The Journal of Nutrition
Nutritional Epidemiology

Urinary Phytoestrogens Are Associated with Subtle Indicators of Semen Quality among Male Partners of Couples Desiring Pregnancy[1–3]

Sunni L Mumford,[4]* Sungduk Kim,[4] Zhen Chen,[4] Dana Boyd Barr,[5] and Germaine M Buck Louis[4]

[4]Division of Intramural Population Health Research, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, NIH, Department of Health and Human Services, Bethesda, MD; and [5]Rollins School of Public Health, Emory University, Atlanta, GA

Abstract

Background: Phytoestrogens have been associated with subtle hormonal changes, although effects on male fecundity are largely unknown.

Objective: We evaluated associations between male urinary phytoestrogen (isoflavone and lignan) concentrations and semen quality.

Methods: This study was a prospective cohort study of 501 male partners of couples desiring pregnancy and discontinuing contraception. Each participant provided up to 2 semen samples that were analyzed for 35 semen quality endpoints the following day. Linear mixed-effects models were used to estimate associations between baseline urinary phytoestrogen concentrations and semen quality parameters, adjusted for age, body mass index (BMI), research site, and serum lipid and cotinine concentrations.

Results: Most associations between urinary phytoestrogens and semen quality parameters were null. However, select individual phytoestrogens were associated with semen quality parameters, with associations dependent on the class of phytoestrogens and modified by BMI. Specifically, genistein and daidzein were associated with a lower percentage of normal sperm and increased abnormalities in semen morphology, with reduced associations observed as BMI increased ($P < 0.05$) [percentages (95% CIs) of normal morphology by WHO traditional criteria: genistein, main effect: −5.61% (−9.42%, −1.79%); interaction: 0.19% (0.06%, 0.31%) per log unit increase; daidzein, main effect: −5.35% (−9.36%, −1.34%); interaction: 0.18% (0.05%, 0.32%) per log unit increase]. Enterolactone was associated with fewer abnormalities in semen morphometry and morphology and decreased DNA fragmentation, with reduced associations observed as BMI increased ($P < 0.05$) [percentages (95% CIs) of abnormalities in the neck and midpiece: enterolactone, main effect: −3.35% (−6.51%, −0.19%); interaction: 0.11% (0.01%, 0.21%) per log unit increase].

Conclusions: These results suggest that male urinary phytoestrogen concentrations characteristic of the US population may be associated with subtle indicators of male fecundity and semen quality but were not associated with couple fecundity. *J Nutr* 2015;145:2535–41.

Keywords: isoflavones, lignans, phytoestrogens, semen quality, male fertility

Introduction

Phytoestrogens are plant-derived compounds that exert both estrogenic and antiestrogenic effects because of their structural similarity to estrogen. As such, phytoestrogen intake and circulating concentrations have been associated with subtle hormonal changes and may influence male fecundity (1, 2). Although animal studies have shown various reproductive effects, exposure to phytoestrogens and effects on semen quality are less clear because some studies have observed reductions in normal and live sperm count in adult male mice (3), whereas others have observed no effects (4–6). There is a paucity of research in humans regarding these associations, and the findings have been conflicting with some reporting effects on idiopathic male infertility (7) or individual semen quality

[1] Supported by the Intramural Research Program of the NIH, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (contracts N01-HD-3-3355, N01-HD-3-3356, and N01-HD-3-3358).

[2] Author disclosures: SL Mumford, S Kim, Z Chen, DB Barr, and GMB Louis, no conflicts of interest.

* To whom correspondence should be addressed. E-mail: mumfords@mail.nih.gov.

[3] Supplemental Figure 1 and Supplemental Tables 1 and 2 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.

Manuscript received April 10, 2015. Initial review completed May 13, 2015. Revision accepted August 31, 2015.
First published online September 30, 2015; doi:10.3945/jn.115.214973.

parameters (7–10) and others reporting no effects on semen quality among small intervention studies of healthy men (11, 12). Dietary intake of soy has been shown to be associated with reduced sperm count and concentration in studies among infertile men or men seeking fertility treatment (8, 9), but these studies have been limited because it is particularly difficult to assess soy intake using traditional dietary assessment tools. Previous studies have also been limited in that they have typically only evaluated associations between isoflavones, with basic semen analyses focused only on sperm count, motility, and morphology despite existing technology that can analyze DNA fragmentation and additional functional measures.

Therefore, the objective of this study was to explore potential associations between urinary phytoestrogen concentrations, including both isoflavone and lignan concentrations, and a comprehensive semen quality assessment in a population-based prospective cohort study. These hypotheses are of great interest given the potential role of dietary factors on male fecundity.

Methods

Design and Study Population. The design and methods of the Longitudinal Investigation of Fertility and the Environment (LIFE) study, a prospective cohort study designed to investigate environmental influences on human fecundity and fertility, have been described in detail previously (13). In brief, 501 male partners of couples discontinuing contraception for the purposes of becoming pregnant were recruited from 16 counties in Michigan and Texas from 2005 to 2009 using sampling frameworks tailored for each state to identify couples planning pregnancy in the near future. Eligible men were aged >18 y, in a committed relationship, able to communicate in English or Spanish, and not surgically or medically sterile. Full participant approval was granted from all participating institutions, and all participants gave informed consent before data collection.

Data Collection. Health, demographic, physical activity, and reproductive histories were obtained from each male partner during in-person interviews at the time of enrollment. All data and biospecimens were collected in the home, and baseline interviews were followed by a standardized anthropometric assessment for determining BMI as conducted by research nurses (14). The nurse obtained nonfasting blood and urine samples for quantifying phytoestrogens and lipids, respectively. Samples were frozen on dry ice at −20°C or colder until shipment to the CDC for analysis.

Measurement of Phytoestrogens. Phytoestrogens were measured in urine at baseline for all participants in which sufficient urine was available for analysis (n = 467), and included the isoflavones genistein, daidzein, *O*-desmethylangolensin, and equol (the latter 2 of which are daidzein metabolites) and lignans enterodiol and enterolactone. Phytoestrogens were analyzed using a 1-mL urine sample spiked with isotopically labeled internal standards to correct for potential procedural losses and to ensure accurate quantitation. Phytoestrogens were measured using a high-performance liquid chromatography electrospray tandem mass spectrometry method (interassay coefficient of variation was <6% based on quality control data acquired during study sample analysis) (15). All machine-observed concentrations were retained in the analysis without substituting or removing concentrations below the limits of detection to avoid introducing biases (16–18).

Urinary creatinine was quantified using a Roche Hitachi model 912 clinical analyzer and analyzed using the Creatinine Plus assay (Roche Diagnostics), which involves the combined use of creatininase, creatinase, and sarcosine oxidase. Serum lipids were quantified using commercially available enzymatic methods (19) and reported as total serum lipids (nanograms per gram of serum) using established calculation methods and individual components (20). Cotinine serum levels were quantified using liquid chromatography-isotope dilution tandem mass spectrometry (21) for assessing baseline exposure to smoking with cut points based on previous literature (22). All analyses were subjected to standard quality assurance procedures, and all reported results were from run charts found to be in control by standard statistical methods.

Semen Collection and Analysis. A semen sample was obtained at baseline and was followed by a second sample approximately 1 mo later irrespective of couples' pregnancy status as previously described (13). Men collected semen samples through masturbation without the use of any lubricant following a recommended 2 d of abstinence using home collection kits (actual abstinence time: median = 3.0 d; mean = 4.1 d) (23, 24) and shipped the samples overnight to the study's andrology laboratory at the National Institute for Occupational Health and Safety for next-day analysis. The integrity of all samples was verified upon arrival at the laboratory. Semen delivered to a central andrology laboratory by overnight mail in insulated mailing kits has been successful in maintaining specimens for other studies (23, 25, 26). Semen analysis after home collection has been reported to be reliable for all semen parameters with the exception of motility parameters (27, 28). A percentage of sperm is alive after 24 h, and a next-day motility assessment can still be made and may provide important information on sperm function and survivability (27).

We quantified 35 semen parameters, including 5 that reflected general characteristics (volume, straw distance, sperm concentration, total sperm count, hypo-osmotic swelling), 8 motility measures, 12 morphometry measures, 8 morphology measures, and 2 sperm chromatin-stability assay measures using established laboratory protocols that included ongoing quality assurance and control procedures (29). Specifics regarding methodology are described elsewhere (30–32). In brief, sperm motility was assessed using the HTM-IVOS (Hamilton Thorne Biosciences) computer-assisted semen analysis system. Sperm concentration was measured using the IVOS system and IDENT stain (33). Sperm viability was conducted using the hypo-osmotic swelling assay (34). Sperm morphology was classified by the 2 widely accepted classification systems: WHO 3rd edition (traditional morphology) and WHO 5th edition (strict morphology) (35–37). Morphometric analyses were conducted using the HTM-IVOS computer-assisted semen analysis system (Hamilton Thorne Biosciences). Progressive sperm motility was assessed by placing a flat capillary tube filled with hyaluronic acid and plugged at 1 end into the fresh ejaculate, and sperm progression was measured when the specimen arrived at the laboratory the next day as a marker of motile sperm at collection (straw distance) (24). The sperm chromatin structure assay (SCSA) procedure was conducted on a Coulter Epics Elite flow cytometer using the SCSA program (SCSA Diagnostics) (38, 39).

The second sample was assessed to affirm azoospermia observed in the first sample and for a second global fecundity assessment and was limited to exclusively measurement of volume, concentration, motility, and sperm head morphology.

Statistical Analysis. Of 473 men with at least 1 semen sample, 5 (1%) were found to be azoospermic on both samples, and 32 men with missing urinary phytoestrogen concentrations were excluded from this analysis (n = 436 included with both the semen quality assessment and phytoestrogen concentrations). A descriptive analysis included the inspection of missing data and influential observations. Differences in characteristics between quartiles of total phytoestrogen concentrations were assessed using ANOVA and chi-square tests where appropriate. Data are presented as means ± SDs or n (%). Geometric mean concentrations with 95% CIs and relevant percentiles were calculated.

Linear mixed-effects models were used to estimate the associations between continuous urinary phytoestrogen concentrations and semen quality parameters. Mixed modeling techniques were used to incorporate the intersample correlations for all semen quality endpoints measured in both samples (volume, concentration, next-day motility, and sperm head morphology). Phytoestrogens were log-transformed for all analyses. Models were adjusted for age (y), cotinine [>40.35 μg/L; cut point based on previous literature (22)], site, and total serum lipids (mg/dL) and included an interaction term with continuous BMI. Models

were also adjusted for abstinence time, sample age, fish consumption, and the sum of polychlorinated biphenyls because these chemicals have been associated with semen quality in previous work among this cohort (31). However, adjusting for these factors did not appreciably change the results and were not included in the final models for parsimony. β coefficients and 95% CIs for the continuous main phytoestrogen effect and the interaction term with continuous BMI are presented. Tertiles of phytoestrogen exposures and restricted spline-based tests for nonlinearity were performed to evaluate the linearity assumption in our continuous models, and the assumption of linearity was empirically supported.

Sensitivity analyses were performed using quartiles of phytoestrogens as the exposure to evaluate the assumption of linearity. Semen quality parameters were also considered with the Box–Cox transformation to achieve normality assumption in the linear mixed models. Following Handelsman (40), we found the optimal transformation parameter for each semen quality outcome, transformed the semen outcome, and reran the analyses to determine whether the obtained results were different from the primary analyses using untransformed semen outcomes. We also performed a sensitivity analysis using multiple imputation to account for missing urinary phytoestrogen concentrations and semen quality parameters to determine the robustness of our findings relative to these missing data. P values < 0.05 were considered statistically significant.

Results

The LIFE study included 436 male partners of couples attempting to become pregnant with measured urinary phytoestrogen concentrations and semen quality that were included in this analysis. Male partners were aged on average 31.8 ± 4.8 y, with an average BMI (in kg/m^2) of 29.9 ± 5.6 (**Table 1**). Most men in the study were college-educated (91%) and self-identified as non-Hispanic white (80%). Men who self-identified as non-Hispanic white were more likely to be in the lowest quartile of total phytoestrogens compared with all other quartiles, whereas men who self-identified as another race were more likely to be in the highest quartile compared with all other quartiles. No differences in age, BMI, education, income, alcohol use, exercise, or baseline supplement use were associated with quartiles of total urinary phytoestrogen concentrations. The distribution and geometric mean concentration of each phytoestrogen is shown in **Table 2**.

Of the 210 associations between individual urinary phytoestrogens and next-day analyses of semen quality parameters evaluated, 21 were observed to be statistically significant (14 with the isoflavones and 7 with the lignans), with significant interactions observed with BMI (**Supplemental Figure 1**, **Table 3**). In particular, genistein and daidzein were associated with a lower percentage of normal sperm [using both the WHO traditional (35) and WHO strict morphology criteria (36)] and increased abnormalities in semen morphology (cytoplasmic droplets and abnormal necks and midpieces), with reduced associations as BMI increased (Supplemental Figure 1, Table 3). *O*-Desmethylangolensin was associated with measures of reduced motility (linearity and straightness), with BMI lessening the associations. Enterolactone was associated with fewer abnormalities in semen morphometry (pyriform and other tail abnormalities) and morphology (neck and midpiece abnormalities), and decreased DNA fragmentation, with reduced associations observed as BMI increased. Details regarding all associations are shown in **Supplemental Tables 1** and **2** and Supplemental Figure 1. A sensitivity analysis was used to evaluate the effects using quartiles of phytoestrogens and the Box–Cox family of transformations for semen quality parameters, yielding similar results (data not shown). Moreover, we observed similar findings using multiple imputations that accounted for missing urinary phytoestrogen concentrations and semen quality parameters.

Discussion

In this study, we observed associations between several select urinary phytoestrogens and semen quality parameters among men from the general population in a cohort of male partners of couples trying to become pregnant. Isoflavone concentrations were associated with a lower percentage of normal sperm and increased abnormalities in semen morphology parameters, and lignan concentrations were associated with fewer abnormalities in morphology and morphometry characteristics. These associations were modified by BMI in that reduced associations were observed as BMI increased. These results suggest that male urinary phytoestrogen concentrations at levels characteristic of the US population may be associated with subtle indicators of male fecundity and semen quality but not with couple fecundity (41).

Previous studies have reported associations between genistein and daidzein and markers of reduced sperm count (7, 9) and sperm concentration (7, 8, 10) using both urinary markers (7, 10) and dietary assessments (8, 9). We observed associations in a similar direction, although our findings were not statistically significant (P values ranged from 0.33 to 0.97). It is worth noting that we also observed associations between these isoflavones and several morphology parameters. One previous study did not find an association between dietary assessment of soy intake and morphology but evaluated morphology using only the WHO strict criteria (8), whereas we measured a comprehensive panel of 8 morphology parameters. Of the previous studies, genistein and daidzein were generally associated with semen quality only among studies of infertile men or men of couples seeking fertility treatment (7–10, 42). Two small intervention studies among healthy volunteers observed no effects on semen quality or other endocrine measurements and testicular volume (11, 12). We did not observe associations between isoflavones and DNA damage as was observed in a previous study (9); however, we did observe an association between enterolactone and reduced DNA fragmentation. Our association of a significant interaction with BMI is in line with a study of dietary soy intake among men presenting at a fertility clinic that observed more pronounced associations with sperm concentration among obese men (8). However, we observed reduced associations as BMI increased among a population of healthy men. Previous studies have typically limited their evaluation to the associations of isoflavones and semen quality, and as such our findings related to enterolactone are novel in this area.

Phytoestrogens are hypothesized to influence semen quality through both estrogenic and antiestrogen effects because of their structural similarity to estrogen and ability to bind and activate both estrogen receptor α and β (43, 44). These impacts on steroid biosynthesis may affect the availability of free bioactive steroid hormones (45) and thus have potential reproductive and endocrine effects on male reproductive function. Interestingly, Adeoya-Osiguwa et al. (46) and Fraser et al. (47) showed that phytoestrogens induce capacitation and a premature acrosome reaction in mouse and human sperm, respectively. These authors showed that the mechanism of action may be caused by unregulated stimulation of cAMP production, which in turn may lead to acrosome loss. These findings point to complicated mechanisms and multiple potential modes of action for associations between phytoestrogens and semen quality. Although lignans

TABLE 1 Characteristics of male partners by quartile of urine total phytoestrogen concentrations, LIFE study, 2005–2009[1]

Characteristics	Total phytoestrogen concentrations				
	Overall	Quartile 1: ≤1080 nmol/L	Quartile 2: 1081–2278 nmol/L	Quartile 3: 2279–5779 nmol/L	Quartile 4: ≥5780 nmol/L	*P*
n (%)	436 (100.0)	109 (25.0)	109 (25.0)	109 (25.0)	109 (25.0)	
Age, y	31.8 ± 4.8	31.7 ± 4.9	31.7 ± 4.9	31.8 ± 4.9	32.0 ± 4.7	0.97
BMI, kg/m^2	29.9 ± 5.8	30.3 ± 5.8	30.2 ± 4.1	30.2 ± 6.2	29.0 ± 4.9	0.33
Abstinence time, d	4.1 ± 4.6	3.9 ± 3.4	4.02 ± 4.6	4.5 ± 6.3	4.0 ± 3.5	0.76
Self-identified race/ethnicity						0.05
Non-Hispanic white	350 (80.3)	91 (83.5)	87 (79.8)	89 (81.7)	83 (76.2)	
Non-Hispanic black	20 (4.6)	2 (1.8)	9 (8.3)	6 (5.5)	3 (2.8)	
Hispanic	36 (8.3)	10 (9.2)	9 (8.3)	9 (8.3)	8 (7.3)	
Other	30 (6.9)	6 (5.5)	4 (3.7)	5 (4.6)	15 (13.8)	
College graduate or higher	398 (91.3)	97 (89.0)	98 (89.9)	102 (93.6)	101 (92.7)	0.70
Household income, $						0.65
29,999	18 (4.1)	3 (2.8)	9 (8.3)	4 (3.7)	2 (1.8)	
30,000–49,999	47 (10.8)	13 (11.9)	9 (8.3)	14 (12.8)	11 (10.1)	
50,000–69,999	80 (18.4)	19 (17.4)	19 (17.4)	23 (21.1)	19 (17.4)	
≥70,000	285 (65.4)	72 (66.1)	70 (64.2)	67 (61.5)	76 (69.7)	
Have health insurance	399 (91.5)	97 (89.0)	98 (89.9)	102 (93.6)	102 (93.6)	0.48
Alcohol intake (per month)						
No	66 (15.1)	17 (15.6)	19 (17.4)	14 (12.8)	16 (14.7)	0.82
Yes	370 (84.9)	92 (84.4)	90 (82.6)	95 (87.2)	93 (85.3)	0.20
<1/mo	25 (6.8)	3 (3.3)	3 (3.3)	11 (11.6)	8 (8.6)	
1/mo	32 (8.7)	12 (13.0)	9 (10.0)	8 (8.4)	3 (3.2)	
2–3 d/mo	69 (18.7)	14 (15.2)	23 (25.6)	18 (19.0)	14 (15.1)	
1/wk	95 (25.7)	29 (31.5)	20 (22.2)	19 (20.0)	27 (29.0)	
2–3 times/wk	113 (30.5)	24 (26.1)	25 (27.8)	30 (31.6)	34 (36.6)	
4–6 times/wk	24 (6.5)	6 (6.5)	6 (6.7)	7 (7.4)	5 (5.4)	
Daily	12 (3.2)	4 (4.4)	4 (4.4)	2 (2.1)	2 (2.2)	
Participated in a vigorous exercise program during the last 12 mo	188 (43.1)	44 (40.4)	47 (43.1)	49 (45.0)	48 (44.0)	0.91
Multivitamin use >1/wk in the past 3 mo	190 (43.6)	54 (49.5)	43 (39.5)	43 (39.5)	50 (45.9)	0.34
Supplement use >1/wk in the past 3 mo	140 (32.1)	38 (34.9)	35 (32.1)	27 (24.8)	40 (36.7)	0.25
Fathered pregnancy before entering study	211 (48.4)	50 (45.9)	51 (46.8)	52 (47.7)	58 (53.2)	0.63
Serum total lipids, mg/dL	735 ± 220	788 ± 240	719 ± 206	734 ± 248	698 ± 172	0.02

[1] Values are means ± SDs and *n* (%). In all, 5 men were found to be azoospermic on both samples and were excluded from this analysis, and 32 men were missing phytoestrogen concentrations. LIFE, Longitudinal Investigation of Fertility and the Environment.

have been shown to have a weaker binding affinity, they also have anticarcinogenic properties that may affect fecundity (48, 49). In addition, the interaction we observed with BMI may stem from the observation that adipose tissue is an endocrine organ (50). Estrogen levels have been hypothesized to increase with increasing BMI because the conversion of androgens to estrogen in adipose tissue may be a primary source of estrogen (51, 52), which in turn may influence estrogen receptor activity and the response to phytoestrogens. Alternatively, because adipose tissue can be a storage site for steroid hormones, it may be that phytoestrogen storage or bioavailability may also be altered by increased body fat.

TABLE 2 Distribution of urine phytoestrogen concentrations among 436 males in the LIFE study, 2005–2009[1]

	Percentile					Geometric	
	5th	25th	50th	75th	95th	mean	95% CI
Genistein, nmol/L	10	44	127	542	2980	151	128, 178
Daidzein, nmol/L	25	91	280	1160	6680	342	291, 403
O-DMA, nmol/L	1	3	18	94	1350	20	17, 25
Equol, nmol/L	4	13	25	57	174	28	25, 31
Enterodiol, nmol/L	7	49	130	315	1040	121	105, 139
Enterolactone, nmol/L	24	231	931	2440	6570	669	566, 789
Total isoflavones, nmol/L	59	197	543	2070	11,700	665	570, 776
Total lignans, nmol/L	47	429	1180	2710	780	959	832, 1110
Total phytoestrogens, nmol/L	271	1080	2280	5780	17,400	2370	2110, 2670

[1] LIFE, Longitudinal Investigation of Fertility and the Environment; *O*-DMA, *O*-desmethylangolensin.

This study has several strengths, including a large number of male participants recruited from the general population with measured urinary phytoestrogen concentrations. Urinary measurements take differences in metabolism and absorption into account and are especially useful because soy products are found in many products and because intake is difficult to assess using traditional dietary assessment tools (53). Moreover, because the mammalian lignans, enterolactone, and enterodiol are converted by the intestinal microflora, there is considerable variability in gut microflora and other factors that may influence metabolism (54–56). As such, urinary concentrations are needed to adequately assess associations with lignans. These markers have been found to be useful biomarkers of dietary intake but are only considered measures of short-term intake. Because only a single measurement was available in this case, we assumed that the

TABLE 3 Significant associations between log-transformed urine phytoestrogens and semen quality parameters, including main effects and interactions with BMI and 95% CIs[1]

Phytoestrogen	Semen quality parameter category	Semen quality parameter[2]	β 95% CI	
			Main effect	Interaction with BMI
Genistein, nmol/L	Overall	Distance sperm traveled in straw[3]	2.51 (0.52, 4.49)	−0.09 (−0.15, −0.02)
	Morphometry	Megalo head	−0.58 (−1.17, 0.005)	0.02 (0.002, 0.04)
	Morphology	Cytoplasmic droplet	1.66 (0.02, 3.31)	−0.06 (−0.11, −0.003)
		Neck and midpiece abnormal	3.89 (0.79, 6.99)	−0.13 (−0.23, −0.03)
		WHO strict criteria (36)	−3.95 (−7.01, −0.89)	0.14 (0.04, 0.24)
		WHO traditional criteria (35)	−5.61 (−9.42, −1.79)	0.19 (0.06, 0.31)
Daidzein, nmol/L	Overall	Distance sperm traveled in straw[3]	2.89 (0.81, 4.97)	−0.10 (−0.17, −0.03)
	Morphology	Cytoplasmic droplet	1.75 (0.02, 3.47)	−0.06 (−0.12, 0.00003)
		Neck and midpiece abnormal	3.61 (0.35, 6.86)	−0.12 (−0.23, −0.01)
		WHO strict criteria (36)	−3.72 (−6.94, −0.50)	0.13 (0.03, 0.24)
		WHO traditional criteria (35)	−5.35 (−9.36, −1.34)	0.18 (0.05, 0.32)
O-DMA, nmol/L	Motility	Linearity	−3.78 (-6.84, -0.72)	0.11 (0.01, 0.21)
		Straightness	−5.83 (−10.52, −1.14)	0.17 (0.01, 0.32)
	Morphology	Cytoplasmic droplet	1.67 (0.22, 3.12)	−0.05 (−0.10, −0.01)
Equol, nmol/L	Morphometry	Coiled tail	6.28 (0.63, 11.93)	−0.21 (−0.39, −0.02)
Enterodiol, nmol/L	Morphometry	Coiled tail	4.07 (0.27, 7.87)	−0.13 (−0.25, −0.003)
Enterolactone, nmol/L	Motility	Linearity	−3.62 (−7.16, −0.08)	0.11 (−0.005, 0.23)
	Morphometry	Round	−0.44 (−0.92, 0.03)	0.02 (0.001, 0.03)
		Pyriform	−2.37 (−4.34, −0.40)	0.07 (0.005, 0.13)
		Other tail abnormalities	−1.68 (−3.01, −0.35)	0.06 (0.01, 0.10
	Morphology	Neck and midpiece abnormal	−3.35 (−6.51, −0.19)	0.11 (0.01, 0.21)
		WHO strict criteria (36)	3.29 (0.16, 6.42)	−0.10 (−0.20, 0.003)
	Sperm chromatin stability	DNA fragmentation	−3.44 (−6.58, −0.30)	0.11 (0.004, 0.21)

[1] Results presented are significant at the $P < 0.05$ level and are adjusted for age, site, serum lipids, and cotinine. Models include a continuous term for phytoestrogens and BMI and an interaction term between continuous phytoestrogens and continuous BMI. Associations between all phytoestrogens and semen quality parameters, including nonsignificant associations, are provided in Supplemental Tables 1 and 2. *O*-DMA, *O*-desmethylangolensin.

[2] Values are percentages unless indicated otherwise.

[3] Units expressed in mm.

measurement reflected usual dietary intake over the time course of a normal spermatogenesis cycle. The extent to which this assumption can be upheld awaits affirmation.

We were also able to adjust for many potential confounders in this study, including fish consumption as a marker of dietary intake and exposure to polychlorinated biphenyls, which have also been shown to exert endocrine-disrupting properties and to be associated with semen quality in this population (31), and to represent the most consistent findings with respect to reductions in couple fecundity (57). Although we were able to adjust for several confounders, residual confounding may be present. We have shown previously, however, that such an unmeasured confounder would need to be strongly associated with the exposure and outcome to explain away the observed associations (32, 41, 58). This exploratory study also involved a detailed semen analysis that evaluated 35 semen quality parameters that give a more comprehensive assessment of semen quality, although we cannot rule out that some of the associations observed may have resulted from multiple comparisons. Moreover, we were also able to evaluate associations with lignans and semen quality, thus further extending research in this area.

However, we were particularly limited in our assessment of next-day motility. The glass straw method results in increased variability in measurement, although the use of next-day analyses has not been shown to introduce any bias because the laboratory staff were blinded to the fecundity status of the male and his phytoestrogen concentrations. Although next-day analyses may not be suitable for clinical purposes, we have utilized them here in a population-based setting to further our understanding of the effects of environmental influences on male fecundity. It should be pointed out that—with the exception of motility (25, 26)—no differences were observed between samples collected at home the night before compared with samples analyzed within 1.5 h with respect to various semen endpoints. Importantly, men in the LIFE study had phytoestrogen concentrations that were fairly comparable to adult men in the NHANES over the study period (59, 60). It is also important to note that changes in semen quality do not necessarily mean accompanying effects on infertility (61–64). Although we observed some associations with semen quality in this study, we previously reported no associations between male urinary phytoestrogen concentrations and couple fecundity as measured by time to pregnancy (41). Similar results were observed in a study among couples attending a fertility center in that soy intake was associated with semen quality parameters (8) but not with in vitro fertilization outcomes (65). Taken together, these results highlight the complexity of studying effects on male fecundity and the need for future research to better understand the implications of changes in semen parameters on couple fecundity. It may also be that the changes in semen quality observed here were not of sufficient magnitude to affect couple fecundity, thus highlighting the need for additional research in this area.

In conclusion, our findings demonstrate that select urinary phytoestrogens at concentrations characteristic of the US

population were associated with markers of semen quality. It is noteworthy that associations depended upon the class of phytoestrogens and were reduced as BMI increased. Although these potential effects did not have an impact on couple fecundity, their impact on sperm quality warrants additional study and highlights the importance of diet on male fecundity (41), especially given the prevalence of phytoestrogens.

Acknowledgments

SLM, DBB, and GMBL conceived and designed the study concept and design; SLM was responsible for conceiving and developing the overall research plan; SK and ZC performed statistical analyses; SLM wrote the manuscript; SLM, SK, ZC, DBB, and GMBL interpreted the data and critically revised the manuscript for important intellectual content; and SLM had primary responsibility for final content. All authors have read and approved the final manuscript.

References

1. Cederroth CR, Auger J, Zimmermann C, Eustache F, Nef S. Soy, phytoestrogens and male reproductive function: a review. Int J Androl 2010;33:304–16.
2. Cederroth CR, Zimmermann C, Nef S. Soy, phytoestrogens and their impact on reproductive health. Mol Cell Endocrinol 2012;355:192–200.
3. Sliwa L, Macura B. Evaluation of cell membrane integrity of spermatozoa by hypoosmotic swelling test - "water test" in mice after intraperitoneal daidzein administration. Arch Androl 2005;51:443–8.
4. Faqi AS, Johnson WD, Morrissey RL, McCormick DL. Reproductive toxicity assessment of chronic dietary exposure to soy isoflavones in male rats. Reprod Toxicol 2004;18:605–11.
5. Glover A, Assinder SJ. Acute exposure of adult male rats to dietary phytoestrogens reduces fecundity and alters epididymal steroid hormone receptor expression. J Endocrinol 2006;189:565–73.
6. Lee BJ, Kang JK, Jung EY, Yun YW, Baek IJ, Yon JM, Lee YB, Sohn HS, Lee JY, Kim KS, et al. Exposure to genistein does not adversely affect the reproductive system in adult male mice adapted to a soy-based commercial diet. J Vet Sci 2004;5:227–34.
7. Xia Y, Chen M, Zhu P, Lu C, Fu G, Zhou X, Chen D, Wang H, Wang B, Wang S, et al. Urinary phytoestrogen levels related to idiopathic male infertility in Chinese men. Environ Int 2013;59:161–7.
8. Chavarro JE, Toth TL, Sadio SM, Hauser R. Soy food and isoflavone intake in relation to semen quality parameters among men from an infertility clinic. Hum Reprod 2008;23:2584–90.
9. Song GK, Andolina E, Herko RC, Brewer KJ, Lewis V. O-115: beneficial effects of dietary intake of plant phytoestrogens on semen parameters and sperm DNA integrity in infertile men. Fertil Steril 2006;86:S49–22.
10. Toshima H, Suzuki Y, Imai K, Yoshinaga J, Shiraishi H, Mizumoto Y, Hatakeyama S, Onohara C, Tokuoka S. Endocrine disrupting chemicals in urine of Japanese male partners of subfertile couples: a pilot study on exposure and semen quality. Int J Hyg Environ Health 2012;215:502–6.
11. Beaton LK, McVeigh BL, Dillingham BL, Lampe JW, Duncan AM. Soy protein isolates of varying isoflavone content do not adversely affect semen quality in healthy young men. Fertil Steril 2010;94:1717–22.
12. Mitchell JH, Cawood E, Kinniburgh D, Provan A, Collins AR, Irvine DS. Effect of a phytoestrogen food supplement on reproductive health in normal males. Clin Sci (Lond) 2001;100:613–8.
13. Buck Louis GM, Schisterman EF, Sweeney AM, Wilcosky TC, Gore-Langton RE, Lynch CD, Boyd Barr D, Schrader SM, Kim S, Chen Z, et al. Designing prospective cohort studies for assessing reproductive and developmental toxicity during sensitive windows of human reproduction and development—the LIFE Study. Paediatr Perinat Epidemiol 2011;25:413–24.
14. Lohman TG, Roche AF, Martorell R. Anthropometric standardization reference manual. Champaign, IL: Human Kinetics Books; 1988.
15. Rybak ME, Parker DL, Pfeiffer CM. Determination of urinary phytoestrogens by HPLC-MS/MS: a comparison of atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI). J Chromatogr B Analyt Technol Biomed Life Sci 2008;861:145–50.
16. Guo Y, Harel O, Little RJ. How well quantified is the limit of quantification? Epidemiology 2010;21 (Suppl 4):S10–6.
17. Harel O, Schisterman EF, Vexler A, Ruopp MD. Monitoring quality control: can we get better data? Epidemiology 2008;19:621–7.
18. Schisterman EF, Vexler A, Whitcomb BW, Liu A. The limitations due to exposure detection limits for regression models. Am J Epidemiol 2006;163:374–83.
19. Akins JR, Waldrep K, Bernert JT, Jr. The estimation of total serum lipids by a completely enzymatic 'summation' method. Clin Chim Acta 1989;184:219–26.
20. Phillips DL, Pirkle JL, Burse VW, Bernert JT, Jr., Henderson LO, Needham LL. Chlorinated hydrocarbon levels in human serum: effects of fasting and feeding. Arch Environ Contam Toxicol 1989;18:495–500.
21. Bernert JT, Jr., Turner WE, Pirkle JL, Sosnoff CS, Akins JR, Waldrep MK, Ann Q, Covey TR, Whitfield WE, Gunter EW, et al. Development and validation of sensitive method for determination of serum cotinine in smokers and nonsmokers by liquid chromatography/atmospheric pressure ionization tandem mass spectrometry. Clin Chem 1997;43:2281–91.
22. Jeemon P, Agarwal S, Ramakrishnan L, Gupta R, Snehi U, Chaturvedi V, Reddy KS, Prabhakaran D. Validation of self-reported smoking status by measuring serum cotinine levels: an Indian perspective. Natl Med J India 2010;23:134–6.
23. Royster MO, Lobdell DT, Mendola P, Perreault SD, Selevan SG, Rothmann SA, Robbins WA. Evaluation of a container for collection and shipment of semen with potential uses in population-based, clinical, and occupational settings. J Androl 2000;21:478–84.
24. Turner TW, Schrader SM. Sperm migration assay as a measure of recently ejaculated sperm motility in specimens shipped overnight. J Androl 2006:56(Suppl S):58.
25. Luben TJ, Olshan AF, Herring AH, Jeffay S, Strader L, Buus RM, Chan RL, Savitz DA, Singer PC, Weinberg HS, et al. The healthy men study: an evaluation of exposure to disinfection by-products in tap water and sperm quality. Environ Health Perspect 2007;115:1169–76.
26. Olshan AF, Perreault SD, Bradley L, Buus RM, Strader LF, Jeffay SC, Lansdell L, Savitz DA, Herring A. The healthy men study: design and recruitment considerations for environmental epidemiologic studies in male reproductive health. Fertil Steril 2007;87:554–64.
27. Stovall DW, Guzick DS, Berga SL, Krasnow JS, Zeleznik AJ. Sperm recovery and survival: two tests that predict in vitro fertilization outcome. Fertil Steril 1994;62:1244–9.
28. Morris RA, Jeffay SC, Strader LF, Evenson DP, Olshan AF, Lansdell LW, Perreault SD. Evaluation of sperm chromatin structure assay (SCSA) in human sperm after simulated overnight shipment. J Androl 2003:24(Suppl):54.
29. American Society of Andrology. Semen analysis: quality control methods for old and new technologies [conference paper on the Internet]; 1996. Semen Analysis-Challenges of Quality Control and New Technology; 1996 Jan 17–20; University of South Florida, Tampa, FL [cited 2015 Sep 15]. Available from: http://godot.urol.uic.edu/~androlog/workshops/wet96.html.
30. Louis GM, Chen Z, Schisterman EF, Kim S, Sweeney AM, Sundaram R, Lynch CD, Gore-Langton RE, Barr DB. Perfluorochemicals and human semen quality: the LIFE study. Environ Health Perspect 2015;123:57–63.
31. Mumford SL, Kim S, Chen Z, Gore-Langton RE, Boyd Barr D, Buck Louis GM. Persistent organic pollutants and semen quality: the LIFE study. Chemosphere 2015;135:427–35.
32. Schisterman EF, Mumford SL, Chen Z, Browne RW, Boyd Barr D, Kim S, Buck Louis GM. Lipid concentrations and semen quality: the LIFE study. Andrology 2014;2:408–15.
33. Zinaman MJ, Uhler ML, Vertuno E, Fisher SG, Clegg ED. Evaluation of computer-assisted semen analysis (CASA) with IDENT stain to determine sperm concentration. J Androl 1996;17:288–92.
34. Jeyendran RS, Van der Ven HH, Perez-Pelaez M, Crabo BG, Zaneveld LJ. Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characteristics. J Reprod Fertil 1984;70:219–28.
35. World Health Organization. Laboratory manual for the examination of human semen and semen-cervical mucus interaction. 3rd ed. Cambridge (United Kingdom): Cambridge University Press; 1992.

36. World Health Organization. Laboratory manual for the examination and processing of human semen. 5th ed. Geneva (Switzerland): World Health Organization Press; 2010.
37. Rothmann SA, Bort AM, Quigley J, Pillow R. Sperm morphology classification: a rational method for schemes adopted by the World Health Organization. Methods Mol Biol 2013;927:27–37.
38. Evenson DP, Jost LK, Baer RK, Turner TW, Schrader SM. Individuality of DNA denaturation patterns in human sperm as measured by the sperm chromatin structure assay. Reprod Toxicol 1991;5:115–25.
39. Evenson DP, Larson KL, Jost LK. Sperm chromatin structure assay: its clinical use for detecting sperm DNA fragmentation in male infertility and comparisons with other techniques. J Androl 2002;23:25–43.
40. Handelsman DJ. Optimal power transformations for analysis of sperm concentration and other semen variables. J Androl 2002;23:629–34.
41. Mumford SL, Sundaram R, Schisterman EF, Sweeney AM, Barr DB, Rybak ME, Maisog JM, Parker DL, Pfeiffer CM, Louis GM. Higher urinary lignan concentrations in women but not men are positively associated with shorter time to pregnancy. J Nutr 2014;144:352–8.
42. Casini ML, Gerli S, Unfer V. An infertile couple suffering from oligospermia by partial sperm maturation arrest: can phytoestrogens play a therapeutic role? A case report study. Gynecol Endocrinol 2006;22:399–401.
43. Hwang CS, Kwak HS, Lim HJ, Lee SH, Kang YS, Choe TB, Hur HG, Han KO. Isoflavone metabolites and their in vitro dual functions: they can act as an estrogenic agonist or antagonist depending on the estrogen concentration. J Steroid Biochem Mol Biol 2006;101:246–53.
44. Kuiper GG, Lemmen JG, Carlsson B, Corton JC, Safe SH, van der Saag PT, van der Burg B, Gustafsson JA. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. Endocrinology 1998;139:4252–63.
45. Déchaud H, Ravard C, Claustrat F, de la Perriere AB, Pugeat M. Xenoestrogen interaction with human sex hormone-binding globulin (hSHBG). Steroids 1999;64:328–34.
46. Adeoya-Osiguwa SA, Markoulaki S, Pocock V, Milligan SR, Fraser LR. 17β-Estradiol and environmental estrogens significantly affect mammalian sperm function. Hum Reprod 2003;18:100–7.
47. Fraser LR, Beyret E, Milligan SR, Adeoya-Osiguwa SA. Effects of estrogenic xenobiotics on human and mouse spermatozoa. Hum Reprod 2006;21:1184–93.
48. Adlercreutz H. Lignans and human health. Crit Rev Clin Lab Sci 2007;44:483–525.
49. Mense SM, Hei TK, Ganju RK, Bhat HK. Phytoestrogens and breast cancer prevention: possible mechanisms of action. Environ Health Perspect 2008;116:426–33.
50. Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. J Clin Endocrinol Metab 2004;89:2548–56.
51. Calle EE, Kaaks R. Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms. Nat Rev Cancer 2004;4:579–91.
52. Simpson ER. Sources of estrogen and their importance. J Steroid Biochem Mol Biol 2003;86:225–30.
53. Arai Y, Uehara M, Sato Y, Kimira M, Eboshida A, Adlercreutz H, Watanabe S. Comparison of isoflavones among dietary intake, plasma concentration and urinary excretion for accurate estimation of phytoestrogen intake. J Epidemiol 2000;10:127–35.
54. Blaut M. Relationship of prebiotics and food to intestinal microflora. Eur J Nutr 2002;41(Suppl 1):I11–6.
55. Kilkkinen A, Pietinen P, Klaukka T, Virtamo J, Korhonen P, Adlercreutz H. Use of oral antimicrobials decreases serum enterolactone concentration. Am J Epidemiol 2002;155:472–7.
56. Kilkkinen A, Stumpf K, Pietinen P, Valsta LM, Tapanainen H, Adlercreutz H. Determinants of serum enterolactone concentration. Am J Clin Nutr 2001;73:1094–100.
57. Buck Louis GM. Persistent environmental pollutants and couple fecundity: an overview. Reproduction 2014;147:R97–104.
58. Schisterman EF, Mumford SL, Browne RW, Barr DB, Chen Z, Louis GM. Lipid concentrations and couple fecundity: the LIFE study. J Clin Endocrinol Metab 2014;99:2786–94.
59. Centers for Disease Control and Prevention. Second national report on biochemical indicators of diet and nutrition in the U.S. population; 2012 [cited 2015 Sep 15]. Available from: http://www.cdc.gov/nutritionreport.
60. Centers for Disease Control and Prevention. Fourth national report on human exposure to environmental chemicals; 2014 [cited 2015 Sep 15]. Available from: http://www.cdc.gov/exposurereport.
61. Practice Committee of the American Society for Reproductive Medicine. The clinical utility of sperm DNA integrity testing: a guideline. Fertil Steril 2013;99:673–7.
62. Buck Louis GM, Sundaram R, Schisterman EF, Sweeney A, Lynch CD, Kim S, Maisog JM, Gore-Langton R, Eisenberg ML, Chen Z. Semen quality and time to pregnancy: the Longitudinal Investigation of Fertility and the Environment Study. Fertil Steril 2014;101:453–62.
63. Milardi D, Grande G, Sacchini D, Astorri AL, Pompa G, Giampietro A, De Marinis L, Pontecorvi A, Spagnolo AG, Marana R. Male fertility and reduction in semen parameters: a single tertiary-care center experience. Int J Endocrinol 2012;2012:649149.
64. Wang C, Chan SY, Ng M, So WW, Tsoi WL, Lo T, Leung A. Diagnostic value of sperm function tests and routine semen analyses in fertile and infertile men. J Androl 1988;9:384–9.
65. Mínguez-Alarcón L, Afeiche MC, Chiu YH, Vanegas JC, Williams PL, Tanrikut C, Toth TL, Hauser R, Chavarro JE. Male soy food intake was not associated with in vitro fertilization outcomes among couples attending a fertility center. Andrology 2015;3:702–8.