American Journal of Epidemiology

Vol. 178, No. 3
DOI: 10.1093/aje/kws591
Advance Access publication:
June 9, 2013

Original Contribution

Hypospadias and Maternal Intake of Phytoestrogens

Suzan L. Carmichael*, Mary E. Cogswell, Chen Ma, Amparo Gonzalez-Feliciano, Richard S. Olney, Adolfo Correa, Gary M. Shaw, and the National Birth Defects Prevention Study

* Correspondence to Dr. Suzan L. Carmichael, Division of Neonatal and Developmental Medicine, Department of Pediatrics, Stanford University School of Medicine, 1265 Welch Road, Room X111, Stanford, CA 94305 (e-mail: scarmichael@stanford.edu).

Initially submitted November 1, 2012; accepted for publication December 27, 2012.

Experimental data indicate that gestational exposures to estrogenic compounds impact risk of hypospadias. We examined whether risk of hypospadias (i.e., a congenital malformation in which the opening of the penile urethra occurs on the ventral side of the penis) was associated with maternal intake of phytoestrogens, given their potential impact on estrogen metabolism. The analysis included data on mothers of 1,250 hypospadias cases and 3,118 controls who delivered their infants from 1997 to 2005 and participated in the National Birth Defects Prevention Study, a multistate, population-based, case-control study. After adjustment for several covariates, high intakes of daidzein, genistein, glycetin, secoisolariciresinol, total isoflavones, total lignans, and total phytoestrogens were associated with reduced risks; odds ratios comparing intakes ≥90th percentile with intakes between the 11th and 89th percentiles ranged from 0.6 to 0.8. For example, the odds ratio for total phytoestrogen intake was 0.7 (95% confidence interval: 0.5, 1.0). This study represents the first large-scale analysis of phytoestrogen intake and hypospadias. The observed associations merit investigation in additional populations before firm conclusions can be reached.

birth defects; diet; hypospadias; nutrition; phytoestrogen

Abbreviation: NBDPS, National Birth Defects Prevention Study.

The birth defect hypospadias occurs when the urethral opening is on the ventral side of the penis. It is a relatively common congenital malformation, affecting 4–6 per 1,000 male births (1). Experimental data indicate that maternal exposures to estrogenic compounds may impact risk of hypospadias by interfering with the production or action of fetal androgens, which are critical to normal urethral closure (2–5).

North and Golding (6) reported a 4-fold increased risk of hypospadias among boys born to vegetarian women. One proposed explanation for the increased risk was higher intake of phytoestrogens among vegetarians, given their estrogenic properties. Subsequent studies have not confirmed an association with a vegetarian diet (7–11), with 1 exception (12). One study specifically estimated phytoestrogen intake and did not find an association with hypospadias, but it included only 56 cases (7).

Phytoestrogens are plant constituents named for their estrogenic properties. The main types are isoflavones, lignans, and coumestans. Lignans have been shown to interfere with conversion of testosterone to dihydrotestosterone (13), which is critical to normal urethral closure, and the isoflavone genistein has been shown to induce hypospadias in mice (14). Genistein has also been associated with reduced anogenital distance in male rats (15). High intake of isoflavones increases 2-hydroxylation of estradiol (16), which results in estrogen derivatives that are mostly non- or weakly estrogenic (17). Thus, isoflavones could protect against hypospadias. Given their varied effects on the levels and activity of sex hormones such as estrogen and testosterone (13, 18), phytoestrogens have been likened to natural selective estrogen receptor modulators (19). As such, it is reasonable to hypothesize that they are associated with hypospadias, but it is not straightforward to predict the direction of such an association.

The objective of this analysis was to investigate whether the congenital malformation hypospadias was associated with intake of phytoestrogens among a large group of women

participating in the National Birth Defects Prevention Study (NBDPS).

MATERIALS AND METHODS

Study design

The NBDPS is a large population-based, multicenter, case-control study of selected structural congenital malformations. Recruitment and data collection began with deliveries that had estimated due dates in October 1997 and are ongoing; the current analysis includes deliveries through the end of 2005 (the year through which the phytoestrogen data are available). Detailed study methods and descriptions of the surveillance systems in the 10 NBDPS study centers have been published (20). In brief, 7 included liveborn, stillborn (fetal deaths at >20 weeks' gestation), and prenatally diagnosed and electively terminated cases (Arkansas, California, Georgia, Iowa, North Carolina, Texas, Utah); 1 included only liveborn and stillborn cases (Massachusetts); 1 included only liveborn cases (New Jersey); and 1 included liveborn cases from 1997 to 1999 and added stillborn cases in 2000 (New York).

Case review and classification

The NBDPS includes second- and third-degree hypospadias; that is, the urethra opened at the penile shaft, scrotum, or perineum. Medical record information (including operative reports) including anatomical descriptions or diagrams was reviewed by a clinical geneticist at each study center who decided about inclusion or exclusion. Cases described as chordee alone, mild (i.e., first degree, coronal, or glanular), hypospadias not otherwise specified, epispadias, or having ambiguous genitalia without further description were excluded. Infants with recognized single-gene disorders or chromosomal abnormalities were excluded. Each case received a final review by 1 clinical geneticist (R.S.O.) to ensure that cases from each study center met eligibility criteria. This geneticist also classified each case as isolated if there was no concurrent major anomaly or only minor anomalies (e.g., sacral/pilonidal dimple) or as nonisolated if there was at least 1 accompanying major anomaly (21).

Control selection

Each participating center randomly selected approximately 150 liveborn controls without birth defects per study year from birth certificates (Arkansas 2000–current, Georgia 2001–current, Iowa, Massachusetts, North Carolina, New Jersey, Utah) or birth hospitals (Arkansas 1997–1999, California, Georgia 1997–2000, New York, Texas) to represent the populations from which cases were derived. The current analysis was restricted to mothers of male controls.

Maternal interviews

Maternal interviews were conducted by using a standardized, computer-based questionnaire, nearly exclusively by telephone, in English or Spanish, from 6 weeks to 24 months after the infant's estimated due date. Exposures to many factors were assessed, relative to the woman's estimated date of conception, which was derived by subtracting 266 days from the woman's expected due date, which was primarily self-reported. Interviews were conducted with mothers of 72% of cases ($n = 1,355$) and 67% of male controls ($n = 3,432$). The median time from actual date of delivery to interview was 12.9 months for cases and 8.8 months for controls.

Food frequency questionnaire

Mothers reported their average intakes of foods using a shortened version of the food frequency questionnaire developed by Willett et al. (22) for the Nurses' Health Study that included 58 food items. Participants reported how often, on

Table 1. Descriptive Characteristics of Mothers of 1,250 Infants With Hypospadias and 3,118 Male Controls, National Birth Defects Prevention Study, 1997–2005

	Cases		Controls	
	No.	%[a]	No.	%[a]
Maternal race/ethnicity				
Non-Hispanic white	909	73	194	62
Non-Hispanic black	152	12	364	12
Hispanic	105	8	609	20
Other	84	7	204	7
Maternal education				
<High school	98	8	454	15
High school	235	19	786	25
>High school	917	73	1,878	60
Maternal age, years				
<25	284	23	1,029	33
25–34	698	56	1,643	53
≥35	268	21	446	14
No. of previous livebirths				
0	685	55	1,272	41
1	371	30	1,050	34
≥2	194	16	796	26
Maternal prepregnancy BMI[b]				
Underweight (<18.5)	61	5	189	6
Normal weight (18.5–<25.0)	678	54	1,736	56
Overweight (25–<30.0)	295	24	702	23
Obese (≥30.0)	216	17	491	16
Folic acid-containing supplement intake[c]				
Yes	1,169	94	2,749	88
No	81	6	369	12

Abbreviation: BMI, body mass index.
[a] Numbers may not add to 100% because of rounding.
[b] BMI: weight (kg)/height $(m)^2$.
[c] During the month before or the first 3 months of pregnancy.

Table 2. Association of Hypospadias With Intake of Phytoestrogens Among Women in the National Birth Defects Prevention Study, 1997–2005

Phytoestrogens, μg/day[a]	Unadjusted Odds Ratio	95% CI	Energy-adjusted Odds Ratio	95% CI	Covariate-adjusted Odds Ratio[b]	95% CI
Daidzein						
≤4.9	1.1	0.9, 1.4	1.0	0.8, 1.2	1.0	0.8, 1.3
5.0–24.1	1.0	Referent	1.0	Referent	1.0	Referent
≥24.2	0.4	0.3, 0.6	0.6	0.4, 0.8	0.7	0.5, 1.0
Genistein						
≤10.7	1.3	1.1, 1.6	1.1	0.9, 1.4	1.1	0.9, 1.4
10.8–41.7	1.0	Referent	1.0	Referent	1.0	Referent
≥41.8	0.4	0.3, 0.6	0.6	0.4, 0.8	0.6	0.4, 0.9
Biochanin A						
≤6.7	1.0	0.8, 1.3	0.8	0.7, 1.0	1.0	0.8, 1.2
6.8–26.0	1.0	Referent	1.0	Referent	1.0	Referent
≥26.1	0.7	0.5, 0.9	1.1	0.8, 1.4	0.9	0.7, 1.3
Formonoetin						
≤2.5	1.0	0.8, 1.2	0.8	0.7, 1.0	1.0	0.8, 1.3
2.6–9.6	1.0	Referent	1.0	Referent	1.0	Referent
≥9.7	0.7	0.6, 0.9	1.1	0.9, 1.5	0.9	0.7, 1.2
Glycetin						
≤3.7	1.2	1.0, 1.5	1.0	0.8, 1.3	1.0	0.8, 1.3
3.8–18.7	1.0	Referent	1.0	Referent	1.0	Referent
≥18.8	0.4	0.3, 0.5	0.5	0.3, 0.6	0.7	0.4, 1.0
Matairesinol						
≤9.1	1.1	0.9, 1.3	0.8	0.7, 1.1	1.0	0.7, 1.2
9.2–39.5	1.0	Referent	1.0	Referent	1.0	Referent
≥39.6	0.9	0.7, 1.1	1.2	0.9, 1.5	1.0	0.8, 1.3

Table continues

average, they consumed food items in the year before they became pregnant. Fifteen response categories were possible ranging from once per month to 6 or more per day. Foods eaten less than once a month were recorded as "never or none." Intakes of breakfast cereals, sodas, food supplements, and caffeinated tea and coffee were assessed by separate, more detailed questions, which covered intake during the 3 months before pregnancy. Because few women (mothers of 10% of cases and 10% of controls) consumed food supplements (which included items such as powdered drink supplements) and nutrient data were not available for many of these products, food supplements were not included in nutrient calculations. The US Department of Agriculture nutrient database, version 20, was the source of nutrient values (23).

Phytoestrogen intake

Phytoestrogen values published by Kuhnle et al. (24–27) were used to estimate maternal phytoestrogen intake. These values are recent, derive from a single laboratory using state-of-the-art techniques (28), are available for a relatively consistent set of specific phytoestrogens across all food items, and were available for most of the foods in this study's food frequency questionnaire (if not, we selected proxies). Phytoestrogen values were assigned to each food item in the food frequency questionnaire, with details available in the report by Carmichael et al. (29). We assessed total intake of specific isoflavones and lignans as well as their totals, intake of coumestans, and total phytoestrogen intake.

Analyses

Mothers with <500 or >5,000 kcal of energy intake and mothers with >1 food item missing (i.e., not queried) from the food frequency questionnaire (54 cases, 105 controls) were excluded, leaving 1,301 cases and 3,327 male controls available for analyses. A total of 1,250 cases and 3,118 controls had complete data on all covariates. Logistic regression analyses were conducted to estimate odds ratios and 95% confidence intervals reflecting the association of hypospadias with phytoestrogen intake. We compared intake ≥90th percentile and intake ≤10th percentile, relative to intakes between the 11th and 89th percentiles, using cutoffs determined from the distribution among control mothers. Bivariate analyses were followed by analyses adjusted only for energy intake (kilocalories, as a continuous variable), to adjust for

Table 2. Continued

Phytoestrogens, μg/day[a]	Unadjusted Odds Ratio	95% CI	Energy-adjusted Odds Ratio	95% CI	Covariate-adjusted Odds Ratio[b]	95% CI
Secoisolariciresinol						
≤40.4	1.0	0.8, 1.2	0.9	0.7, 1.1	1.0	0.8, 1.3
40.5–225.0	1.0	Referent	1.0	Referent	1.0	Referent
≥225.1	0.5	0.4, 0.6	0.6	0.5, 0.8	0.6	0.4, 0.9
Coumestrol						
≤3.6	1.2	1.0, 1.4	1.0	0.8, 1.2	1.1	0.9, 1.4
3.7–17.3	1.0	Referent	1.0	Referent	1.0	Referent
≥17.4	0.7	0.5, 0.9	0.9	0.7, 1.2	1.0	0.7, 1.3
Total isoflavones						
≤25.7	1.1	0.9, 1.4	1.0	0.8, 1.2	1.0	0.8, 1.3
25.8–103.4	1.0	Referent	1.0	Referent	1.0	Referent
≥103.5	0.4	0.3, 0.6	0.6	0.4, 0.8	0.7	0.5, 1.0
Total lignans						
≤50.8	0.9	0.7, 1.1	0.8	0.6, 1.0	0.9	0.7, 1.2
50.9–258.6	1.0	Referent	1.0	Referent	1.0	Referent
≥258.7	0.5	0.4, 0.7	0.7	0.6, 1.0	0.8	0.6, 1.1
Total phytoestrogens						
≤86.6	1.0	0.8, 1.3	0.9	0.7, 1.1	1.0	0.8, 1.2
86.7–376.3	1.0	Referent	1.0	Referent	1.0	Referent
≥376.4	0.4	0.3, 0.6	0.6	0.4, 0.8	0.7	0.5, 1.0

Abbreviation: CI, confidence interval.

[a] Nutrient values reflect intakes ≤10th percentile and ≥90th percentile, with intakes between the 11th and 89th percentiles as reference. These categories were determined from nutrient intake levels among control mothers.

[b] Adjusted for maternal age (years), parity (ordinal), race/ethnicity, education, body mass index (weight (kg)/height $(m)^2$), study center, folic acid–containing supplement intake, and energy intake (kcal).

the overall caloric density of the diet. We adjusted analyses for the following covariates, which were selected a priori on the basis of potential associations with hypospadias and/or nutritional status: maternal race/ethnicity (non-Hispanic white, Hispanic, African American, other); education (less than, equal to, or greater than high school); age (years); number of previous livebirths; body mass index (weight (kg)/height $(m)^2$); intake of folic acid–containing supplements during the month before or the first 3 months of pregnancy; and study center.

RESULTS

Mothers of cases were more likely to be non-Hispanic white, have higher education, be older and nulliparous, and take folic acid–containing supplements, relative to control mothers (Table 1).

For each phytoestrogen, intake ≥90th percentile was associated with reduced risk of hypospadias in unadjusted models, with odds ratios ranging from 0.4 to 0.7 and 95% confidence intervals excluding 1.0 with the exception of matairesinol (odds ratio = 0.9) (Table 2). After adjustment for energy intake, high intakes of biochanin A, formonoetin, and coumestrol were not associated with reduced risks. After adjustment for additional covariates, high intakes of daidzein, genistein, glycetin, secoisolariciresinol, total isoflavones, total lignans, and total phytoestrogens were associated with reduced risks, although the odds ratios tended to be slightly closer to 1 than before adjustment (ranging from 0.6 to 0.8), and some of the confidence intervals included 1.0.

DISCUSSION

This study found that higher intake of some phytoestrogens was associated with reduced risks of delivering infants with hypospadias (second and third degree), even after adjustment for several covariates. This finding applied to overall intake of phytoestrogens as well as intake of specific phytoestrogens. Lower intake, however, did not tend to be associated with increased risk.

The study that first reported an association between hypospadias and a maternal vegetarian diet also reported increased risk associated with intake of soy products and legumes, but estimates were imprecise and not adjusted for any covariates (6). Another small study reported that legume intake was not associated with hypospadias (9). A third small study administered a phytoestrogen-specific food questionnaire and quantified intake of lignans and isoflavones; phytoestrogen intake was not associated with hypospadias (7). Several factors may

contribute to the differences in the findings of the current study relative to the previous one, including in the previous study, the use of a different food frequency questionnaire, the use of a different database to estimate phytoestrogen content of the queried foods, the examination of tertiles rather than more extreme ends of the distribution, and chance.

Our previous investigations of hypospadias with nutritional factors, also using NBDPS data, have not found an association with vegetarian diet, frequency of consumption of animal products, general diet quality, or intake of vitamin supplements (11, 30). The mechanism by which phytoestrogens may protect against hypospadias is not clear. Phytoestrogens may protect against various other health outcomes, such as certain cancers, diabetes, and cardiovascular disease (13, 31–35). Their endocrine-related activities as well as their antiproliferative and antioxidant properties may contribute to these associations (31, 36). Further, several recent experimental studies in rodents have suggested either teratogenic or protective effects of phytoestrogens with respect to fetal development (14, 37–40). Nonetheless, our findings warrant replication.

A critical strength of this study is its ability to estimate phytoestrogen intake from a variety of foods, rather than relying only on food groups that are high in phytoestrogens as proxies for intake, as has occurred in previous studies. We developed a database that reflects the phytoestrogen content of foods that were queried for this study (29), using recent values that were generated by using state-of-the-art measurement techniques, for multiple specific phytoestrogens (26). Our study is also strengthened by its large sample size, population-based control selection, and careful case review. A limitation is incomplete or absent collection of data on intake of certain items that contribute to phytoestrogen intake, such as coffee and tea (NBDPS assessed only caffeinated versions), alfalfa sprouts, flax seed, food supplements, and specific soy-containing products, as well as lack of information on bioavailability or absorption. These limitations could have led to misclassification, which we expect to be nondifferential. Foods with modest phytoestrogen content but high frequency of intake tended to be primary contributors to phytoestrogen intake in this study population (29), which may stem from lack of information on high-phytoestrogen foods like soy products. The current study cannot determine whether more extreme phytoestrogen intake, for example, among women consuming large quantities of soy products, is associated with hypospadias, either positively or negatively. This subset of women, although important, is likely to be small. On the basis of recent NBDPS data collection, which involved a modified questionnaire (but insufficient hypospadias cases for a separate analysis), only 3%–4% of control mothers consumed items such as soy milk or tofu at least once per week (29). In addition, we could not validate our phytoestrogen estimates against a "gold standard," such as serum values. However, previous studies of phytoestrogen intake derived from food frequency questionnaires have demonstrated good validity when compared with serum or urine values (41, 42). The study relied on women's recall of dietary intake 1 year before pregnancy. The quality of self-reported information and the predictive value of such information specifically for gestational intake may be subject to error. If this error is unrelated to case status, it would tend to bias effects toward 1.0. We should also note that the NBDPS excluded mild (first-degree) cases by design, so the generalizability of our findings to less severe cases is uncertain.

This study represents the first large-scale analysis of phytoestrogen intake and hypospadias (second and third degree). The observed protective associations merit investigation in additional study populations before firm conclusions can be reached.

ACKNOWLEDGMENTS

Author affiliations: Division of Neonatal and Developmental Medicine, Department of Pediatrics, Stanford University, Stanford, California (Suzan L. Carmichael, Chen Ma, Gary M. Shaw); Division for Heart Disease and Stroke Prevention, Centers for Disease Control and Prevention, Atlanta, Georgia (Mary E. Cogswell); Science Applications International Corporation, Immunization Services Division, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia (Amparo Gonzalez-Feliciano); National Center on Birth Defects and Developmental Disabilities, Centers for Disease Control and Prevention, Atlanta, Georgia (Richard S. Olney, Adolfo Correa); and University of Mississippi Medical Center, Jackson, Mississippi (Adolfo Correa).

This project was partially supported by grants R01 ES017060 and R03 HD058873 from the National Institutes of Health and by grant 6U01DD000489 from the Centers for Disease Control and Prevention; through cooperative agreements under PA 96043, PA 02081, and FOA DD09-001 from the Centers for Disease Control and Prevention to the Centers for Birth Defects Research and Prevention participating in the National Birth Defects Prevention Study; and by grant DK56350 from the Nutrition Epidemiology Core of the University of North Carolina Clinical Nutrition Research Center.

We thank the Maternal Child and Adolescent Health Division, California Department of Public Health, for providing data.

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention or the California Department of Public Health.

Conflict of interest: none declared.

REFERENCES

1. Carmichael SL, Shaw GM, Lammer EJ. Environmental and genetic contributors to hypospadias: a review of the epidemiologic evidence. *Birth Defects Res A Clin Mol Teratol*. 2012;94(7):499–510.
2. Yucel S, Cavalcanti AG, Desouza A, et al. The effect of oestrogen and testosterone on the urethral seam of the developing male mouse genital tubercle. *BJU Int*. 2003; 92(9):1016–1021.
3. Kim KS, Torres CR Jr, Yucel S, et al. Induction of hypospadias in a murine model by maternal exposure to synthetic estrogens. *Environ Res*. 2004;94(3): 267–275.

4. Gray LE Jr, Ostby J, Furr J, et al. Toxicant-induced hypospadias in the male rat. *Adv Exp Med Biol.* 2004;545:217–241.
5. Hsieh MH, Grantham EC, Liu B, et al. In utero exposure to benzophenone-2 causes hypospadias through an estrogen receptor dependent mechanism. *J Urol.* 2007;178(4 Pt 2): 1637–1642.
6. North K, Golding J. A maternal vegetarian diet in pregnancy is associated with hypospadias. The ALSPAC Study Team. Avon Longitudinal Study of Pregnancy and Childhood. *BJU Int.* 2000;85(1):107–113.
7. Pierik FH, Burdorf A, Deddens JA, et al. Maternal and paternal risk factors for cryptorchidism and hypospadias: a case-control study in newborn boys. *Environ Health Perspect.* 2004;112(15):1570–1576.
8. Brouwers MM, Feitz WF, Roelofs LA, et al. Risk factors for hypospadias. *Eur J Pediatr.* 2006;166(7):671–678.
9. Giordano F, Carbone P, Nori F, et al. Maternal diet and the risk of hypospadias and cryptorchidism in the offspring. *Paediatr Perinat Epidemiol*. 2008;22(3):249–260.
10. Ormond G, Nieuwenhuijsen MJ, Nelson P, et al. Endocrine disruptors in the workplace, hair spray, folate supplementation, and risk of hypospadias: case-control study. *Environ Health Perspect.* 2009;117(2):303–307.
11. Carmichael SL, Ma C, Feldkamp ML, et al. Nutritional factors and hypospadias risks. *Paediatr Perinat Epidemiol.* 2012; 26(4):353–360.
12. Akre O, Boyd HA, Ahlgren M, et al. Maternal and gestational risk factors for hypospadias. *Environ Health Perspect.* 2008;116(8):1071–1076.
13. Adlercreutz H. Lignans and human health. *Crit Rev Clin Lab Sci.* 2007;44(5-6):483–525.
14. Vilela ML, Willingham E, Buckley J, et al. Endocrine disruptors and hypospadias: role of genistein and the fungicide vinclozolin. *Urology*. 2007;70(3):618–621.
15. Levy JR, Faber KA, Ayyash L, et al. The effect of prenatal exposure to the phytoestrogen genistein on sexual differentiation in rats. *Proc Soc Exp Biol Med*. 1995; 208(1):60–66.
16. Xu X, Duncan AM, Merz BE, et al. Effects of soy isoflavones on estrogen and phytoestrogen metabolism in premenopausal women. *Cancer Epidemiol Biomarkers Prev*. 1998;7(12): 1101–1108.
17. Bradlow HL, Jernstrom H, Sepkovic DW, et al. Comparison of plasma and urinary levels of 2-hydroxyestrogen and 16α-hydroxyestrogen metabolites. *Mol Genet Metab.* 2006; 87(3):135–146.
18. Walcott FL, Hauptmann M, Duphorne CM, et al. A case-control study of dietary phytoestrogens and testicular cancer risk. *Nutr Cancer*. 2002;44(1):44–51.
19. Setchell KD. Soy isoflavones—benefits and risks from nature's selective estrogen receptor modulators (SERMs). *J Am Coll Nutr.* 2001;20(5 suppl):354S–362S.
20. Yoon PW, Rasmussen SA, Lynberg MC, et al. The National Birth Defect Prevention Study. *Public Health Rep.* 2001; 116(suppl 2):32–40.
21. Rasmussen SA, Olney RS, Holmes LB, et al. Guidelines for case classification for the National Birth Defects Prevention Study. *Birth Defects Res A Clin Mol Teratol*. 2003;67(3): 193–201.
22. Willett WC, Sampson L, Stampfer MJ, et al. Reproducibility and validity of a semiquantitative food frequency questionnaire. *Am J Epidemiol*. 1985;122(1):51–65.
23. Agricultural Research Service, Department of Agriculture. USDA national nutrient database for standard reference, release 20. Washington, DC: Department of Agriculture; 2007. (http://www.ars.usda.gov/Services/docs.htm?docid=17476). (Accessed December 13, 2012).
24. Kuhnle GG, Dell-Aquila C, Aspinall SM, et al. Phytoestrogen content of beverages, nuts, seeds, and oils. *J Agric Food Chem*. 2008;56(16):7311–7315.
25. Kuhnle GG, Dell-Aquila C, Aspinall SM, et al. Phytoestrogen content of foods of animal origin: dairy products, eggs, meat, fish, and seafood. *J Agric Food Chem*. 2008;56(21): 10099–10104.
26. Kuhnle GGC, Dell-Aquila C, Aspinall SM, et al. Phytoestrogen content of fruits and vegetables commonly consumed in the UK based on LC-MS and ^{13}C-labelled standards. *Food Chem.* 2009;116:542–554.
27. Kuhnle GGC, Dell-Aquila C, Aspinall SM, et al. Phytoestrogen content of cereals and cereal-based foods consumed in the UK. *Nutr Cancer*. 2009;61(3):302–309.
28. Kuhnle GG, Dell-Aquila C, Low YL, et al. Extraction and quantification of phytoestrogens in foods using automated solid-phase extraction and LC/MS/MS. *Anal Chem*. 2007; 79(23):9234–9239.
29. Carmichael SL, Gonzalez-Feliciano A, Ma C, et al. Estimated dietary phytoestrogen intake and major food sources among women during the year before pregnancy. *Nutr J*. 2011;10:105.
30. Carmichael SL, Yang W, Correa A, et al. Hypospadias and intake of nutrients related to one-carbon metabolism. *J Urol*. 2009;181(1):315–321.
31. Helferich WG, Andrade JE, Hoagland MS. Phytoestrogens and breast cancer: a complex story. *Inflammopharmacology*. 2008;16(5):219–226.
32. Cano A, Garcia-Perez MA, Tarin JJ. Isoflavones and cardiovascular disease. *Maturitas.* 2010;67(3):219–226.
33. Ward HA, Kuhnle GG, Mulligan AA, et al. Breast, colorectal, and prostate cancer risk in the European Prospective Investigation into Cancer and Nutrition-Norfolk in relation to phytoestrogen intake derived from an improved database. *Am J Clin Nutr.* 2010;91(2):440–448.
34. Nanri A, Mizoue T, Takahashi Y, et al. Soy product and isoflavone intakes are associated with a lower risk of type 2 diabetes in overweight Japanese women. *J Nutr.* 2010; 140(3):580–586.
35. Park SY, Murphy SP, Wilkens LR, et al. Legume and isoflavone intake and prostate cancer risk: The Multiethnic Cohort Study. *Int J Cancer*. 2008;123(4):927–932.
36. Harper A, Kerr DJ, Gescher A, et al. Antioxidant effects of isoflavonoids and lignans, and protection against DNA oxidation. *Free Radic Res*. 1999;31(2):149–160.
37. Zhao H, Liang J, Li X, et al. Folic acid and soybean isoflavone combined supplementation protects the post-neural tube closure defects of rodents induced by cyclophosphamide in vivo and in vitro. *Neurotoxicology*. 2010;31(2):180–187.
38. Xing L, Xu Y, Xiao Y, et al. Embryotoxic and teratogenic effects of the combination of bisphenol A and genistein on in vitro cultured postimplantation rat embryos. *Toxicol Sci.* 2010;115(2):577–588.
39. Jefferson WN, Padilla-Banks E, Goulding EH, et al. Neonatal exposure to genistein disrupts ability of female mouse reproductive tract to support preimplantation embryo development and implantation. *Biol Reprod.* 2009;80(3):425–431.
40. Ruhlen RL, Howdeshell KL, Mao J, et al. Low phytoestrogen levels in feed increase fetal serum estradiol resulting in the "fetal estrogenization syndrome" and obesity in CD-1 mice. *Environ Health Perspect.* 2008;116(3):322–328.

41. French MR, Thompson LU, Hawker GA. Validation of a phytoestrogen food frequency questionnaire with urinary concentrations of isoflavones and lignan metabolites in premenopausal women. *J Am Coll Nutr*. 2007;26(1):76–82.

42. Heald CL, Bolton-Smith C, Ritchie MR, et al. Phyto-oestrogen intake in Scottish men: use of serum to validate a self-administered food-frequency questionnaire in older men. *Eur J Clin Nutr*. 2006;60(1):129–135.