A Pilot Study of Urinary Estrogen Metabolites (16α-OHE, and 2-OHE) in Postmenopausal Women with and without Breast Cancer

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The two main pathways for metabolizing estrogen are via 16α-hydroxylation and 2-hydroxylation. The 16α-hydroxy metabolites are biologically active; the 2-hydroxy metabolites are not. It is suggested that women who metabolize a larger proportion of their endogenous estrogen via the 16α-hydroxy pathway may be at significantly elevated risk of breast cancer compared with women who metabolize proportionally more estrogen via the 2-hydroxy pathway. In particular, it is suggested that the ratio of urinary 2-hydroxyestrone (2-OHE1) to 16α-hydroxyestrone (16α-OHE1) is an index of reduced breast cancer risk. This pilot study compared this ratio in postmenopausal women diagnosed with breast cancer to those of healthy controls. Urinary concentrations of estrone (E1), 17β-estradiol (E2) and estriol (E3) were also quantified. White women who were subjects in a previous breast cancer case-control study at our institution were eligible for inclusion. All participants provided a sample of their first morning urine. The results from the first 25 cases and 23 controls are presented here. The ratio of 2-OHE1 to 16α-OHE1 was 12% lower in the cases (p=0.58). However, urinary E1 was 30% higher (p=0.10), E2 was 58% higher (p=0.07), E3 was 15% higher (p=0.48), and the sum of E1, E2, and E3 was 22% higher (p=0.18) in the cases. These preliminary results do not support the hypothesis that the ratio of the two hydroxylation metabolites (2-OHE1/16α-OHE1) is an important risk factor for breast cancer or that it is a better predictor of breast cancer risk than levels of E1, E2 and E3 measured in urine. — Environ Health Perspect 105(Suppl 3):601–606 (1997)

Key words: estrogen metabolism, 16α-hydroxyestrone, 2-hydroxyestrone, breast cancer, urinary estrogen metabolites

Introduction

Overwhelming evidence supports a role of ovarian hormones in the etiology of breast cancer (1). At menopause circulating estrogens decline sharply, explaining in large part, and possibly completely, the decreased breast cancer risk associated with early menopause (2). In postmenopausal women, the major source of estrogen arises from the peripheral conversion of androstenedione in adipose tissue (3). This, together with decreased sex hormone-binding globulin levels, is the most probable explanation for the higher breast cancer risk in obese postmenopausal women (4). Both elevated serum estrogen levels (5–16) and increased urinary excretion rates of estrone (E1), 17β-estradiol (E2) and estriol (E3) have been found in breast cancer cases as compared with controls (17–24).

The two main pathways for metabolizing estrogen are via 16α-hydroxylation and 2-hydroxylation, and the major estrogen metabolites excreted in urine are 2-hydroxy products [2-hydroxyestrone (2-OHE1), 2-hydroxyestradiol (2-OH-E2), 2-methoxyestrone (2-MeO-E1)], nonmetabolized E1, 16α-hydroxy products [E2, 16α-hydroxyestrone (16α-OHE1)] and E2 (25). The 16α-metabolites are biologically active (26,27); the 2-hydroxy metabolites are not (28).

The extent to which estrogen is metabolized via the 16α-hydroxylation pathway may be associated with breast cancer risk (29–31). Increased 16α-hydroxylation activity, but not 2-hydroxylation activity, has been observed in mice strains with high spontaneous mammary tumor formation (29). In humans, the extent of biotransformation of 3H-E2 via the 16α-hydroxylation pathway was 4.6-fold higher in terminal duct lobular units in breast tissue from breast cancer cases than in breast tissue from reduction mammoplasty controls (32). Two other epidemiologic studies suggested that the extent of 16α-hydroxylation was higher in women with breast cancer (33) and in women with high familial risk of breast cancer (34) than in controls. However, a third study found no elevation of 16α-hydroxylation in breast cancer cases compared with controls (25).

We selected women interviewed in a previous population-based epidemiologic study to determine whether postmenopausal women with breast cancer have a lower ratio of urinary 2-OHE1 to 16α-OHE1 than controls. We report here the data from the first 25 cases and 23 controls.

Methods

This study was approved by the local Institutional Review Board. Written informed consent was obtained from each participant.

Eligible cases were identified from women between 55 and 64 years of age...
diagnosed with histologically confirmed breast cancer, identified through the Los Angeles County Cancer Surveillance Program (a National Cancer Institute Surveillance, Epidemiology, and End Results Program Registry), who had participated in a previous breast cancer case–control study conducted at our institution (35). The dates of diagnosis were 1 March 1987 through 31 December 1989. Only women diagnosed with incident cancer at stage II or less [tumor size ≤T2, nodes ≤N1, and no distant metastasis (M0), or T3, N0, M0] were included (36). Eligible controls were participants in the same case–control study who had not been diagnosed with breast cancer. Subjects had to be English-speaking whites (including Hispanics), and residents of Los Angeles County.

Cases and controls were contacted; the most recent interviewees were contacted first. Eligibility was determined based on a phone interview. Subjects who had used medications during the previous 6 months that may have interfered with estrogen metabolism (specifically, cimetidine, thyrroxine, estrogen, progesterone, tamoxifen, or 63 fatty acid supplements) (37–40) were eliminated from the study. Subjects who had general anesthesia in the previous 3 months or weighed more than 200 lb (90 kg) were also excluded.

A box containing a 100-ml urine vial with a 100-mg ascorbate tablet, a small cooler with an ice pack, an informed consent form, and a questionnaire on recent intake of medication, alcohol, and specific foods was shipped to each eligible woman who agreed to participate. First morning urine samples were collected, aliquoted, and frozen at −70°C within 6 hr after specimens were produced.

Urine samples were sent to two different laboratories. Batches of 30 samples (15 from cases, 15 from controls, including 10% duplicates) were coded and shipped on dry ice. The only identifiers on the samples were code numbers ensuring that the laboratories were blinded as to case or control status of the individual samples and to the identity of duplicates.

**Enzyme Immunoassay of 16α-OHE1 and 2-OHE1**

Measurements of urinary 16α-OHE1 and 2-OHE1 were carried out using commercially available competitive enzyme immunoassay (EIA) kits (Estramet, Immuna Care Corporation, Bethlehem, PA) to measure 2-OHE1 and 16α-OHE1 directly in urine. The two metabolites were measured simultaneously to avoid interassay variation. This method has been described in detail by Klug et al. (41). In brief, monoclonal antibodies to the estrogen metabolites were immobilized directly to the solid phase, and the metabolite standards were conjugated to alkaline phosphatase enzyme. Each urine sample was acidified and subjected to β-glucuronidase/aryl sulfatase hydrolysis before assay.

The 16α-OHE1 and 2-OHE1 EIA kits were validated by comparing values obtained with these kits to values obtained by gas chromatography–mass spectroscopy (41). The inter- and intraassay coefficients of variation for 2-OHE1 and 16α-OHE1 were between 7 and 13% (41). Creatinine values above 0.20 mg/ml are considered necessary to obtain adequate reproducibility of the 2-OHE1 and 16α-OHE1 assays (HL Bradlow, personal communication).

**Radioimmunoassay of Urinary E1, E2, and E3**

Measurements of urinary E1, E2, and E3 were carried out using high-performance liquid chromatography–radioimmunoassay (HPLC–RIA). Each urine sample was acidified and subjected to β-glucuronidase/aryl sulfatase hydrolysis before assay.

Following the addition of approximately 10000 dpm of 3H-E1, 3H-E2, and 3H-E3, which served as internal standards to follow procedural losses, solid phase extraction was performed. Ethyl acetate was used to extract the estrogens, the organic solvent was evaporated and the extract was subjected to HPLC. A reverse-phase HPLC column (C18) was used to elute E3, E2, and E1 in a gradient of acetonitrile:water:acetic acid (40:60:0.1) at a flow rate of 1 ml/min. The retention times for E3, E2, and E1 were 4.13, and 16 min, respectively.

The E1, E2, and E3 fractions were quantified by RIA, using methods previously described by Katagiri et al. (42), Stanczyk et al. (43), and Cassdenti et al. (44). Appropriate quality controls were used with each set of samples that was assayed to monitor assay reliability.

**Statistical Analysis**

All directly measured hormone variables were lognormally distributed, and the statistical significance of the difference in these variables between cases and controls was evaluated using t tests of the natural logs of these values. The statistical significance of the differences in 2-OHE1/16α-OHE1 between cases and controls was evaluated using Wilcoxon's nonparametric rank sum test. Statistical analyses were conducted using SAS (SAS Institute, Cary, NC).

**Results**

The full study will include almost 100 cases and 100 controls. We reported here results from the first subset of the women enrolled in the study.

The results for the first two batches of urine samples were available for the analyses reported here. These represented 27 cases, 27 controls, and 6 duplicate samples. We excluded six samples with low creatinine values. Among the remaining 25 cases and 23 controls, the mean 16α-OHE1 was 8.0% higher and the mean 2-OHE1 was 3.9% lower in cases than in controls (Table 1). The ratio of 2-OHE1 to 16α-OHE1 was 12.0% lower in cases. None of these differences were statistically significant. The individual values of 2-OHE1/16α-OHE1 are plotted in Figure 1.

Ratios of 2-OHE1/16α-OHE1 below 2.0 have been suggested as an index of high risk of breast cancer (HL Bradlow, personal communication). However, in this study, nearly all cases and controls had at least this low ratio; 20 of 23 controls and 24 of 25 cases had ratios less than 2.0.

E2 was 30% higher (p = 0.10) and E2 was 58% higher (p = 0.07) in cases than in controls. E2 was 15% higher and the sum of E1, E2, and E3 was 22% higher in cases; neither result was statistically significant.

### Table 1. Mean levels of estrogen metabolites in postmenopausal breast cancer cases and controls.

| Urinary metabolite* | Cases, n = 25 | Controls, n = 23 | Difference, †% | p value |
|---------------------|--------------|-----------------|----------------|--------|
| 2-OHE1              | 7.09 ± 0.89  | 7.38 ± 0.77     | −3.9           | 0.89   |
| 16α-OHE1            | 5.27 ± 0.47  | 4.88 ± 0.57     | 8.0            | 0.61   |
| 2-OHE1/16α-OHE1     | 1.39 ± 0.10  | 1.58 ± 0.20     | −12            | 0.59   |
| E1                  | 3.14 ± 0.34  | 2.42 ± 0.34     | 30             | 0.10   |
| E2                  | 0.87 ± 0.14  | 0.55 ± 0.06     | 58             | 0.07   |
| E3                  | 5.63 ± 0.66  | 4.90 ± 0.49     | 15             | 0.48   |
| E1+E2+E3            | 9.64 ± 0.94  | 7.87 ± 0.81     | 22             | 0.16   |

*ng/mg creatinine. †[(cases mean value)/(controls mean value)] × 100.
The coefficients of variation for the six blind duplicates were 13% for 2-OHE$_1$, 20% for 16α-OHE$_1$, 13% for E$_1$, 14% for E$_2$, and 24% for E$_3$.

**Discussion**

Our results confirm previous studies that E$_1$ and E$_2$ are higher in urine of postmenopausal breast cancer cases than controls (17–24). However, we found only small differences between cases and controls in urinary levels of 16α-OHE$_1$, 2-OHE$_1$, and the ratio of the two.

The epidemiologic data addressing the 2-OHE$_1$/16α-OHE$_1$ hypothesis are sparse. Schneider and co-workers used a radiometric method to determine the extent of 2- and 16α-hydroxylation (33). They injected 33 peri- and postmenopausal breast cancer patients and 10 postmenopausal controls with E$_2$ tracers labeled with $^3$H in the 17α, C-2, and 16α position. They drew serial blood samples before and after isotope administration and determined the rate and extent of the oxidative metabolism at positions 17α, C-2, and 16α. Cases had 60% higher extent of 16α-hydroxylation than controls; this difference was statistically significant. However, the two groups did not differ significantly in the extent of 2-hydroxylation, which was only 5% higher among cases. The ratio of the average level of 16α-hydroxylation to the average level of 2-hydroxylation was 52% greater in the breast cancer cases than in the controls. No data on total estrogen values were provided.

The only other published study of 16α-/2-hydroxylation in breast cancer patients was performed by Adlercreutz et al. (25). They examined estrogen metabolites in young Finnish premenopausal breast cancer cases ($n = 10$) and control women on an omnivorous normal Finnish diet ($n = 12$) or on a lacto-vegetarian diet ($n = 11$). There was no statistically significant difference in 2-OHE$_1$, 16α-OHE$_1$, or total urinary estrogens (E$_1$, E$_2$, E$_3$, 2-OHE$_1$, 16α-OHE$_1$, and eight other estrogen metabolites) between breast cancer patients and omnivores or breast cancer patients and lacto-vegetarians.

Both of the above-mentioned studies measured metabolites after breast cancer diagnosis. In an attempt to determine whether an elevated ratio of 16α- to 2-hydroxylation precedes diagnosis, Osborne and co-workers used radiometric methods to study estrogen metabolism in premenopausal women presumed to be at high or low risk of breast cancer (34). They found that women at high risk of breast cancer (family history of breast cancer or epithelial atypia in a previous biopsy) had a significantly higher (22%) extent of 16α-hydroxylation than women without high-risk lesions or a family history (low-risk controls). High-risk women had a similarly elevated extent of 16α-hydroxylation of E$_2$ as the breast cancer patients in the study by Schneider et al. (33). Translated to relative risks, the data of Osborne et al. (34) suggest that one standard deviation increase in the extent of 16α-hydroxylation from the level of low-risk controls may result in a 3-fold elevation of breast cancer risk. No data on total estrogen values were provided.

Several factors could also have affected our results. We studied a select group of women with few extraneous factors that might influence estrogen metabolism. With this approach we excluded a large number of women. Based on the first 300 women identified, we excluded 55 to 60% for a variety of reasons: 10% were above 200 lb, 15% were smokers, 25% of controls were taking estrogen replacement therapy, 10% were on other medications, and at least 20% of the cases were on tamoxifen. However, none of these exclusions appear likely to introduce any biases in any direction because they were applied equally to cases and controls.

The intraassay coefficients of variation for the assays used in this study were 13 and 20%, respectively. These values are somewhat higher than the published values of approximately 10% (41). It is, however, unclear whether the original reproducibility tests were conducted in pre- or postmenopausal women. Ziegler (45) addresses reproducibility problems elsewhere in this volume. She found that the reproducibility of this assay was low when testing urines with low estrogen concentrations. As a result of these findings, both the 2-OHE$_1$ and 16α-OHE$_1$ tests are being adjusted to improve reproducibility at low concentrations (HL Bradlow, personal communication).

The evidence is rather clear that certain diets influence the extent of 16α- and 2-hydroxylation (46–49). Recent dietary changes in cases-controls could obscure or accentuate the differences between these groups. We addressed this issue by asking participants whether they have changed their diet in the past 10 years, and we will include a complete analysis of these data in a subsequent report on the completed study.

It is not known whether the onset of cancer may affect 2- and 16α-hydroxylation. We are therefore conducting another study examining the association between the extent of 2- and 16α-hydroxylation and familial risk of breast cancer in healthy young women.

In conclusion, our preliminary results from this case–control study of breast cancer in postmenopausal women do not support the hypothesis that the ratio of urinary 2-OHE$_1$ to 16α-OHE$_1$ is a better predictor of breast cancer risk than urinary E$_1$, E$_2$, and E$_3$.
REFERENCES

1. Henderson BE, Ross RK, Bernstein L. Estrogens as a cause of human cancer. In: The Richard and Hinda Rosenthal Foundation Award Lecture. Cancer Res 48:246-253 (1988).

2. Trichopoulos D, MacMahon B, Cole P. Menopause and breast cancer risk. J Natl Cancer Inst 48:605-613 (1972).

3. Grodin JM, Siriri PK, MacDonald PC. Source of estrogen production in postmenopausal women. J Clin Endocrinol Metab 36:307-314 (1973).

4. Lubin F, Ruder AM, Wax Y, Modan B. Overweight and changes in weight throughout adult life in breast cancer etiology. Am J Epidemiol 122:579-588 (1985).

5. England PC, Skinner LG, Cottrell KM, Sellwood RA. Serum estradiol-17β in women with benign and malignant breast disease. Br J Cancer 30:571-576 (1974).

6. McFadyen IJ, Prescott RJ, Groom GV, Forrest APM, Golder MP, Fahmy DR. Circulating hormone concentrations in women with breast cancer. Lancet ii:1100-1102 (1976).

7. Malarchey WB, Schroeder LL, Stevens VC, James AG, Lane R. Two-hour postprandial endocrine profiles in women with benign and malignant breast disease. Cancer Res 37:4655-4659 (1977).

8. Adami HO, Johansson EDB, Vegelius J, Victor A. Serum concentrations of estrone, androstenedione, testosterone and sex-hormone-binding globulin in postmenopausal women with breast cancer and in age-matched controls. Upsala J Med Sci 84:259-274 (1979).

9. Drafta D, Schindler AF, Milicu M, Keller E, Stroo E, Horodniceanu E, Balanescu I. Plasma hormones in pre- and postmenopausal breast cancer. J Steroid Biochem 43:793-802 (1980).

10. Moore JW, Clark GMG, Bulbrook RD, Hayward JL, Murai JT, Hammond GL, Siriri PK. Serum concentrations of total and non-protein-bound oestradiol in patients with breast cancer and in normal controls. Int J Cancer 29:17-21 (1982).

11. Reed MJ, Cheng RW, Noel CT, Dudley HAF, James VHT. Plasma levels of estrone, estrone sulfate, and estradiol and the percentage of unbound estradiol in postmenopausal women with and without breast disease. Cancer Res 43:3940-3943 (1983).

12. Reed MJ, Beranek PA, Cheng RW, Gilchik MW, James VHT. The distribution of oestradiol in plasma from postmenopausal women with or without breast cancer: relationships with metabolic clearance rates of oestradiol. Int J Cancer 35:457-460 (1985).

13. Secreto G, Racchione C, Cavalleri A, Miraglia M, Dati V. Circulating levels of testosterone, 17β-estradiol, luteinising hormone and prolactin in postmenopausal breast cancer patients. Br J Cancer 47:269-275 (1983).

14. Bruning PF, Bonner JMG, Hart AAM. Non-protein bound oestradiol, sex hormone binding globulin and breast cancer risk. Br J Cancer 51:479-484 (1985).

15. Siriri PK, Simberg N, Muri J. Estrogens and breast cancer. Ann NY Acad Sci 464:100-105 (1986).

16. Wysowski DK, Comstock GW, Helsing KJ, Lau HL. Sex hormone levels in serum in relation to the development of breast cancer. Am J Epidemiol 25:791-799 (1987).

17. Persson BH, Risholm L. Oophorectomy and cortisone treatment as a method of eliminating estrogen production in patients with breast cancer. Acta Endocrinol 47:15-26 (1964).

18. Marmorton J, Crowley LG, Myers SM, Stern E, Hopkins CE. II: Urinary excretion of estrone, estradiol, and estriol by patients with breast cancer and benign breast disease. Am J Obstet Gynecol 4:460-467 (1965).

19. Arguelles AE, Hoffman C, Poggi UL, Chekhemandi M, Saborida C, Blanchard O. Endocrine profiles and breast cancer. Lancet i:165-167 (1973).

20. Gronroos M, Aho AJ. Estrogen metabolism in postmenopausal women with primary and recurrent breast cancer. Eur J Cancer 4:523-527 (1968).

21. Grattarola R, Secreto G, Recchione C, Castellini W. Androgens in breast cancer. Am J Obstet Gynecol 118:173-178 (1974).

22. Thijsen JHH, Poortman J, Schwarz F. Androgens in postmenopausal breast cancer: excretion, production and interaction with estrogens. J Steroid Biochem 6:729-734 (1975).

23. Morreal CE, Dao TL, Neneto T, Lonergan PA. Urinary excretion of estrone, estradiol, and estriol in postmenopausal women with primary breast cancer. J Natl Cancer Inst 63:1171-1174 (1979).

24. Bernstein L, Ross RK, Pike MC, Brown JB, Henderson BE. Hormone levels in older women: a study of post-menopausal breast cancer patients and healthy population controls. Br J Cancer 61:298-302 (1990).

25. Adlercreutz H, Fotsis T, Hockerstedt K, Hämäläinen E, Bannwart C, Bloigu S, Valtosen A, Ollus A. Diet and urinary estrogen profile in premenopausal omnivorous and vegetarian women and in premenopausal women with breast cancer. J Steroid Biochem 34:527-530 (1989).

26. Clark JH, Paszko Z, Peck EJ Jr. Nuclear binding and retention of the receptor estrogen complex: relation to the agonistic and antagonistic properties of estriol. Endocrinol 100:91-96 (1977).

27. Fishman J, Martucci C. Biological properties of 16α-hydroxy-oestrone: implications in estrogen physiology and pathophysiology. J Clin Endocrinol Metab 51:611-615 (1980).

28. Martucci C, Fishman J. Direction of estradiol metabolism as a control of its hormonal action—uterotrophic activity of estradiol metabolites. Endocrinol 101:1709-1715 (1977).

29. Bradlow HL, Hershcopf RJ, Fishman JF. Oestriol 16α-hydroxylation: a risk marker for breast cancer. Cancer Surv 5:574-583 (1986).

30. Bradlow HL, Hershcopf R, Martucci C, Fishman J. 16α-Hydroxylation of estradiol: a possible risk marker for breast cancer. Ann NY Acad Sci 464:138-151 (1986).

31. Bradlow HL, Hershcopf R, Martucci CP, Fishman J. Estradiol 16α-hydroxylation in the mouse correlates with mammary tumor incidence and presence of murine mammary tumor virus: a possible model for the hormonal etiology of breast cancer in humans. Proc Natl Acad Sci USA 82:6295-6299 (1985).

32. Osborne MP, Bradlow HL, Wong GYC, Telang NT. Upregulation of estradiol C16α-hydroxylation in human breast tissue: a potential biomarker of breast cancer risk. J Natl Cancer Inst 85:1917-1920 (1993).

33. Schneider J, Kinne D, Fracchia A, Pierce V, Bradlow HL, Fishman J. Abnormal oxidative metabolism of estradiol in women with breast cancer. Proc Natl Acad Sci USA 79:3047-3051 (1982).

34. Osborne MP, Karmali RA, Hershcopf RJ, Bradlow HL, Kourides IA, Williams WR, Rosen PP, Fishman J. Omega-3 fatty acids: modulation of estrogen metabolism and potential for breast cancer prevention. Cancer Invest 8:629-631 (1988).

35. Longnecker MP, Paganini-Hill A, Ross RK. Lifetime alcohol consumption and breast cancer risk among postmenopausal women in Los Angeles. Cancer Epidemiol Biomarkers Prev 4:721-725 (1995).

36. Bland KI, Copeland EM III. The Breast: Comprehensive Management of Benign and Malignant Diseases. Philadelphia: W.B. Saunders, 1991.

37. Fishman J, Hellman L, Zumoff B, Gallagher TF. Influence of thyroid hormone on estrogen metabolism in man. J Clin Endocrinol Metabol 22:389-392 (1962).

38. Galbraith RA, Michnovicz JJ. The effects of cimetidine on the oxidative metabolism of estradiol. N Engl J Med 321:269-274 (1989).

39. Osborne MP, Telang NT, Kaur S, Bradlow HL. Influence of chemopreventive agents on estradiol metabolism and mammarypreneoplasia in the C3H mouse. Steroids 55:114-119 (1990).
40. Niwa T, Bradlow HL, Fishman J, Swaneck GE. Induction and inhibition of estradiol hydroxylase activities in MCF-7 human breast cancer cells in culture. Steroids 55:297–302 (1990).

41. Klug TL, Bradlow HL, Sepkovic DW. Monoclonal antibody-based enzyme immunoassay for simultaneous quantitation of 2- and 16α-hydroxyestrone in urine. Steroids 59:648–655 (1994).

42. Katagiri H, Stanczyk FZ, Goebelsmann U. Estriol in pregnancy III: Development, comparison and use of specific antisera for rapid radioimmunoassay of unconjugated estriol in pregnancy plasma. Steroids 24:225–238 (1974).

43. Stanczyk FZ, Shoupe D, Nunez V, Macias-Gonzales P, Vijod MA, Lobo RA. A randomized comparison of nonoral estradiol delivery in postmenopausal women. Am J Obstet Gynecol 159:1540–1546 (1988).

44. Cassidenti DL, Vijod AG, Vijod MA, Stanczyk FZ, Lobo RA. Short-term effects of smoking on the pharmacokinetic profiles of micronized estradiol in postmenopausal women. Am J Obstet Gynecol 163:1953–1960 (1990).

45. Ziegler RG, Rossi SC, Fears TR, Bradlow HL, Aldercreutz H, Sepkovic D, Kiuru P, Wahala K, Vaught JB, Falk RT, Donaldson JL, Gail MH, Siiteri PK, Hoover RN. Quantifying estrogen metabolism: establishing the reproducibility and validity of assays for 2-hydroxyestrone and 16α-hydroxyestrone in urine. Environ Health Perspect 105(Suppl 3):607–614 (1997).

46. Musey PJ, Collins DC, Bradlow HL, Gould KG, Preedy JRK. Effect of diet on oxidation of 17β-estradiol in vivo. J Clin Endocrinol Metab 65:792–795 (1987).

47. Longcope C, Gorbach S, Goldin B, Woods M, Dwyer J, Morrill A, Warram J. The effect of a low-fat diet on estrogen metabolism. J Clin Endocrinol Metab 64:1246–1250 (1987).

48. Michnovicz JJ, Bradlow HL. Altered estrogen metabolism and excretion in humans following consumption of indole-3-carbinol. Nutr Cancer 16:59–66 (1991).

49. Hoffman AR, Majchrowicz E, Poth A, Paul SM. Ethanol reduces hepatic estrogen-2-hydroxylase activity in the male rat. Life Sciences 29:789–794 (1981).