

Characteristics	Total isoflavonoids*	Daidzein	Genistein	Glycitein	O-DMA	Dihydrodaidzein	Dihydrogenistein	Equol	Enterodiol	Enterolactone
BMI (continuous)†	-0.04 (-0.08,0.01)	-0.02 (-0.06,0.03)	-0.01 (-0.05,0.03)	-0.01 (-0.05,0.03)	-0.01 (-0.05,0.03)	-0.05 (-0.09,-0.01)§	-0.05 (-0.09,-0.01) §	-0.07 (-0.11,-0.03)§	-0.05 (-0.10,0.00)§	-0.08 (-0.13,-0.03)§
WHR (continuous)‡	0.00 (-0.04,0.04)	0.00 (-0.04,0.04)	0.01 (-0.03,0.06)	0.01 (-0.03,0.05)	0.01 (-0.03,0.05)	-0.01 (-0.05,0.04)	0.00 (-0.04,0.05)	0.00 (-0.04,0.04)	-0.02 (-0.07,0.03)	-0.03 (-0.08,0.02)
Menopausal status‡	0.03 (-0.02,0.07)	0.04 (-0.01,0.08)	0.03 (-0.01,0.07)	0.01 (-0.03,0.05)	0.03 (-0.01,0.08)	-0.01 (-0.05,0.04)	0.04 (0.00,0.08)	-0.05 (-0.09,-0.01)§	0.07 (0.02,0.12)§	-0.01 (-0.06,0.04)
Exercise participation (Yes/No)‡	0.06 (0.02,0.11)§	0.05 (0.01,0.10)§	0.05 (0.01,0.09)§	0.04 (0.00,0.09)§	0.07 (0.02,0.11)§	0.05 (0.01,0.09)§	0.05 (0.00,0.09)§	0.00 (-0.04,0.05)	0.03 (-0.02,0.08)	0.00 (-0.05,0.05)
Overall physical activity (MET)‡	0.00 (-0.06,0.07)	0.02 (-0.05,0.09)	0.01 (-0.06,0.08)	-0.01 (-0.08,0.06)	0.00 (-0.07,0.06)	0.02 (-0.04,0.09)	0.00 (-0.06,0.07)	-0.02 (-0.08,0.05)	0.00 (-0.08,0.09)	0.03 (-0.05,0.11)
Current smoking (Yes/No)‡	0.02 (-0.02,0.07)	0.04 (0.00,0.09)§	0.03 (-0.01,0.07)	0.03 (-0.01,0.07)	0.01 (-0.03,0.05)	0.04 (0.00,0.08)	-0.01 (-0.05,0.03)	0.01 (-0.04,0.05)	-0.02 (-0.07,0.03)	-0.01 (-0.06,0.04)
Alcohol consumption (Yes/No)‡	0.00 (-0.05,0.04)	0.00 (-0.04,0.04)	0.00 (-0.04,0.05)	-0.01 (-0.05,0.03)	0.00 (-0.04,0.04)	0.00 (-0.04,0.04)	-0.01 (-0.05,0.03)	-0.03 (-0.07,0.01)	-0.02 (-0.07,0.03)	-0.01 (-0.06,0.03)
Tea consumption (Yes/No)‡	0.02 (-0.02,0.07)	0.03 (-0.01,0.07)	0.00 (-0.04,0.05)	0.04 (-0.01,0.08)	0.01 (-0.03,0.05)	0.01 (-0.03,0.06)	0.00 (-0.04,0.04)	0.04 (0.00,0.08)	0.00 (-0.05,0.05)	0.06 (0.01,0.11) ‡
Vitamin supplement use (Yes/No)‡	0.04 (0.00,0.09)§	0.05 (0.01,0.09)§	0.03 (-0.01,0.07)	0.02 (-0.02,0.06)	0.02 (-0.02,0.06)	0.02 (-0.02,0.06)	0.01 (-0.03,0.06)	-0.02 (-0.06,0.02)	0.01 (-0.04,0.06)	0.00 (-0.05,0.05)

*Total isoflavonoids including daidzein, genistein, glycitein, O-DMA, dihydrodaidzein, dihydrogenistein, and equol; values in nmol/mg; †Adjusted for age at study enrollment; ‡Adjusted for age at study enrollment, and BMI; §*P* < 0.05.

ous studies have indicated that high soy food consumption may reduce the risk of breast cancer, and soy food intake was significantly associated with decreased risk of death and recurrence among women with breast cancer [4, 5, 25]. The inverse association between soy intake and breast cancer risk was found in Asian but not Western populations in a meta-analysis by Wu et al., which suggests that protection against breast cancer may require that women consume levels of soy typically seen in Asian diets [26, 27].

Relationships between isoflavones and intakes of fruits and vegetables in previous studies have been inconsistent. In an analysis of 96 postmenopausal American women, plasma genistein levels were positively related to total servings of fruits and vegetables [28]. Stumpf et al. did not find any change in serum daidzein or genistein concentrations among 85 middle-aged Finnish participants in a dietary intervention study after markedly increasing consumption of vegetables, fruit, and berries [29]. In our study, we found that urinary excretions of total isoflavonoids, daidzein, genistein, glycitein, and O-DMA were weakly and inversely related to fruit intake, and genistein was also inversely associated with vegetable intake among middle-aged and elderly Chinese women. The inverse correlation between isoflavone level and fruit intake may be explained by the fact that soy food consumption is a part of traditional Chinese diet and is very affordable while fruit consumption is part of an affluent lifestyle. The reason for the inverse correlation between genistein and total vegetable intake remains to be investigated.

Urinary isoflavanoid excretion was higher among older women in our study, which is in agreement with previous reports that have suggested that older women may consume more soy-based foods for the purpose of improving their health [28]. In our study population, urinary isoflavonoid levels were also higher among women who engaged in regular exercise, supporting the healthy lifestyle hypothesis. On the other hand, our results could also be due to older women being more likely to adhere to a traditional Chinese dietary pattern.

In accordance with the findings of Chun et al. that flavonoid-rich diets were consumed more frequently among vitamin supplement users [30], we found that urinary excretions of total isoflavonoids and daidzein were positively associated with vitamin supplement use. Chun et al. also found that isoflavone intake was inversely associated with BMI [31]. Although there was no significant relationship between urinary excretion of genistein or daidzein and BMI in our study, we found that urinary excretion of dihydrodaidzein, equol, dihydrogenistein, and the metabolites of daidzein and genistein were inversely related to body mass index in our study. The metabolization of daidzein occurs in the gut through the action of a particular class of bacteria Clostridium sp, Eubacterium ramulus, Bacteroides ovatus, Bifidobacterium breve, etc [8]. The gut bacterial flora in obese individuals is known to differ from that in non-obese individuals. The lack of a direct association between daidzein and BMI could suggest that the inverse association between daidzein metabolites and BMI is a result of obesity. On the other hand, we could not exclude the possibility that daidzein and its metabolites may have a different effect on obesity. More research in this field is needed.

Equol, which possesses a more potent estrogenic activity than its precursor, daidzein, has been proposed as one of the most important components of isoflavones for disease prevention [8, 32, 33]. Not all humans (only 30-60%) possess the gut flora that can produce equol [18]. Besides the composition and activity of the intestinal microbiota, the diet has been suggested to contribute to the ability to harbor equol-producing bacteria [8]. Asians, who are usually habitual soy consumers, seem to have a greater ability to change daidzein to equol than Western populations [34]. In our study, 1,895 women (87.5%) had a urinary daizein level higher than 1 nmol/mg creatinine. Among these women, we estimated the prevalence of equol producers using the recommended ratio of urinary equol:daidzein higher than 0.018 as the cut-point for being an equol producer [35]. We found that 50.3% women in our population were equol producers, which is much higher than 33% reported in a Western population but is comparable to 55.7% reported among a Japanese population in which soy product intake is also high [32, 36]. In our study population, there was a slightly higher percentage of equol producers among pre-menopausal women (53.2%) than post-menopausal women (48.7%).

In our study both enterodiol and enterolactone concentrations were weakly and inversely associated with BMI. Lignans can pass through the preadipocyte cell membrane [37, 38]; therefore, lignan concentrations in obese women may be diluted by rapid transport into cells, resulting in lower urinary excretions [37, 38]. However, this inverse correlation also suggests a true association. Johnsen et al. has reported that high fat intake decreased serum enterolactone levels [39]. In contrast, we found a positive correlation between fat intake and urinary enterolactone excretion.

Tea contains high levels of lignans [40]. In our study, urinary enterolactone concentration was positively related to tea consumption. Enterolactone concentration was higher among younger women, consistent with an earlier report that young, vegetarian women had much higher lignan excretion than their older counterparts [41]. Urinary enterodiol excretion was higher among post-menopausal than among pre-menopausal women in our study.

Because urinary excretion of creatinine depends on muscle mass, which is influenced by body weight, gender, and age, we also estimated the urinary excretions of phytoestrogens applying the suggested body weight- and age-specific creatinine excretion rates (mg-creatinine/kg BW /day ratio [in nmol/mg creatinine] multiplied by body weight) [42]. We found that the levels of excretion of urinary phytoestrogens derived from this method were highly correlated with levels derived without applying weight-age specific creatinine excretion levels ($0.99 \leq r \leq 1.00$). Furthermore, we found that associations between urinary phytoestrogen concentrations and lifestyle factors and dietary intakes remained largely unchanged, with the exception of the association for BMI. The correlation with BMI carries no biological meaning, however, because body weight was the major determinant for both BMI measurement and the adjustment method described above for estimating phytoestogen concentrations.

The cross-sectional study design is the major limitation of this study and makes the interpretation of the underlying mechanisms responsible for the correlations observed in our study difficult to determine. Another limitation is that a single spot urine sample may not capture the overall levels of isoflavones well given the short half-life of phytoestrogens, which is in the range of 3-10 hours [43]. Nevertheless, we have previously shown that, in our study population, urinary isoflavone level from a single spot urine sample was relatively stable across a one-year period of time [44]. Furthermore, we found good correlation between dietary isoflavone intake and urinary excretion level.

In summary, in this analysis of a large group of Chinese women aged 40-70 years, we found that urinary levels of isoflavones are substantially higher among Chinese women than their counterparts in the U.S., while levels of lignans for these two populations were more comparable. Urinary excretion of isoflavones significantly correlated with soy food intake but was inversely associated with fruit intake. Our finding of different correlation patterns for parent isoflavones and metabolites of isoflavones with BMI calls for further study in order to understand the nature of this association and to investigate the full spectrum of the health effects related to dietary phytoestrogen intake.

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