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Urinary Phytoestrogens and Relationship to Menstrual Cycle Length and Variability Among Healthy, Eumenorrheic Women

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Context: Phytoestrogens may influence fecundability, although biological mechanisms remain elusive. Since it is hypothesized that phytoestrogens may act through influencing hormone levels, we investigated associations between phytoestrogens and menstrual cycle length, a proxy for the hormonal milieu, in healthy women attempting pregnancy.

Design: A population-based prospective cohort of 326 women ages 18 to 40 with self-reported cycles of 21 to 42 days were followed until pregnancy or for 12 months of attempting pregnancy.

Methods: Urinary genistein, daidzein, O-desmethylangolensin, equol, enterodiol, and enterolactone were measured upon enrollment. Cycle length was determined from fertility monitors and daily journals. Linear mixed models assessed associations with continuous cycle length and were weighted by the inverse number of observed cycles. Logistic regression models assessed menstrual regularity (standard deviation > 75th vs ≤ 75th percentile). Models were adjusted for age, body mass index, race, creatinine, exercise, supplements, lipids, lead, cadmium, cotinine, parity, alcohol, and other phytoestrogens.

Results: Individual phytoestrogens were not associated with cycle length, although total phytoestrogens were associated with shorter cycles (−0.042 days; 95% confidence interval [CI], −0.080 to −0.003, per 10% increase). Each 1 nmol/L increase in enterolactone (odds ratio [OR] 0.88; 95% CI, 0.79-0.97) and total lignans (OR 0.85; 95% CI, 0.76-0.95) was associated with reduced irregularity, and each 1 nmol/L increase in genistein with irregularity (OR 1.19; 95% CI, 1.02-1.38).

Conclusion: Phytoestrogens were not meaningfully associated with cycle length but may be associated with menstrual regularity, among women with self-reported regular cycles. These results highlight differences between isoflavones and lignans and are reassuring for women attempting pregnancy.

Key Words: phytoestrogens, lignans, menstrual cycle, menstrual regularity

Abbreviations: Cyp19, cytochrome P450 19 aromatase; CI, confidence interval; ERβ, estrogen receptor β; HSD, 17β-hydroxysteroid dehydrogenase; IQR, interquartile range; LIFE study, Longitudinal Investigation of Fertility and the Environment study; NHANES, National Health and Nutrition Examination Survey; O-DMA, O-desmethylangolensin; OR, odds ratio; SHBG, sex hormone–binding globulin.

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1. Introduction

Phytoestrogens are plant-derived compounds abundant in soybeans, whole grains, cruciferous vegetables, flaxseed, and berries. Both classes of phytoestrogens, the isoflavones and the lignans, are structurally and functionally similar to mammalian estrogens, so they may have reproductive health implications [1]. Although precise mechanisms are not fully understood, the estrogenic and anti-estrogenic properties of phytoestrogens—specifically, the preferential binding to estrogen receptor β (ERβ) [2]—suggest the potential of phytoestrogens to induce downstream hormonal changes [3, 4]. Furthermore, dietary isoflavones have been associated with higher sex hormone–binding globulin (SHBG) levels in some studies [5, 6].

Whether phytoestrogen intake and observed alterations in the hormonal milieu are sufficient to influence subsequent reproductive events is generally unknown. Prospective data indicate that urinary lignan concentrations are positively associated with fecundability [7], perhaps through effects on menstrual cycle length and function. Menstrual cycle function is suggested to be a sentinel of fecundity, irrespective of pregnancy intentions [8, 9], and short menstrual cycles have been linked to a longer time to pregnancy, ranging from 11% to 36% [10–12]. Understanding the relationship between phytoestrogens and cycle length may elucidate mechanisms that underlie previous findings linking higher urinary lignan concentrations with shorter time to pregnancy [7].

To date, studies of phytoestrogens and menstrual cycle characteristics have been limited to phytoestrogen intake from supplements, and data are mixed [13, 14]. Importantly, no prior studies have evaluated menstrual cycle length with regard to urinary phytoestrogens, which captures combined exposure via diet and supplementation. Therefore, the objective of this study was to investigate the relationship between urinary phytoestrogens and menstrual cycle length and regularity among women in the Longitudinal Investigation of Fertility and the Environment (LIFE) Study, a large population-based cohort with phytoestrogen concentrations characteristic of the US population.

2. Participants and Methods

A. Study Population and Data Collection

The LIFE study was designed to assess relationships between environmental influences, including endocrine-disrupting chemicals, on human fecundity and fertility; details regarding study design have been published elsewhere [15]. In brief, 501 couples discontinuing contraception for the purposes of becoming pregnant were recruited from Texas and Michigan from 2005 to 2009, using a prospective cohort design. The study included English- or Spanish-speaking couples whose female partners were aged 18 to 40 years, with self-reported menstrual cycles from 21 to 42 days in length, no history of hormonal contraception injections in the past year, discontinuation of oral contraception usage less than 2 months prior to enrollment, and no sterilization procedures or physician-diagnosed infertility. Women were followed for up to 12 months of trying to conceive or until pregnant.

Among the 501 women enrolled in the LIFE study, 157 women did not have complete cycle length information available because they either became pregnant during (n = 105) or before (n = 45) the first full menstrual cycle in which they were enrolled, or they withdrew from the study during their first cycle (n = 7). Complete menstrual cycle length data were available for 344 women (69% of women enrolled), and 326 of these had urinary phytoestrogen measurements available (95%). Menstrual cycles until the first study pregnancy were included in this analysis (1548 observed menstrual cycles contributed to the analysis during 12 months of attempting pregnancy); conception cycles were excluded from the analysis (n = 209 cycles excluded).

Upon enrollment, in-person interviews were conducted to ascertain health, demographic, and reproductive histories as well as physical activity and supplement use, followed by standardized anthropometric assessment, including the measurement of height and weight

to calculate body mass index (BMI, calculated as the weight in kilograms divided by height in meters squared). Daily journals were completed to capture lifestyle behaviors relevant to fecundity and sexual intercourse, as well as menstruation and pregnancy test results. Women were provided with and instructed in the use of the Clearblue Easy fertility monitors, commencing on cycle day 6 for tracking daily concentrations of estrone-3-glucoronide and luteinizing hormone. Women also used the digital Clearblue Easy home pregnancy test upon enrollment to ensure the absence of pregnancy at study start and on the expected day of menses for each cycle. During the baseline interview, nonfasting urine, blood, and serum samples were obtained and frozen at −20°C or colder until shipment on dry ice to the Centers for Disease Control and Prevention (CDC) for analysis. Full human participants approval was granted from all collaborating institutions before obtaining informed consent from all couples.

B. Phytoestrogen Measurement

Phytoestrogens were measured in urine samples collected at baseline for women with sufficient volume for analysis (n = 471; 94%). Measured phytoestrogens included the isoflavones genistein, daidzein, O-desmethylangolensin (O-DMA), and equol (the latter 2 being metabolites of daidzein) [16], and the lignans enterodiol and enterolactone. Phytoestrogens were analyzed in a 2 mL urine sample with added isotopically labeled internal standards to correct for potential procedural losses and to ensure accurate quantitation. Phytoestrogens were measured using high performance liquid chromatography electrospray tandem mass spectrometry method (interassay coefficient of variation was < 6%, based on quality control data acquired during the analysis of the study samples) [17]. Urinary creatinine was quantified using a Roche/Hitachi Model 912 clinical analyzer and the Creatinine Plus Assay, which involves the combined use of creatininase, creatinase, and sarcosine oxidase.

C. Menstrual Cycle Length Assessment

Using daily journals and information from the fertility monitors, a menstrual cycle was defined as the interval or number of days between the first day of observed bleeding followed by at least 2 days of increasing bleeding intensity to the first day of bleeding in the next cycle. Cycle length was grouped into 3 categories: < 26 days, 26 to 35 days, and > 35 days.

We calculated the average cycle length and standard deviation for each woman and used this variability as a marker of menstrual irregularity [18]. We categorized the standard deviations as less variable (or "regular") if the standard deviation was ≤ 75th percentile (4.25 days), and more variable ("irregular") if the standard deviation was > 75th percentile. This analysis was restricted to the 262 women with at least 2 menstrual cycles recorded.

D. Covariate Assessment

Total serum lipids (ng/g of serum) were quantified using commercially available enzymatic methods [19] and established calculation methods and individual components [20]. Cotinine concentration (ng/mL) was quantified in 1 mL of serum using liquid chromatography-isotope dilution tandem mass-spectrometry [21]. Blood lead and cadmium were analyzed at the CDC's National Center for Environmental Health using inductively coupled plasma mass spectrometry. Machine-observed concentrations of urinary and serum analytes below the limits of detection were not removed or substituted [21–24]. All analyses were subjected to standard quality assurance procedures, and all reported results were from samples found to be in control by standard statistical methods.

E. Statistical Analysis

We characterized demographic factors and reproductive history by category of women who had average cycle lengths of < 26 days, 26 to 35 days, and > 35 days, and compared

them using ANOVA or Chi-square test as appropriate among the 326 women with cycle length and phytoestrogen data available. We also assessed demographic characteristics by quartiles of total urinary phytoestrogens. Additionally, the demographic characteristics of women with missing data were compared with those for whom we had complete data. Concentrations of urinary phytoestrogens were log-transformed during all analyses. Concentrations of genistein, daidzein, O-DMA, and equol were summed to determine total isoflavones; enterodiol and enterolactone were summed to determine total lignans; and all 6 phytoestrogens were summed to determine total phytoestrogens. Medians and interquartile ranges (IQR) of each phytoestrogen, as well as the total isoflavones, total lignans, and total phytoestrogens, were calculated and compared by cycle length category, using the Wilcoxon-Mann-Whitney test. Geometric mean phytoestrogen concentrations were compared with data available for women of reproductive age (ie, 20 to 39 years) in the 2005–2006 National Health and Nutrition Examination Survey (NHANES) to assess the comparability of the concentrations observed in our study with those of a nationally representative population [25].

Among women with cycle length data available, we imputed missing values for phytoestrogen concentrations (n = 30, 6.0%) and covariates (BMI, 0.2%; race, 0.6%; urinary creatinine, 9.6%; serum lipids, 2.6%; serum cotinine, 2.4%; blood lead, 9.4%; blood cadmium, 9.4%; and frequency of alcohol consumption, 0.4%). A multiple imputation model with 20 imputations and the fully conditional specification method was used.

We used 2 different approaches to investigate associations between urinary phytoestrogen concentrations and menstrual cycle length. First, repeated measures multinomial logistic regression models were used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for the associations between phytoestrogens and cycle lengths of < 26 days or > 35 days, with cycle lengths of 26 to 35 days serving as the referent category. Models were weighted by the inverse of the number of contributed cycles. All cycles for each participant with cycle length information available, excluding conception cycles, were included in the analysis. Second, linear mixed models were used to analyze continuous cycle length, again accounting for repeated measures and weighted for the number of contributed cycles. In models of continuous cycle length, β estimates were divided by 10 to account for use of a log-transformed exposure and non–log-transformed outcome, and they are interpreted as a change in cycle length (in days), per 10% increase in phytoestrogen concentrations. Models were adjusted for age (years), BMI (kg/m^2), race (non-Hispanic white, non-Hispanic black, Hispanic, or other), urinary creatinine (continuous, mg/dL), exercise (followed a regular vigorous exercise program in the past year, yes or no), supplement use (fish oil, echinacea, ginkgo biloba, kava kava, St. John's wort, protein shakes, steroids, or creatine; yes or no), total serum lipids (mg/dL), serum cotinine (ng/mL), parity conditional on gravidity, alcohol consumption (frequency in the past year), blood cadmium (μg/L), blood lead (μg/dL), and sum of the remaining individual phytoestrogens. We adjusted models for blood lead and cadmium concentrations as women could be concurrently exposed through ingestion of food items with high phytoestrogens from contaminated soil and these metals could also impact the menstrual cycle [26, 27].

To estimate associations between urinary phytoestrogens and menstrual regularity, we used logistic regression models for cycle length variability > 75th percentile comparing with ≤ 75th percentile (ie, regularly cycling women) serving as the referent category. Regularity was not modeled continuously due to the nonnormal distribution of standard deviations. These models were adjusted for the covariates previously indicated.

We conducted 2 sensitivity analyses: 1) we assessed associations between urinary phytoestrogen concentrations and average cycle length, and 2) we assessed potential nonlinearity of associations between phytoestrogen levels and cycle length using restricted cubic splines by comparing the model with and without the cubic spline terms in addition to the linear terms in the model [28]. SAS version 9.4 (SAS Institute, Cary, NC) was used for all statistical analysis.

3. Results

For the 326 women included in the analysis, median (25th percentile, 75th percentile) concentrations of total baseline urinary phytoestrogens were 2114 (868, 4731) nmol/L, 540 (191, 2068) nmol/L for total isoflavones, and 967 (311, 2590) nmol/L for total lignans. The average cycle length was 29.6 (standard deviation 6.7) days. Eighty-one (25%) women had an average cycle length < 26 days and 421 (29%) cycles were < 26 days. Thirty-two (10%) women had an average cycle length > 35 days and 141 (10%) cycles were > 35 days.

Women with cycles < 26 days tended to be older, and women with cycles > 35 days tended to have a higher BMI than women with cycles of 26 to 35 days (Table 1). Self-reported current smokers tended to have cycles < 26 or > 35 days. Of note, women with average cycle length < 26 days and 26 to 35 days were more likely to report at baseline that they experienced menstrual regularity, while women with cycles > 35 days were more likely to report irregularity. Neither average cycle length nor total phytoestrogen levels varied by self-reported supplement usage. Average menstrual cycle length did not otherwise vary between races, level of education, physical activity, parity, and other baseline demographic characteristics. Women with the highest quartile of total urinary phytoestrogens were nonsmokers and more likely to exercise. Self-reported menstrual regularity did not vary between quartiles of total phytoestrogens. The 326 women included in the analysis have similar characteristics to those with missing data (data not shown).

Urinary concentrations of isoflavones, except equol, were higher among the LIFE participants compared with those measured in the NHANES survey during the same time period, whereas levels of lignan were lower among LIFE participants. The comparisons, in terms of geometric mean in nmol/L (95% CI) are genistein: LIFE 134.5 (107.3, 161.7) vs NHANES 90.8 (73.9, 107.7), $P < 0.01$; daidzein: LIFE 296.7 (237.3, 356.1) vs NHANES 223.8 (181.8, 265.8), $P < 0.05$; O-DMA: LIFE 22.5 (16.6, 28.3) vs NHANES 12.9 (9.7, 16.0), $P < 0.01$; equol: LIFE 26.0 (22.2, 29.7) vs NHANES 31.9 (26.9, 37.0), $P = 0.07$; enterodiol: LIFE 93.4 (76.2, 110.6) vs NHANES 126.1 (103.8, 148.3), $P < 0.05$; and enterolactone: LIFE 583.4 (476.2, 690.5) vs NHANES 922.0 (757.0, 1086.9), $P < 0.001$.

There were no significant differences in the distribution of urinary phytoestrogen concentrations by cycle length category (Table 2). Of the isoflavones, genistein and daidzein were most abundant, and of the lignans, enterolactone was most abundant. Restricted cubic splines did not indicate a significant nonlinear relationship between urinary phytoestrogens and cycle length.

In multinomial logistic regression models based on all cycles for each woman, urinary phytoestrogen concentrations were not associated with cycles of < 26 days or >35 days compared with cycles of 26 to 35 days, in either unadjusted nor adjusted models (Table 3). When cycle length was modeled as a continuous outcome, total phytoestrogens were associated with slightly shorter cycle length both in unadjusted (−0.043 days shorter; 95% CI, −0.080 to −0.007) and adjusted models (−0.042 days shorter; 95% CI, −0.080 to −0.003), per 10% increase in total phytoestrogens. Some individual phytoestrogens (equol and enterolactone), as well as total lignans, showed associations with shorter menstrual cycles in the unadjusted models, but the associations were attenuated and not significant after adjusting for potential confounders. Similar results were observed in models of average cycle length (Table 4).

The mean difference between the longest and shortest cycle was 27.8 days among women in the highest quartile of menstrual variability (standard deviation > 75th percentile), whereas the mean difference was 4.8 days among women in the “regular” group (standard deviation ≤ 75th percentile). In adjusted models of cycle variability, women with higher urinary concentrations of enterolactone (OR 0.88; 95% CI, 0.79-0.97, per 1 nmol/L increase) and total lignans (OR 0.85; 95% CI, 0.76-0.95, per 1 nmol/L increase) were less likely to have highly variable cycles (standard deviation > 75th percentile versus ≤ 75th percentile) (Table 5). On the other hand, higher concentrations of genistein were associated with increased menstrual variability or irregularity (OR 1.19; 95% CI, 1.02-1.38, per 1 nmol/L increase).

Table 1. Demographic Characteristics of Study Participants by Categories of Average Menstrual Cycle Length: the LIFE Study, 2005–2009

		Average Menstrual Cycle Length					Total Urinary Phytoestrogens		
		Included Cohort	<26 days	≥26 and ≤35 days	>35 days	*P*	Q1	Q4	*P*
n		326	81	213	32		86	80	
Age, y (mean ± SD)		31 ± 4	32 ± 4	30 ± 4	29 ± 5	0.0006	31 ± 4	31 ± 4	0.87
BMI, kg/m^2 (mean ± SD)		28 ± 7	28 ± 7	27 ± 7	31 ± 11	0.03	28 ± 7	27 ± 7	0.45
Race/ethnicity, n (%)	Non-Hispanic white	249 (77)	64 (79)	163 (77)	22 (69)	0.31	63 (74)	62 (78)	0.97
	Non-Hispanic black	17 (5)	5 (6)	12 (6)	0 (0)		5 (6)	5 (6)	
	Hispanic	37 (11)	8 (10)	22 (10)	7 (22)		11 (13)	8 (10)	
	Other	21 (6)	3 (4)	15 (7)	3 (9)		6 (7)	5 (6)	
Current smoker, n (%)	No	290 (89)	67 (83)	195 (92)	28 (88)	0.09	69 (80)	71 (89)	0.01
	Yes	36 (11)	14 (17)	18 (8)	4 (13)		17 (20)	9 (11)	
Followed a vigorous exercise program in the last 12 mos, n (%)	No	193 (59)	46 (57)	132 (62)	15 (47)	0.24	59 (69)	43 (54)	0.05
	Yes	133 (41)	35 (43)	81 (38)	17 (53)		27 (31)	37 (46)	
Education, n (%)	<High school/equivalent	18 (6)	3 (4)	12 (6)	3 (9)	0.80	7 (8)	2 (3)	0.23
	Some college or technical school	67 (21)	18 (22)	42 (20)	7 (22)		22 (26)	14 (18)	
	College graduate or higher	238 (74)	59 (73)	157 (74)	22 (69)		56 (66)	63 (80)	
Household income, n (%)	<$29 999	16 (5)	4 (5)	7 (3)	5 (16)	0.14	4 (5)	4 (5)	0.96
	$30 000 - $49 999	41 (13)	8 (10)	29 (14)	4 (13)		14 (17)	9 (12)	
	$50 000 - $69 999	46 (14)	11 (14)	30 (14)	5 (16)		12 (14)	9 (12)	
	≥$70 000	215 (68)	56 (69)	141 (66)	18 (56)		53 (64)	55 (71)	
Number of previous live births, n (%)	0	170 (52)	37 (46)	112 (53)	21 (66)	0.42	49 (57)	38 (48)	0.18
	1	106 (33)	31 (38)	68 (32)	7 (22)		20 (23)	29 (36)	
	≥2	50 (15)	13 (16)	33 (15)	4 (13)		17 (20)	13 (16)	
Self-reported menstrual regularity, n (%)	Regular	276 (85)	72 (89)	185 (87)	19 (59)	0.0009	71 (83)	68 (85)	0.31
	Not regular	23 (7)	4 (5)	12 (6)	7 (22)		9 (10)	4 (5)	
	Varies	27 (8)	5 (6)	16 (8)	6 (19)		6 (7)	8 (10)	
Health insurance, n (%)	Yes	296 (91)	74 (91)	195 (92)	27 (84)	0.33	74 (87)	75 (94)	0.42
	No	28 (9)	6 (7)	17 (8)	5 (16)		11 (13)	5 (6)	
Multivitamin use more than once per week in past 3 mos, n (%)	Yes	209 (65)	54 (67)	131 (62)	24 (75)	0.28	47 (55)	57 (71)	0.18
	No	115 (35)	26 (32)	81 (38)	8 (25)		38 (45)	23 (29)	
Supplement use more than once per week in past 3 mos, n (%)	Yes	123 (38)	33 (41)	80 (38)	10 (31)	0.64	29 (34)	32 (40)	0.84
Total lipids, mg/dL (mean ± SD)		628 ± 132	617 ± 128	626 ± 127	672 ± 172	0.13	639 ± 138	612 ± 129	0.6
Urinary creatinine, mg/dL (mean ± SD)		93 ± 72	93 ± 71	94 ± 75	88 ± 55	0.92	67 ± 55	118 ± 78	<0.0001
Age at menarche, y (mean ± SD)		13 ± 2	13 ± 2	13 ± 2	13 ± 2	0.96	13 ± 2	12 ± 2	0.45

Values are means ± SDs or *n* (%), where noted. *P* values were derived by ANOVA and Fisher's exact test comparing characteristics by cycle length. Abbreviations: BMI, body mass index; mos, months; SD, standard deviation.

Table 2. Distribution of Baseline Urinary Concentrations of Phytoestrogens by Category of Average Menstrual Cycle Length

		<26 days	26–35 days	>35 days	
		Median (IQR)	**Median (IQR)**	**Median (IQR)**	***P***
N		81	213	32	
Urinary isoflavones, *nmol/L*	Genistein	167 (61, 418)	104 (31, 470)	152 (52, 514)	0.38
	Daidzein	340 (123, 947)	242 (71, 1030)	311 (85, 727)	0.68
	O-DMA	21 (3, 100)	22 (4, 132)	31 (2, 105)	0.98
	Equol	23 (10, 63)	23 (11, 52)	17 (10, 48)	0.86
	Total isoflavones	575 (225, 1690)	490 (162, 1960)	638 (254, 1634)	0.68
Urinary lignans, *nmol/L*	Enterodiol	128 (42, 301)	94 (40, 257)	77 (31, 271)	0.45
	Enterolactone	982 (166, 2630)	754 (230, 1973)	521 (149, 2387)	0.58
	Total lignans	1216 (262, 2950)	866 (335, 2474)	647 (237, 2839)	0.49
Total phytoestrogens, *nmol/L*		2486 (1086, 5009)	2046 (802, 4730)	2569 (880, 3985)	0.48

P-values calculated using the Wilcoxon-Mann-Whitney test. Abbreviations: IQR, interquartile range; O-DMA, O-desmethylangolensin.

4. Discussion

Urinary levels of phytoestrogens were not meaningfully associated with prospectively observed menstrual cycle length among healthy women with self-reported regular cycles. Although there was a statistically significant association between total phytoestrogens and shorter menstrual cycle length, this association is likely not clinically relevant, as it is less than 1 day. However, our data do suggest associations between lignans and menstrual regularity, and genistein and irregularity. The associations with lignans and menstrual regularity may provide a potential explanation for previously observed associations between lignans and increased fecundability in this population [7, 29]. As cycle length and regularity can be used as indicators of reproductive health and ovarian function [8, 9], these results may shed light on the link between phytoestrogens and other reproductive health outcomes, highlighting the importance of distinguishing between classes of phytoestrogens.

Our results are largely consistent with prior studies examining the association between phytoestrogens and menstrual cycle characteristics, though, importantly, these prior studies assessed effects of various phytoestrogen supplements. These studies—1 using a lignan supplement [30] and 5 using an isoflavone supplement [13, 14, 31–33]—mostly detected an unchanged cycle length [13, 14, 31, 33], yet 2 studies noted increased luteal [30] or follicular [13] phase length and no changes in overall length. Our study extends the previous work in this area by assessing urinary phytoestrogens among women attempting pregnancy with regular cycles.

To our knowledge, the association between phytoestrogens and menstrual regularity has not previously been assessed, so it is difficult to compare our findings with prior results. Menstrual irregularity has previously been associated with a variety of negative health outcomes, including increased risk for type 2 diabetes mellitus [34] and coronary heart disease [35]. Our findings that genistein was associated with increased irregularity may suggest a deleterious role of isoflavones on reproductive health as well. However, our findings that enterolactone and total lignans were associated with reduced menstrual variability may suggest a beneficial role of lignans. Two previous studies have examined associations between menstrual variability and fecundability, which may also provide context for the relationship between lignans and fecundability. One study found that menstrual variability, as measured by standard deviation greater than 2.3 days, was associated with a reduced fecundability, independent of a woman's age and average cycle length [18]. Another study reported slightly reduced fecundability (fecundability OR 0.93; 95% CI, 0.81-1.06) among women whose cycles never regularized after menarche [11]. We previously found that higher levels of urinary lignans were associated with increased fecundability in this population [7].

Table 3. Associations Between Urinary Log Phytoestrogen Concentrations (nmol/L) and Length of All Menstrual Cycles During Follow-Up

		<26 days				>35 days			Continuous		
		OR	95% CI		26–35 days	OR	95% CI		β[b]	95% CI[b]	
Genistein	Unadjusted	1.01	0.88	1.16	Ref	1.01	0.83	1.21	-0.007	-0.033	0.020
	Adjusted[a]	1.02	0.85	1.22	Ref	1.02	0.71	1.47	0.007	-0.003	0.040
Daidzein	Unadjusted	1.00	0.88	1.15	Ref	0.98	0.81	1.18	-0.018	-0.044	0.009
	Adjusted[a]	0.99	0.83	1.19	Ref	0.97	0.67	1.40	-0.008	-0.041	0.025
O-DMA	Unadjusted	0.98	0.87	1.10	Ref	0.96	0.82	1.13	-0.020	-0.042	0.003
	Adjusted[a]	0.96	0.82	1.11	Ref	1.01	0.75	1.35	0.0005	-0.028	0.027
Equol	Unadjusted	1.03	0.85	1.25	Ref	0.99	0.75	1.30	**-0.044**	**-0.082**	**-0.006**
	Adjusted[a]	1.00	0.77	1.27	Ref	1.00	0.59	1.69	-0.039	-0.086	0.009
Total Isoflavones	Unadjusted	1.01	0.87	1.17	Ref	0.99	0.81	1.21	-0.016	-0.044	0.012
	Adjusted[a]	1.02	0.87	1.20	Ref	0.91	0.66	1.27	-0.016	-0.046	0.013
Enterodiol	Unadjusted	1.00	0.85	1.17	Ref	0.96	0.77	1.19	-0.029	-0.060	0.002
	Adjusted[a]	0.98	0.81	1.19	Ref	1.01	0.69	1.48	-0.013	-0.049	0.022
Enterolactone	Unadjusted	1.02	0.88	1.19	Ref	0.95	0.78	1.16	**-0.034**	**-0.060**	**-0.006**
	Adjusted[a]	1.04	0.87	1.24	Ref	0.96	0.69	1.34	-0.016	-0.046	0.015
Total Lignans	Unadjusted	1.03	0.87	1.21	Ref	0.94	0.75	1.17	**-0.040**	**-0.072**	**-0.009**
	Adjusted[a]	1.03	0.85	1.25	Ref	0.94	0.65	1.35	-0.023	-0.056	0.011
Total Phytoestrogens	Unadjusted	1.04	0.86	1.26	Ref	0.93	0.72	1.21	**-0.043**	**-0.080**	**-0.007**
	Adjusted[a]	1.07	0.87	1.32	Ref	0.85	0.55	1.29	**-0.042**	**-0.080**	**-0.003**

Bold values indicate $P < 0.05$.

Abbreviations: CI, confidence interval; O-DMA, O-desmethylangolensin; OR, odds ratio.

[a]Models adjusted for age, BMI, race, urinary creatinine, exercise (questionnaire), supplement use (yes/no), lipids, lead, cadmium, cotinine, parity conditional on gravidity, frequency of alcohol consumption, and sum of the other phytoestrogens (log).

[b]β and 95% CIs calculated by β/10; interpreted as: the change in cycle length (by day) per 10% increase in phytoestrogen concentration.

Table 4. Associations Between Urinary Phytoestrogens and Average Menstrual Cycle Length

		<26 days				>35 days			Continuous		
		OR	95% CI		26–35 Days	OR	95% CI		β[b]	95% CI[b]	
Genistein	Unadjusted	1.07	0.93	1.23	Ref	1.08	0.88	1.32	-0.006	-0.046	0.033
	Adjusted[a]	1.04	0.86	1.25	Ref	1.12	0.83	1.50	0.006	-0.044	0.055
Daidzein	Unadjusted	1.06	0.92	1.22	Ref	1.03	0.84	1.27	-0.017	-0.057	0.022
	Adjusted[a]	1.02	0.85	1.23	Ref	1.02	0.76	1.37	-0.008	-0.058	0.042
O-DMA	Unadjusted	0.98	0.87	1.10	Ref	0.98	0.83	1.17	-0.020	-0.053	0.014
	Adjusted[a]	0.90	0.78	1.05	Ref	1.00	0.79	1.26	0.005	-0.041	0.041
Equol	Unadjusted	1.06	0.87	1.29	Ref	0.94	0.69	1.28	-0.044	-0.100	0.013
	Adjusted[a]	0.97	0.76	1.24	Ref	0.92	0.59	1.44	-0.039	-0.110	0.030
Total Isoflavones	Unadjusted	1.05	0.90	1.21	Ref	1.05	0.85	1.30	-0.017	-0.058	0.027
	Adjusted[a]	1.03	0.88	1.22	Ref	1.03	0.80	1.33	-0.016	-0.060	0.028
Enterodiol	Unadjusted	1.09	0.93	1.30	Ref	0.98	0.77	1.24	-0.028	-0.075	0.018
	Adjusted[a]	1.08	0.89	1.32	Ref	1.02	0.76	1.36	-0.013	-0.066	0.039
Enterolactone	Unadjusted	1.07	0.92	1.25	Ref	0.96	0.78	1.18	-0.034	-0.077	0.008
	Adjusted[a]	1.07	0.89	1.28	Ref	1.02	0.79	1.31	-0.016	-0.061	0.030
Total Lignans	Unadjusted	1.08	0.91	1.29	Ref	0.95	0.75	1.20	-0.040	-0.087	0.007
	Adjusted[a]	1.08	0.88	1.31	Ref	1.00	0.76	1.32	-0.023	-0.074	0.029
Total Phytoestrogens	Unadjusted	1.11	0.91	1.35	Ref	0.97	0.74	1.29	-0.043	-0.098	0.012
	Adjusted[a]	1.11	0.89	1.37	Ref	0.95	0.69	1.30	-0.041	-0.099	0.016

Abbreviations: CI, confidence interval; O-DMA, O-desmethylangolensin; OR, odds ratio.

[a]Models adjusted for age, BMI, race, urinary creatinine, exercise (questionnaire), supplement use (yes/no), lipids, lead, cadmium, cotinine, parity conditional on gravidity, frequency of alcohol consumption, sum of the other phytoestrogens (log).

[b]β and 95% CIs calculated by β/10; interpreted as: the change in cycle length (by day) per 10% increase in phytoestrogen concentration.

Table 5. Associations Between Urinary Log Phytoestrogen Concentrations (nmol/L) and Prospective Menstrual Regularity (Measured by Standard Deviation)[a]

		SD Above vs Below [ref] the 75th percentile		
		OR	95% CI	
Genistein	Unadjusted	**1.10**	**1.00**	**1.19**
	Adjusted[b]	**1.19**	**1.02**	**1.38**
Daidzein	Unadjusted	1.02	0.93	1.12
	Adjusted[b]	1.07	0.91	1.25
O-DMA	Unadjusted	**0.90**	**0.84**	**0.97**
	Adjusted[b]	0.91	0.82	1.01
Equol	Unadjusted	0.92	0.83	1.03
	Adjusted[b]	0.88	0.75	1.04
Total Isoflavones	Unadjusted	1.05	0.96	1.16
	Adjusted[b]	1.08	0.95	1.23
Enterodiol	Unadjusted	**0.90**	**0.83**	**0.98**
	Adjusted[b]	0.97	0.87	1.09
Enterolactone	Unadjusted	**0.85**	**0.78**	**0.92**
	Adjusted[b]	**0.88**	**0.79**	**0.97**
Total Lignans	Unadjusted	**0.82**	**0.75**	**0.89**
	Adjusted[b]	**0.85**	**0.76**	**0.95**
Total Phytoestrogens	Unadjusted	0.95	0.85	1.07
	Adjusted[b]	1.00	0.87	1.16

Bold values indicate $P < 0.05$.
Abbreviations: CI, confidence interval; O-DMA, O-desmethylangolensin; OR, odds ratio; SD, standard deviation.
[a]Analyses limited to women who contributed at least 2 menstrual cycles.
[b]Models adjusted for age, BMI, race, urinary creatinine, exercise (questionnaire), supplement use (yes/no), lipids, lead, cadmium, cotinine, frequency of alcohol consumption, parity conditional on gravidity, sum of the other phytoestrogens (log).

Thus, it is possible that our findings of enterolactone and total lignans with increased menstrual regularity could help explain these previous findings, though it is also important to note that women needed to have at least 2 cycles in order to contribute to this analysis, which may have excluded the most fecund women.

Although the mechanisms through which phytoestrogens influence menstrual cycle regularity, and in turn fecundability, are complex and poorly understood, there are a variety of proposed explanations. First, various studies have investigated the effects of phytoestrogens on estrogen biosynthesis, with some reporting altered levels of estrogen, progesterone [33], luteinizing hormone, and follicle stimulating hormone following increased phytoestrogen consumption [31, 36]. Decreased endogenous estrogen concentrations after phytoestrogen consumption may be explained by the interference of phytoestrogens with enzymes integral in synthesizing estrogens, specifically cytochrome P450 19 aromatase (Cyp19) and 17β-hydroxysteroid dehydrogenase (HSD) [37]. These enzymes facilitate the interconversion of relatively inactive androstenedione and estrone to testosterone and estrogen [38, 39]. Genistein and daidzein have been found to suppress both expression of *CYP19* mRNA and HSD activity in human luteal granulosa cells [40–42]. Perhaps the observed association of genistein with menstrual irregularity can be explained by its inhibition of enzymes integral to estrogen biosynthesis. Genistein has also been shown to have a high affinity for ERβ [43], and in high concentrations, may compete with endogenous estrogen to bind ERβ, thereby blocking the action of endogenous estrogen [3, 44]. Genistein's affinity for ERβ could perhaps lead to an antiestrogenic effect, consistent with our observation of its association with menstrual irregularity.

Furthermore, phytoestrogens have been observed to affect synthesis of SHBG [36, 45], a protein that regulates free and bound estrogen levels. Enterolactone has been shown to bind

SHBG, increasing SHBG synthesis in liver cancer cells [46] and inhibiting estrogen biosynthesis in vitro [47, 48]. Altered SHBG concentrations would increase plasma concentrations of bound estrogens and decrease concentrations of free estrogens [3, 5]. Thus, SHBG fluctuations induced by phytoestrogens could perhaps explain enterolactone's association with menstrual regularity.

This study has several strengths and limitations. First, urinary measurements of phytoestrogens have been validated as biomarkers of dietary intake, and urinary measurements account for individual variability in metabolism, absorption, and activity of the gut microbiome [49]—variability that may be undetected in dietary intake data alone. Urinary measures also capture intake from supplements, although specific data on dosage and types of supplements containing phytoestrogens used by participants was not available, and overall use of supplements was low in this cohort [50]. Moreover, these measures are reflective of intake from all potential sources of phytoestrogens, which is useful as phytoestrogens are found in many food products and are otherwise difficult to assess using typical dietary assessment. Second, the design of the LIFE study ensured that cycle length and variability were prospectively evaluated [51], eliminating potential for recall bias [52]. Third, as the LIFE study is population-based, our sample population is representative of the general US population seeking pregnancy, although we observed somewhat higher isoflavone, and lower lignan, concentrations compared with women of similar reproductive age in the US general population [25]. Finally, we adjusted the models for serum lead and cadmium levels to account for a potential impact of estrogenic chemicals on the menstrual cycle, which could have been concurrently ingested via phytoestrogen-rich foods from contaminated soil. However, this study is limited in that urinary measurements were taken one time at baseline, so the observed concentrations of phytoestrogens may not be representative of habitual levels of dietary intake and may have fluctuated over time depending on the timing of intake, especially considering the short half-life of phytoestrogens in the body system. We assume that the measurements are reflective of usual intake, although additional research is needed to verify this assumption. Furthermore, direct measurement of reproductive hormones was not available. Lastly, although phytoestrogens are increasingly common in the Western diet as well as in typical Asian diets, it is possible that we observed an association between lignans and regularity, and not with most isoflavones, due to differences in food sources and metabolism.

To conclude, our findings suggest that urinary levels of phytoestrogens are likely not associated with menstrual cycle length, although lignans may be associated with menstrual regularity and genistein with irregularity in healthy, reproductive-aged women with phytoestrogen levels typical of the US population. These results highlight the need to elucidate differences between classes of phytoestrogens and may provide a mechanism for the previously observed association between lignans and increased fecundability in this population. Considering the concern regarding increasing consumption of soybeans and soy-derived products, these results are likely reassuring for women with regular cycles attempting pregnancy.

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