Published in final edited form as:
Nutr Cancer. 2005 ; 52(1): 22–28.

Urinary Sex Steroid Excretion Levels During a Soy Intervention Among Young Girls: A Pilot Study

Gertraud Maskarinec, **Yukiko Morimoto**, and **Adrian A. Franke**
The Cancer Research Center of Hawaii, Honolulu, HI 96813.

Rachel Novotny
The University of Hawaii at Manoa, Human Nutrition, Food and Animal Sciences, Honolulu, HI 96822.

Frank J. Nordt
The Rhein Consulting Laboratories, Portland, OR 97225.

Frank Z. Stanczyk
The University of Southern California, Los Angeles, CA 90033.

Abstract

Soy intake early in life may protect against breast cancer later in life, possibly by altering sex hormone metabolism. We evaluated the feasibility of assessing urinary sex steroid excretion among 20 young girls aged 8–14 yr in an 8-wk trial. The girls consumed one daily soy serving, collected weekly overnight urine samples, and reported Tanner stages for breast and pubic hair development. Sex steroid excretion was measured in duplicate by gas chromatography-mass spectrometry and adjusted for urinary creatinine. The respective coefficients of variation for estrone, estradiol, estriol, testosterone, pregnanediol were 11.4%, 10.4%, 8.4%, 12.8%, and 4.6%. The statistical analysis included t*-tests, Spearman's correlations, and analysis of variance. Seventeen girls completed the study and showed good compliance with the intervention strategy. We observed nonsignificant increases in total androgens (0.11* μ*g/mg creatinine) and total estrogens (0.001* μ*g/mg creatinine) and a nonsignificant decrease in pregnanediol (−0.03* μ*g/mg creatinine) during the study period. Higher Tanner stages for pubic hair development were associated with ninefold higher estrogen, fourfold higher androgen, and twofold higher pregnanediol excretions* ($P = 0.01$, $P < 0.001$, *and* $P = 0.047$, *respectively). Similar differences were observed after stratification by breast development and menarcheal status. The association of sex steroid levels with pubertal development supports the validity of the sex steroid measurements.*

Introduction

A number of reports offer strong support for a protective effect of soy against breast cancer (1-4) despite some conflicting results (5,6). As one possible mechanism of action, an effect of soy isoflavones on circulating estrogen levels has been investigated in premenopausal women (7,8), but recent results indicate that there is probably no such effect (9-11). It appears that soy exposure early in life may play a more important role in the development of breast cancer than soy foods consumed later in life. Two case-control studies showed that women who consumed soy during adolescence experienced protection against breast cancer as adults (12,13). Experimental evidence strongly supports this hypothesis (14-16). So far, no dietary intervention has investigated the effect of soy consumption on sex hormones early in life, and only a few studies have investigated the relation of diet with hormones during adolescence

Address correspondence to Gertraud Maskarinec, MD, PhD, Cancer Research Center of Hawaii, 1236 Lauhala St., Honolulu, HI 96813. Phone: (808) 586–3078. FAX: (808) 586–2984. E-mail: Gertraud@crch.hawaii.edu..

(17-21). One study evaluated the effect of dietary fat consumption on serum concentrations of sex hormones among prepubertal girls and observed a significant decrease in estrogens and a significant increase in testosterone in the low-fat intervention group compared with the controls (21). Other studies indicated that a diet rich in vegetable fiber and a vegetarian dietary pattern might influence serum sex hormone levels and subsequent pubertal development (17,20). Although these findings support the idea that dietary intake affects sex hormone metabolism, intake of soy was not evaluated among these predominantly non-Asian populations. Thus, there is a need for intervention studies to evaluate the possible protective effects of soy against breast cancer through its effect on sex hormone metabolism. As a pilot study, we conducted an 8-wk soy intervention trial in a small sample of young girls to investigate the feasibility of conducting a nutritional intervention in this age group and to test the methodology of detecting urinary sex steroids at such low levels.

Materials and Methods

Subjects and Study Procedures

We recruited 20 free-living girls, ages 8–14 yr, through flyers distributed to staff at our center and to premenopausal women who had participated in a soy trial. The University of Hawaii Committee on Human Studies approved the study protocol; all participants and their parents signed informed consent. During the 8-wk intervention period, subjects consumed one daily serving of sweetened plain soymilk (8.5 oz) or honey-roasted soy nuts (1.0 oz) in individual packages or 3/4 cup (6.7 oz) of tofu. These serving sizes provided approximately 30 mg isoflavones and were chosen from a recently completed soy intervention study in premenopausal women, after adjustment to the lower body weight in girls (11,22). Details of the intervention strategy are described elsewhere(23).

Data Collection

At baseline and at the end of the study, the girls provided information on their demographic background, pubertal development, menarche, first and last menstrual period, and anthropometric characteristics and completed 3-day food records. At the same time, they reviewed drawings of Tanner stages for breast and pubic hair development and selected the stages that most closely resembled their current developmental status. Tanner stages categorize the appearance of breast and pubic hair development into five stages according to sexual maturation (24). Self-assessment of Tanner stages by adolescents was found to be highly comparable with those performed by physicians (25). In addition, those girls who had experienced menarche kept a menstrual calendar and reported their cycle information at follow-up contacts.

Urine Collection and Analysis

The girls collected their overnight urine on the most convenient weekend day at baseline and after each week of intervention. The weekly urine samples were stored in airtight plastic containers in the home freezer until the time of the final visit when all nine samples (baseline, Weeks 1–7, and final) were brought to our center. A mixture of boric and ascorbic acid had been added to the containers to stabilize the isoflavones as well as to prevent bacterial growth that may break down the isoflavones.

The urine specimens were analyzed for isoflavonoid content using high-pressure liquid chromatography with photo diode array and coulometric detection (26,27). We measured urinary creatinine in 0.01 ml of urine using a Jaffé-based test kit from Sigma Company (Kit555, Sigma, St. Louis, MO), adjusted urine concentrations of isoflavones for creatinine levels, and expressed final values per milligram of creatinine as reported previously (28,29). Although the girls collected nine urine samples, due to budget constraints we were only able to evaluate three

urine specimens per girl for sex steroids: baseline, final, and a mixture of seven samples from Weeks 1–7. From each specimen, 12 ml was analyzed; the combined sample of Weeks 1–7 consisted of 1.71 ml from each week.

Steroid excretion profiles were determined via gas chromatography-mass spectrometry (GC-MS) utilizing typical GC-MS conditions analogous to those described by Shackleton (30). All samples were analyzed together. Briefly, for quantitation of total steroids, representing the sum of unconjugated, sulfated, and glucuronidated steroids, urine was extracted using C-18 (octadecylsilyl) solid phase extraction (SPE) columns (500-mg extraction bed, Varian Instruments, Palo Alto, CA) and eluted with methanol. The eluate, after drying, was subjected to hydrolysis using a mixture of β-glucuronidase/aryl sulfatase (*Helix pomatia*, Roche Biochemicals, Indianapolis, IN) and crude β-glucuronidase (*H. pomatia*, Sigma) in sodium acetate buffer, pH 4.8. This digest was again extracted with the C-18 SPE cartridge, and the adsorbed steroids were eluted with methanol, dried down, and derivatized to the methyloxime-trimethylsilyl (MO-TMS) ether. Successful profiling of steroids via GC-MS depends on efficacious derivatization, which this step affords. Derivatization products were purified by taking them up in a small volume of cyclohexane and passing them through a Lipidex column (Hydroxyalkoxypropyldextran, Type IX, Sigma). Finally, an aliquot (≤2μl) of the concentrated eluate in cyclohexane was injected into a nonpolar capillary GC column (for example, HP-1MS, 30 m, Agilent, Palo Alto, CA) housed in a temperature-programmed HP-6890 gas chromatograph. Eluting steroids were identified and quantified using an Agilent 5973 mass spectral detector based on single-ion monitoring with HP-Chemstation software (Agilent) for integration of peak areas. The steroids 5α-androstane-3α,17α-diol, stigmasterol, and cholesteryl butyrate served as internal standards. The latter does not derivatize and was used to monitor efficacy of the column. Recovery standards were added immediately prior to derivatization of the extracted steroids to the MO-TMS ethers. Pooled urine samples (normal and abnormal) served as quality-control samples, which were prepared in-house and were run along with every batch of unknown samples. After adjusting all hormone concentrations for urinary creatinine excretion (expressed as μg/mg creatinine), we added the results for the five androgenic steroids [androsterone, 17β-dihydroandrosterone (5α-androstane-3α,17β-diol), androstenedione, dehydroepiandrosterone, and testosterone] and for the six estrogenic steroids [estrone, estradiol, estriol, 2-hydroxyestrone (2OH-estrone), 16α-hydroxyestrone (16α-OH estrone), and 4-hydroxyestrone (4-OH estrone)] to estimate total androgen and total estrogen excretions.

Statistical Methods

The statistical analysis was performed using Microsoft® Excel 2000/XLSTAT©-Pro (Version 7.5, Addinsoft, Brooklyn, NY); the significance level was set at $P < 0.05$. Sex steroid excretion was analyzed using analysis of variance, *t*-tests, and Spearman's rank order correlation coefficients to detect any differences among the girls' urine samples from baseline, Weeks 1–7, and the final week. Coefficients of variation (CVs) were calculated as the ratio of the standard deviation (SD) and the mean for replicate samples. Sex steroid excretion at baseline was evaluated by pubertal stages and menarcheal status using two-sample *t*-tests.

Results

Of the 17 girls, aged 10.7 ± 2.0 yr, who completed the 8-wk protocol, 6 subjects were exclusively Asian, 8 had at least one parent who was of Asian descent, and 3 girls reported Caucasian ancestry only. The 17 girls reported a mean weekly soy intake of 6.28 servings out of a maximum of 7 servings (31). Three-day food records and urinary isoflavone excretion confirmed a significant increase in soy intake during the intervention period (27.2 mg/day, $P < 0.01$, and 118.8 nmol/mg creatinine, $P = 0.02$, respectively) (32). The mean intra-assay CVs for estrone, estradiol, estriol, testosterone, and pregnanediol were 11.4%, 10.4%, 8.4%, 12.8%,

and 4.6%. Pregnanediol reflects progesterone present during the luteal phase of the menstrual cycle.

The excretion levels of androgens were more than 100 times greater than the excretion of estrogens (Table 1). Androsterone was the most commonly excreted sex steroid; its excretion was lower in Weeks 1–7 (0.80 ± 0.67 μg/mg creatinine) than at baseline (1.04 ± 0.82 μg/mg creatinine) and in the final week (1.14 ± 1.14 μg/mg creatinine). However, this difference was not statistically significant ($P = 0.53$). The large SDs for all the analytes reflect the strong variation among girls. Despite the very low levels of all metabolites, we observed statistically significant correlations among hormones measured at the three points in time for the majority of sex steroids. The correlations were higher for total androgens (0.91–0.93) than for the more cycle-dependent estrogens (0.56–0.77) and pregnanediol (0.62–0.77).

During the study period, we observed a mean increase of 0.11 μg/mg creatinine in the urinary excretion of total androgens ($P = 0.29$), a mean increase of 0.001 μg/mg creatinine in total estrogens ($P = 0.87$), and a mean decrease of 0.03 μg/mg creatinine in pregnanediol ($P = 0.18$). The changes varied greatly among girls and were not statistically significant (Table 1) except for dehydroepiandrosterone ($P = 0.007$). The increase in estrone excretion was close to statistical significance ($P = 0.09$) but not that of estradiol ($P = 0.36$). Interestingly, marked differences in hormonal excretion were noted among girls according to their developmental status. The eight girls reporting Tanner Stages 3 and 4 for pubic hair development excreted significantly higher levels of sex hormones than the nine girls in Stage 1 (Table 2a). The differences were ninefold for total estrogens ($P = 0.01$), fourfold for total androgens ($P < 0.001$), and twofold for pregnanediol ($P = 0.047$). Similar differences were observed after stratification by breast development (Table 2b). Total androgens and total estrogens were three times higher for girls in Stages 2 and 3 than for girls in Stage 1 ($P = 0.003$ and $P = 0.12$, respectively); pregnanediol was twice as high in the more-developed girls. Baseline androgen and estrogen excretion was approximately twice as high in the 4 girls who had experienced menarche as in the 13 girls who were not yet menstruating (Table 3), but these results were also not statistically significant.

Discussion

This study is the first to demonstrate the feasibility of collecting regular urine samples and of evaluating sex hormone excretion among young girls during a soy intervention. The fact that we detected little change in overall sex hormone excretion as a result of 8 wk of soy intake is not surprising considering the small sample size and the short duration of the intervention; in a low-fat intervention among girls, changes in hormone levels were most pronounced in Year 5 (33). Longer trials are needed to examine the effects of soy consumption on sex hormones in young girls. The few significant changes in hormone excretion were probably a result of chance given the low statistical power because of the limited sample size and the great variability in sex hormone excretion among girls. The changes in estrogens were in the opposite direction expected for a protective effect from soy, most likely due to increasing hormone levels in this age group as a consequence of sexual maturation. This illustrates the need for a control group in future studies. However, there is no evidence at this time whether pubertal hormone levels play a role in developing breast cancer later in life, although sex hormones were linked to increased breast cancer risk among postmenopausal women (34). Pubertal soy intake may confer a protective effect against breast cancer through nonhormonal mechanisms.

There are three reasons that support the soundness of our urinary findings. First, the intra-assay CVs of estrogens, androgens, and pregnanediol were all below 13%, indicating good reproducibility of the sex hormone assessments using GC-MS. Second, high correlations among urinary excretions over 9 wk, particularly for androgens, corroborate the accuracy of

the methodology. Finally, distinct differences in hormonal excretion after stratification by Tanner stages and by menarcheal status confirm the biological validity of our measurements. Our results agree with an investigation among 56 girls aged 4.6–16.5 yr that showed high levels of androgens relative to estrogens during early puberty (35). As mentioned previously, only a few studies have investigated the effect of diet on hormonal levels in young girls (17,20,36). Although all these studies collected blood samples to analyze circulating sex hormone levels, our current study evaluated sex steroid excretion in overnight urine samples as a more noninvasive approach. For long-term studies, we consider that the only practical procedure. One major limitation of our current study was the use of overnight urine samples. Obviously, 24-h urine collections would have been better in that they might have produced fewer undetectable hormone levels and a better comparison with levels in adult women. However, it would be very challenging, if not impossible, for free-living, school-age girls to collect urine over a 24-h period. Considering the diurnal rhythm of testosterone secretion in girls (35), it appears that a morning urine collection may capture the high levels at that time.

To compare the hormone levels of girls with those of adult women, we estimated hormone levels in girls per 24 h by multiplying the measured hormone levels with a reference value for daily creatinine excretion according to age (8–30 mg/kg body weight/day) (37). The respective values for total estrogens and pregnanediol were 2.85–10.68 μg/day and 55–209 μg/day. The reference ranges using the same GC-MS method as in this study are 23–158 μg/day for total estrogens and 800–3,290 μg/day for pregnanediol in premenopausal women during the luteal phase. This indicates that mean estrogen excretion in our group of young girls is approximately 10-fold lower than in adult women. The difference in pregnanediol was more than 15-fold due to the lack of a luteal phase in the majority of girls. Based on a crude, nonadjusted comparison with published results (38) from 24-h urine collections, it appears that the relative urinary sex steroid excretion pattern of girls was comparable with that of premenopausal women. In the girls, all the estrogens were excreted in similar proportions; only the relative amounts of estradiol and 4-OH estrone appeared somewhat lower than those of other estrogens.

The very low and sometimes nondetectable excretion levels of sex steroids pose the greatest challenge to this type of research. Even this very sensitive and expensive GC-MS method could not detect all metabolites in all girls, a fact that will discourage the use of faster and cheaper assays in future studies. Due to financial considerations, we had to combine the urine samples from Weeks 1–7 instead of examining them individually. The pooling of multiple samples may have affected our results in that it may have reduced the strong variability of individual hormone measurements due to the cyclic pattern of hormone excretion. This phenomenon may be responsible for the 20–30% lower excretion of total androgens in Weeks 1–7 than at baseline and in the final week. In the presence of numerous undetectable hormone levels, mean levels from the combined sample probably provided a more stable assessment than hormone excretion levels obtained from a single urine specimen. Furthermore, the frequency of undetectable hormone values may have contributed to an overestimate of correlation coefficients between collection times. Another limitation of this study is that we did not ask the girls who had reached menarche to collect timed urine samples. As a result, the samples were collected at various time points during the menstrual cycle. Moreover, there were fewer subjects in the menarcheal (N=4) than in the nonmenarcheal group (N = 13). These factors contributed to a relatively large within-group variation in mean hormone excretion levels among the girls who had experienced menarche and were probably responsible for the nonsignificant difference in sex steroid excretion levels between the two groups.

The present study demonstrated that urinary sex hormone excretion levels from young girls were measurable in a single urine sample with acceptable reproducibility and high sensitivity and that they showed a significant association with pubertal development. Self-assessed Tanner stages for pubertal development were associated with a clear difference in sex hormone levels

among young girls. Stratification by menarcheal status also suggested that, despite the inconsistency in the timing of urine collection during the menstrual cycle, the differences in sex hormone levels between menarcheal and nonmenarcheal girls were detectable through the evaluation of urinary excretion levels. These preliminary findings will be useful in future studies investigating the effect of soy consumption on sex hormone levels during adolescence, but, given the small number of girls in this pilot study and the large variation in urinary measurements, our results need to be validated in a much larger population exposed to soy foods over a longer time period.

Acknowledgments and Notes

We are grateful to the girls who participated in this study. This investigation was supported by a Research Centers in Minority Institutions award, P20 RR11091, from the National Center for Research Resources, National Institutes of Health. Thank you to DrSoy Nutrition, Aloha Tofu Factory, and Foodland Supermarkets for supplying us with soy foods. We would also like to acknowledge our dedicated staff members for their efforts.

References

1. Lee HP, Gourley L, Duffy SW, Esteve J, Lee J, et al. Dietary effects on breast-cancer risk in Singapore. Lancet 1991;337:1197–1200. [PubMed: 1673746]
2. Wu AH, Ziegler RG, Horn-Ross PL, Nomura AM, West DW, et al. Tofu and risk of breast cancer in Asian-Americans. Cancer Epidemiol Biomarkers Prev 1996;5:901–906. [PubMed: 8922298]
3. Dai Q, Shu XO, Jin F, Potter JD, Kushi LH, et al. Population-based case-control study of soyfood intake and breast cancer risk in Shanghai. Br J Cancer 2001;85:372–378. [PubMed: 11487268]
4. Yamamoto S, Sobue T, Kobayashi M, Sasaki S, Tsugane S. Soy, isoflavones, and breast cancer risk in Japan. JNCI 2003;95:906–913. [PubMed: 12813174]
5. Yuan JM, Wang QS, Ross RK, Henderson BE, Yu MC. Diet and breast cancer in Shanghai and Tianjin, China. Br J Cancer 1995;71:1353–1358. [PubMed: 7779738]
6. Key TJ, Sharp GB, Appleby PN, Beral V, Goodman MT, et al. Soya foods and breast cancer risk: a prospective study in Hiroshima and Nagasaki, Japan. Br J Cancer 1999;81:1248–1256. [PubMed: 10584890]
7. Nagata C, Takatsuka N, Inaba S, Kawakami N, Shimizu H. Effect of soymilk consumption on serum estrogen concentrations in premenopausal Japanese women. JNCI 1998;90:1830–1835. [PubMed: 9839524]
8. Wu AH, Stanczyk FZ, Hendrich S, Murphy PA, Zhang C, et al. Effects of soy foods on ovarian function in premenopausal women. Br J Cancer 2000;82:1879–1886. [PubMed: 10839307]
9. Maskarinec G, Williams AE, Stanczyk FZ, Stanczyk FZ, Franke AA. A randomized isoflavone intervention among premenopausal women. Cancer Epidemiol Biomarkers Prev 2002;11:195–201. [PubMed: 11867507]
10. Kumar NB, Cantor A, Allen K, Riccardi D, Cox CE. The specific role of isoflavones on estrogen metabolism in premenopausal women. Cancer 2002;94:1166–1174. [PubMed: 11920488]
11. Maskarinec G, Franke AA, Williams AE, Hebshi S, Oshiro C, et al. Effects of a 2-year randomized soy intervention on sex hormone levels in premenopausal women. Cancer Epidemiol Biomarkers Prev 2004;13:1736–1744. [PubMed: 15533901]
12. Shu XO, Jin F, Dai Q, Wen W, Potter JD, et al. Soyfood intake during adolescence and subsequent risk of breast cancer among Chinese women. Cancer Epidemiol Biomarkers Prev 2001;10:483–488. [PubMed: 11352858]
13. Wu AH, Wan P, Hankin J, Tseng CC, Yu MC, et al. Adolescent and adult soy intake and risk of breast cancer in Asian-Americans. Carcinogenesis 2002;23:1491–1496. [PubMed: 12189192]
14. Lamartiniere CA, Moore J, Holland M, Barnes S. Neonatal genistein chemoprevents mammary cancer. Proc Soc Exp Biol Med 1995;208:120–123. [PubMed: 7892285]
15. Murrill WB, Brown NM, Zhang JX, Manzolillo PA, Barnes S, et al. Prepubertal genistein exposure suppresses mammary cancer and enhances gland differentiation in rats. Carcinogenesis 1996;17:1451–1457. [PubMed: 8706248]

16. Hilakivi-Clarke L, Onojafe I, Raygada M, Cho E, Skaar T, et al. Prepubertal exposure to zearalenone or genistein reduces mammary tumorigenesis. Br J Cancer 1999;80:1682–1688. [PubMed: 10468283]

17. Persky VW, Chatterton RT, Van Horn LV, Grant MD, Langenberg P, et al. Hormone levels in vegetarian and nonvegetarian teenage girls: potential implications for breast cancer risk. Cancer Res 1992;52:578–583. [PubMed: 1531038]

18. Gray GE, Williams P, Gerkins V, Brown JB, Armstrong B, et al. Diet and hormone levels in Seventh-Day Adventist teenage girls. Prev Med 1982;11:103–107. [PubMed: 7079242]

19. Wilson DW, Turkes A, Jones R, Danutra V, Read GF, et al. A comparison of menstrual cycle profiles of salivary progesterone in British and Thai adolescent girls. Eur J Cancer 1992;28A:1162–1167. [PubMed: 1627388]

20. de Ridder CM, Thijssen JH, van't Veer P, van Duuren R, Bruning PF, et al. Dietary habits, sexual maturation, and plasma hormones in pubertal girls: a longitudinal study. Am J Clin Nutr 1991;54:805–813. [PubMed: 1951150]

21. Dorgan JF, Hunsberger SA, McMahon RP, Kwiterovich PO Jr, Lauer RM, et al. Diet and sex hormones in girls: findings from a randomized controlled clinical trial. JNCI 2003;95:132–141. [PubMed: 12529346]

22. Maskarinec G, Robbins C, Riola B, Kane-Sample L, Franke A, et al. Three measures show high compliance in soy intervention among premenopausal women. J Am Diet Assoc 2003;103:861–866. [PubMed: 12830025]

23. Adlercreutz H, Brown J, Collins W, Goebelsman U, Kellie A, et al. The measurement of urinary steroid glucuronides as indices of the fertile period in women. World Health Organization, Task Force on Methods for the Determination of the Fertile Period, special programme of research, development and research training in human reproduction. J Steroid Biochem 1982;17:695–702. [PubMed: 7176659]

24. Tanner, JM. Growth at Adolescence, with a General Consideration of the Effects of Hereditary and Environmental Factors upon Growth and Maturation from Birth to Maturity. Blackwell Scientific; Oxford: 1962.

25. Duke PM, Litt IF, Gross RT. Adolescents' self-assessment of sexual maturation. Pediatrics 1980;66:918–920. [PubMed: 7454482]

26. Franke AA, Custer LJ, Wang W, Shi SJ. HPLC analysis of isoflavonoids and other phenolic agents from foods and from human fluids. Proc Soc Exp Biol Med 1998;217:263–273. [PubMed: 9492334]

27. Franke AA, Custer LJ. High-performance liquid chromatographic assay of isoflavonoids and coumestrol from human urine. J Chromatography B 1994;662:47–60.

28. Zheng W, Dia Q, Custer LJ, Shu XO, Wen WQ, et al. Urinary excretion of isoflavonoids and the risk of breast cancer. Cancer Epidemiol Biomarkers Prev 1999;8:35–40. [PubMed: 9950237]

29. Dai Q, Franke AA, Jin F, Shu XO, Hebert JR, et al. Urinary excretion of phytoestrogens and risk of breast cancer among Chinese women in Shanghai. Cancer Epidemiol Biomarkers Prev 2002;11:815–821. [PubMed: 12223424]

30. Shackleton CH. Profiling steroid hormones and urinary steroids. J Chromatogr 1986;379:91–156. [PubMed: 3525596]

31. Oshiro C, Maskarinec G, Petitpain D, Hebshi S, Novotny R. A soy intervention in adolescent girls: design and implementation. J Nutr Educ 2004;36:204–208.

32. Maskarinec G, Oshiro C, Morimoto Y, Hebshi S, Novotny R, et al. Urinary isoflavone excretion as a compliance measure in a soy intervention among young girls: a pilot study. Eur J Clin Nutr 2004;59:369–375. [PubMed: 15523482]

33. Dorgan JF, Hunsberger SA, McMahon RP, Kwiterovich PO Jr, Lauer RM, et al. Diet and sex hormones in girls: findings from a randomized controlled clinical trial. JNCI 2003;95:132–141. [PubMed: 12529346]

34. The Endogenous Hormones and Breast Cancer Collaborative Group. Endogenous sex hormones and breast cancer in postmenopausal women: reanalysis of nine prospective studies. JNCI 2002;94:606–616. [PubMed: 11959894]

35. Ankarberg C, Norjavaara E. Diurnal rhythm of testosterone secretion before and throughout puberty in healthy girls: correlation with 17beta-estradiol and dehydroepiandrosterone sulfate. J Clin Endocrinol Metab 1999;84:975–984. [PubMed: 10084582]

36. Dorgan JF, Hunsberger SA, McMahon RP, Kwiterovich PO Jr, Lauer RM, et al. Diet and sex hormones in girls: findings from a randomized controlled clinical trial. JNCI 2003;95:132–141. [PubMed: 12529346]

37. Burtis, CA.; Ashwood, ER. Tietz Textbook of Clinical Chemistry. WB Saunders; Philadelphia: 1998.

38. Xu X, Duncan AM, Merz BE, Kurzer MS. Effects of soy isoflavones on estrogen and phytoestrogen metabolism in premenopausal women. Cancer Epidemiol Biomarkers Prev 1998;7:1101–1108. [PubMed: 9865428]

Table 1

Sex Hormone Excretion Among 17 Young Girls During a Soy Intervention[a]

Sex Hormone (μg/mg creatinine)	Baseline	Weeks 1–7 Mean ± SD	Final	P[b]	Baseline and Final		Baseline and Weeks 1–7		Baseline and Final		Weeks 1–7 and Final	
					Change[c]	P	r[d]	P	r[d]	P	r[d]	P
Total androgens	1.10 ± 0.83	0.87 ± 0.66	1.21 ± 1.16	0.54	0.11	0.29	0.92	<**0.001**	0.93	<**0.001**	0.91	<**0.001**
Androsterone	1.04 ± 0.82	0.80 ± 0.67	1.14 ± 1.14	0.53	0.10	0.33	0.95	<**0.001**	0.92	<**0.001**	0.97	<**0.001**
17β-Dihydroandrosterone	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.16	−0.0008	0.71	0.68	**0.003**	0.68	**0.003**	0.74	<**0.001**
Dehydroepiandrosterone	0.03 ± 0.03	0.05 ± 0.08	0.05 ± 0.03	0.54	0.02	0.007	0.54	**0.03**	0.81	<**0.001**	0.74	<**0.001**
Androstenedione	0.002 ± 0.001	0.001 ± 0.003	0.0004 ± 0.002	0.55	−0.002	0.23	−0.13	0.61	0.64	**0.01**	−0.09	0.73
Testosterone	0.002 ± 0.003	0.003 ± 0.002	0.002 ± 0.004	0.73	0.0009	0.85	0.17	0.52	0.67	**0.003**	0.41	0.10
Total estrogens	0.01 ± 0.012	0.009 ± 0.008	0.011 ± 0.013	0.94	0.001	0.87	0.56	**0.02**	0.59	**0.01**	0.77	<**0.001**
Estrone	0.002 ± 0.003	0.003 ± 0.003	0.004 ± 0.005	0.43	0.002	0.09	0.73	**0.001**	0.77	<**0.001**	0.87	<**0.001**
Estradiol	0.0006 ± 0.001	0.0007 ± 0.001	0.001 ± 0.002	0.59	0.0004	0.36	0.29	0.26	0.81	<**0.001**	0.37	0.14
Estriol	0.001 ± 0.002	0.002 ± 0.004	0.002 ± 0.004	0.77	0.001	0.25	0.69	**0.002**	0.81	<**0.001**	0.86	<**0.001**
2-OH Estrone	0.002 ± 0.001	0.003 ± 0.004	0.003 ± 0.002	0.64	0.001	0.52	0.73	**0.001**	0.56	**0.02**	0.79	<**0.001**
16α-OH Estrone	0.004 ± 0.010	ND	0.0004 ± 0.001	0.13	−0.004	0.20	NA	NA	−0.17	0.52	NA	NA
4-OH Estrone	0.0002 ± 0.0002	0.0004 ± 0.0004	0.0005 ± 0.0009	0.22	0.0003	0.16	0.03	0.92	−0.03	0.91	0.66	**0.004**
Pregnanediol	0.19 ± 0.15	0.19 ± 0.17	0.16 ± 0.10	0.80	−0.03	0.18	0.67	**0.003**	0.62	**0.008**	0.77	<**0.001**

[a] Bold signifies statistical significance. Abbreviations are as follows: ND, nondetectable; NA, not applicable.

[b] Analysis of variance among baseline, Weeks 1–7, and final.

[c] Change in hormones from baseline to final (μg/mg creatinine).

[d] Spearman's correlation coefficient.

Table 2a

Mean Sex Hormone Excretion by Tanner Stage for Pubic Hair Development

Sex Hormone (μg/mg creatinine)	Stage 1	Stages 3 and 4	*P* Value[a]
Subjects (*N*)	9	8	
Total androgens	0.47 ± 0.24	1.80 ± 0.67	<**0.001**
Androsterone	0.43 ± 0.24	1.72 ± 0.68	<**0.001**
17β-Dihydroandrosterone	0.02 ± 0.01	0.03 ± 0.01	**0.009**
Dehydroepiandrosterone	0.02 ± 0.02	0.05 ± 0.03	0.08
Androstenedione	0.002 ± 0.006	0.001 ± 0.004	0.83
Testosterone	0.002 ± 0.002	0.003 ± 0.004	0.51
Total estrogens	0.002 ± 0.003	0.018 ± 0.013	0.01
Estrone	0.0002 ± 0.0006	0.004 ± 0.003	0.02
Estradiol	0.0003 ± 0.0008	0.001 ± 0.0015	0.28
Estriol	0.0005 ± 0.001	0.002 ± 0.002	**0.006**
2-OH Estrone	0.0008 ± 0.0005	0.004 ± 0.001	0.01
16α-OH Estrone	0.0005 ± 0.001	0.007 ± 0.013	0.21
4-OH Estrone	0.0001 ± 0.0001	0.0003 ± 0.0003	0.09
Pregnanediol	0.12 ± 0.05	0.28 ± 0.18	**0.047**

[a]Two-sample *t*-test. Bold signifies statistical significance.

Table 2b

Mean Sex Hormone Excretion by Tanner Stage for Breast Development

Sex Hormone (μg/mg creatinine)	Stage 1	Stages 2 and 3	*P* Value[a]
Subjects (*N*)	9	8	
Total androgens	0.54 ± 0.39	1.72 ± 0.76	**0.003**
Androsterone	0.50 ± 0.37	1.64 ± 0.76	**0.003**
17β-Dihydroandrosterone	0.02 ± 0.01	0.03 ± 0.01	0.10
Dehydroepiandrosterone	0.02 ± 0.02	0.05 ± 0.03	0.10
Androstenedione	0.002 ± 0.006	0.001 ± 0.004	0.83
Testosterone	0.002 ± 0.002	0.003 ± 0.005	0.51
Total estrogens	0.005 ± 0.009	0.015 ± 0.014	0.12
Estrone	0.0002 ± 0.0006	0.004 ± 0.003	**0.02**
Estradiol	0.0005 ± 0.0008	0.0008 ± 0.0015	0.57
Estriol	0.0008 ± 0.001	0.0035 ± 0.0024	**0.02**
2-OH Estrone	0.0006 ± 0.0005	0.0024 ± 0.0015	**0.007**
16α-OH Estrone	0.003 ± 0.008	0.004 ± 0.012	0.83
4-OH Estrone	0.0002 ± 0.0003	0.0002 ± 0.0002	0.92
Pregnanediol	0.14 ± 0.09	0.25 ± 0.18	0.15

[a]Two-sample *t*-test. Bold signifies statistical significance.

Table 3

Mean Hormone Excretion by Menarcheal Status[a]

Hormone (μg/mg creatinine)	No Menarche	Menarche	*P* Value[b]
Subjects (*N*)	13	4	
Total androgens	0.87 ± 0.68	1.83 ± 0.95	0.14
Androsterone	0.81 ± 0.65	1.77 ± 0.97	**0.036**
17(β-Dihydroandrosterone	0.02 ± 0.01	0.02 ± 0.005	0.85
Dehydroepiandrosterone	0.034 ± 0.032	0.037 ± 0.028	0.86
Androstenedione	0.002 ± 0.006	ND	NA
Testosterone	0.003 ± 0.004	ND	NA
Total estrogens	0.007 ± 0.01	0.018 ± 0.017	0.57
Estrone	0.001 ± 0.003	0.004 ± 0.003	0.14
Estradiol	0.0008 ± 00013	ND	0.24
Estriol	0.0018 ± 0.0024	0.0029 ± 0.0013	0.41
2-OH Estrone	0.001 ± 0.001	0.002 ± 0.001	0.20
16(α-OH Estrone	0.002 ± 0.007	0.009 ± 0.017	0.52
4-OH Estrone	0.0002 ± 0.0003	0.0002 ± 0.0002	0.93
Pregnanediol	0.17 ± 0.10	0.29 ± 0.25	0.16

[a] Abbreviations are as follows: ND, nondetectable; NA, not applicable.

[b] Two-sample *t*-test. Bold signifies statistical significance.