Relationships of Age and Reproductive Characteristics with Plasma Estrogens and Androgens in Premenopausal Women

Joanne F. Dorgan,[1] Marsha E. Reichman,[2] Joseph T. Judd, Charles Brown, Christopher Longcope, Arthur Schatzkin, William S. Campbell, Charlene Franz, Lisa Kahle, and Philip R. Taylor

Division of Cancer Prevention and Control, National Cancer Institute, Bethesda, Maryland 20852 [J. F. D., M. E. R., C. B., A. S., W. S. C., P. R. T.]; Beltsville Human Nutrition Research Center, Agricultural Research Service, United States Department of Agriculture, Beltsville, Maryland 20705 [J. T. J.]; Departments of Obstetrics and Gynecology and Medicine, University of Massachusetts Medical School, Worcester, Massachusetts 01655 [C. L., C. F.]; and Information Management Services, Inc., Silver Spring, Maryland 20904 [L. K.]

Abstract

We used data from a cross-sectional study of 107 premenopausal women to evaluate the relation of age, menarcheal age, parity, and age at first live birth with plasma estrogen and androgen levels in premenopausal women. Fasting blood specimens were collected on each of days 5–7, 12–15, and 21–23 of menstrual cycles of the participants and pooled to create follicular, midcycle, and luteal phase samples, respectively, for each woman. Age was associated significantly and positively with plasma estradiol levels during the follicular phase [percentage difference/year = 2.6; 95% confidence interval (CI) = 1.0–4.2] and midcycle (percentage difference/year = 2.7; 95% CI = 0.9–4.7) but not the luteal phase (percentage difference/year = −0.4; 95% CI = −1.9–1.3) of the menstrual cycle. The relation of age to plasma estradiol varied by parity, with significant interactions during midcycle and luteal phase. Among nulliparous women, plasma estradiol levels increased with age midcycle and during the luteal phase, but among parous women estradiol levels decreased with age during these phases of the menstrual cycle. Plasma estrone increased with age in all women during the follicular phase of the menstrual cycle (percentage difference/year = 1.5; 95% CI = 0.2–2.8). During the luteal phase there was a significant interaction with parity; estrone levels in nulliparous women varied only slightly with age, but levels in parous women decreased significantly as age increased. The androgens, androstenedione and dehydroepiandrosterone sulfate decreased, and sex hormone-binding globulin increased as age increased. The results of this cross-sectional study suggest that pregnancy may modify age-related changes in plasma estrogen levels.

Introduction

Reproductive history is reported to affect breast cancer risk in the majority of epidemiological studies. Early age at menarche increases risk (1–9), whereas pregnancy before age 35 years decreases lifelong risk (6–8, 10–19). Both young age at first pregnancy (4–8, 10–22) and multiparity (4–14, 16–23) are reported to be protective. The mechanisms by which reproductive characteristics influence breast cancer risk are not known. Young age at menarche has been hypothesized to increase risk by increasing the lifelong duration of exposure to ovarian estrogens (1). Pregnancy, on the other hand, has been hypothesized to decrease risk by causing terminal differentiation of breast epithelium, thereby decreasing susceptibility to carcinogens (24).

Women with an early age at menarche may have different hormonal profiles as adults compared to women whose menarche occurs at an older age. In a prospective study, Apter *et al.* (25) reported that in women 20–31 years of age, follicular phase serum estradiol concentrations were higher and SHBG[3] concentrations were lower among those who had an early menarche. MacMahon *et al.* (26) also reported inverse associations between age at menarche and follicular phase urine estradiol and estrone levels in adults; Moore *et al.* (27) reported higher luteal phase serum estradiol in Japanese but not British women with early menarche. Bernstein *et al.* (28) and Ingram *et al.* (29), however, found little evidence for an association between age at menarche and serum estrogen levels during adulthood.

Pregnancy also may result in long-term endocrine changes. Here again, however, results are conflicting. In a study by Bernstein *et al.* (30), parous women had significantly lower urine and plasma estradiol levels compared to nulliparous women on day 11 of their menstrual cycles. Parity and age at first pregnancy, however, were not associated with luteal phase serum estradiol levels in a study by Ingram *et al.* (29). Musey *et al.* (31) also did not detect changes in follicular phase serum estradiol, estrone, estrone sulfate, or testosterone levels from before to 7–19 months after a pregnancy. Serum estriol was significantly higher and DHEAS was significantly lower following the pregnancy.

Hormones, particularly the estrogens, are believed to play a key role in the etiology of breast cancer (32), and age at menarche and parity could affect breast cancer risk through endocrine effects. We therefore used data from a cross-sectional study to evaluate the relation of these reproductive characteristics to plasma hormones in premenopausal adult women.

Received 10/27/94; revised 3/15/95; accepted 3/16/95.

[1] To whom requests for reprints should be addressed, at NIH, National Cancer Institute, Department of Health and Human Services, 6130 Executive Boulevard, EPN, Room 211, Rockville, MD 20852.

[2] Present address: MERJRE Associates, Bethesda, MD 20817.

[3] The abbreviations used are: SHBG, sex hormone-binding globulin; DHEAS, dehydroepiandrosterone sulfate; RIA, radioimmunoassay.

Materials and Methods

Participants for the cross-sectional study were recruited by posters and newspaper advertisements from communities around Beltsville, Maryland, during 1988–1990. Only premenopausal women 20–40 years of age who met the following criteria were eligible: (*a*) weight for height 85–130% of desirable based on 1983 Metropolitan Life Insurance tables (33); (*b*) usual menstrual cycle length of not more than 35 days; (*c*) not pregnant or lactating during the past 12 months and not taking oral contraceptives during the past 6 months; (*d*) no history of cancer, diseases of the reproductive or endocrine systems, chronic liver or gastrointestinal disease, hypertension, diabetes, nephrolithiasis, gout, or hyperlipidemia; (*e*) not taking any medications other than an occasional analgesic; (*f*) not following a vegetarian diet; (*g*) not routinely participating in strenuous activites; and (*h*) not smoking. Because the primary objective of the study was to evaluate the association of alcohol ingestion with plasma hormones, women with a wide range of drinking patterns were recruited.

All data and blood specimens were collected during a single menstrual cycle. Blood was collected in the morning after an overnight fast. Equal volumes of plasma from each of days 5–7, 12–15, and 21–23 of the menstrual cycle were pooled to create follicular, midcycle, and luteal phase specimens, respectively, for each woman. All plasma specimens were stored at −70°C until hormone analyses were performed. Estrone, estradiol, and androstenedione in plasma were measured by RIA following solvent extraction and celite chromatography (34). Estrone sulfate also was measured by RIA after solvolysis, extraction of hydrolyzed estrone, and celite chromatography (34). DHEAS and progesterone were measured by RIA kits (ICN Biomedicals, Costa Mesa, CA), and SHBG was measured by an immunoradiometric assay kit (Farmos Group Ltd., Oulunsalo, Finland). Percentage unbound and albumin-bound estradiol were measured with the use of centrifugal ultrafiltration (35), and SHBG-bound estradiol was calculated. Coefficients of variation for replicate quality control samples averaged 9.5% for estrone, 11.1% for estradiol, 4.6% for estrone sulfate, 16.7% for androstenedione, 10.8% for DHEAS, 9.4% for progesterone, 10.6% for SHBG, and 10.6% for percentage unbound and 8.6% for albumin-bound estradiol.

We used linear regression to evaluate the relations of age and reproductive history to plasma hormone levels (36). To improve normality all plasma hormone concentrations were converted to the $\log_{10}$ scale before analysis. Percentage differences were calculated from parameter estimates as $(10^b - 1) \times 100$. All models were adjusted for hormone analysis batch by including a set of categorical (dummy) variables. In a previous analysis of these data we found significant associations between height and follicular phase estradiol and weight and plasma SHBG levels (37). Because height confounded associations of age and reproductive history with follicular phase estradiol, and weight confounded associations with SHBG, we included height in models when follicular phase plasma estradiol was the dependent variable, and weight in models when SHBG was the dependent variable. We also reported significant associations between alcohol ingestion and plasma androstenedione levels (38). Because inclusion of alcohol in models for androstenedione did not alter findings for age and reproductive history, results reported are from the more parsimonious models that do not include alcohol. We analyzed the relation of plasma hormones to age, age at menarche, and age at first pregnancy as continuous variables and after categorizing. Categorization did not reveal any significant nonlinear associations; therefore, only results of analyses of continuous variables are presented. For analysis of parity, women were categorized as nulliparous if they had never given birth to a living child or parous if they had given birth to one or more children. Interactions of reproductive history variables with age were tested by including cross-product terms in models. All analyses were performed with the use of SAS statistical software (39).

Results

The 107 participants in this cross-sectional study had a mean age of 29.6 ± 5.1 (SD) years. Their recalled age at menarche averaged 12.7 ± 1.4 years. Seventy-three women were nulliparous and 34 were parous. Fifteen of the women had 1 child, 13 had 2, 4 had 3, and 2 women had 6 children. The mean age of the women at the birth of their first children was 23.0 ± 4.8 years. Hormone levels have been reported previously (37, 38).

Associations of age and reproductive history with plasma estrogens and androgens are summarized in Tables 1 and 2. Age was positively and significantly related to plasma estradiol levels of all women during the follicular phase and midcycle but not during the luteal phase of the menstrual cycle. As shown in Fig. 1, adjusted for age, parous women tended to have higher follicular and lower luteal phase estradiol levels compared to nulliparous women, but differences were not significant.

Fig. 2 presents scatter plots of age by hormone level separately for nulliparous and parous women for each menstrual cycle phase. Plasma estradiol levels increased with age during the follicular phase in both nulliparous and parous women. Among nulliparous women, plasma estradiol levels also increased with age midcycle and during the luteal phase, but among parous women estradiol levels decreased with age during these phases of the menstrual cycle. Interactions between parity and age were significant in relation to both midcycle and luteal phase plasma estradiol. Results were similar when analyses were performed for the different estradiol fractions.

Plasma estrone levels also increased significantly with age during the follicular phase for all women. After adjusting for age, plasma estrone levels of parous and nulliparous women were similar at the different phases of the menstrual cycle. During the luteal phase, however, there was a significant (P = 0.004) interaction between age and parity in relation to estrone. Estrone levels of nulliparous women varied only slightly with age (percentage difference/year = 0.01; 95% CI = −1.6–1.6), whereas estrone levels of parous women decreased significantly (P = 0.04) as age increased (percentage difference/year= −4.5; 95% CI = −8.4 to −0.5). Interactions between age and parity were not significant in relation to plasma estrone during other menstrual cycle phases.

Estrone sulfate increased slightly or did not change with age during the follicular phase and midcycle but decreased significantly with increasing age during the luteal phase of the menstrual cycle. Estrone sulfate levels did not differ by parity, and no tests for interactions between age and parity in relation to estrone sulfate were significant at $P \leq 0.05$. Significant inverse associations between age at first live birth and estrone sulfate levels were observed midcycle and during the luteal phase of the menstrual cycle.

The androgens, androstenedione and DHEAS, averaged across the menstrual cycle phases, decreased significantly as age increased. Levels did not differ, however, by parity. Age-adjusted geometric means for nulliparous and parous women were 9.1 ± 1.03 (SE) and 8.5 ± 1.05 nmol/liter, respectively,

Table 1 Percentage difference/year (95% confidence interval) in plasma estrogen levels by menstrual cycle phase related to age and reproductive history[a]

	Follicular		Midcycle		Luteal	
	% Difference	95% CI	% Difference	95% CI	% Difference	95% CI
Estradiol (pmol/liter)						
Age (yr)	2.6[b,c]	1.0–4.2	2.7[c]	0.9–4.7	−0.4	−1.9–1.3
Age at menarche (yr)	−2.3[b]	−7.8–3.6	0.3	−6.2–7.3	5.6	−0.3–11.9
Age at first live birth (yr)	0.3[b]	−3.0–3.7	−2.2	−6.2–2.0	−2.7	−6.3–1.0
Estrone (pmol/liter)						
Age (yr)	1.5[d]	0.2–2.8	1.2	−0.3–2.6	−1.1	−2.4–0.3
Age at menarche (yr)	1.5	−3.1–6.4	−2.8	−7.7–2.4	0.1	−4.9–5.3
Age at first live birth (yr)	−1.4	−4.9–2.3	−3.3	−7.0–0.5	−1.9	−5.6–1.8
Estrone sulfate (pmol/liter)						
Age (yr)	1.9	−0.1–3.8	0.4	−2.1–2.9	−2.4[d]	−4.6 to −0.1
Age at menarche (yr)	−5.7	−12.0–1.0	−5.6	−13.7–3.2	−4.2	−12.0–4.3
Age at first live birth (yr)	−2.8	−6.9–1.6	−5.7[d]	−10.3 to −1.0	−6.3[c]	−10.1 to −2.5

[a] Estimates from linear regression models including age and hormone analysis batch.
[b] Adjusted for height.
[c] $P \leq 0.01$.
[d] $P \leq 0.05$.

Table 2 Percentage difference/year (95% confidence interval) in plasma androstenedione, DHEAS, and SHBG levels averaged across the menstrual cycle related to age and reproductive history[a]

	Androstenedione (nmol/liter)		DHEAS (μmol/liter)		SHBG (nmol/liter)[b]	
	% Difference	95% CI	% Difference	95% CI	% Difference	95% CI
Age (yr)	−1.7[c]	−2.7 to −0.8	−2.9[c]	−4.5 to −1.4	1.9[d]	0.5–3.3
Age at menarche (yr)	−0.3	−3.8–3.3	−5.3	−10.5–0.1	0.3	−5.0–5.8
Age at first live birth (yr)	−0.1	−2.3–2.2	0.9	−3.1–5.1	1.4	−2.8–5.8

[a] Estimates from linear regression models including age and hormone analysis batch.
[b] Adjusted for weight.
[c] $P \leq 0.001$.
[d] $P \leq 0.01$.

for androstenedione, and 7.7 ± 1.05 and 7.6 ± 1.08 μmol/liter, respectively, for DHEAS. Tests for interactions between age and parity were not significant for either androgen.

Adjusted for body weight, SHBG levels, averaged across the menstrual cycle, increased with age. Geometric mean SHBG levels of nulliparous women were 38.1 ± 1.05 (SE) nmol/liter compared to 39.6 ± 1.07 nmol/liter in parous women. Tests for differences in means of nulliparous and parous women and interactions with age were not significant at $P \leq 0.05$.

As shown in Table 3, older women were significantly ($P = 0.003$) more likely to have a luteal phase progesterone rise; the odds increased by 27% (95% CI = 9–49%) for each additional year of age. Although after adjusting for age parity was not associated significantly with the probability of a progesterone rise, only 1 of 34 (2.9%) parous women failed to have a progesterone rise compared to 16 of 73 (21.9%) nulliparous women. The association of age with the probability of a progesterone rise was unchanged when we included only nulliparous women in the analysis.

When we restricted analyses to the 90 women who had a progesterone rise, the relation of age with follicular phase plasma estradiol was diminished (percentage difference/year = 1.9; 95% CI = 0.1–3.6) but remained significant ($P = 0.04$). SHBG-bound estradiol was the only fraction that was significantly positively associated with age. Age also no longer was associated significantly with follicular phase plasma estrone (percentage difference/year = 0.9; 95% CI = −0.6–2.5). Midcycle, the relation of age with plasma estradiol in all women who had a progesterone rise was decreased (percentage difference/year = 0.6; 95% CI = −1.4–2.6) and not significant for any of the fractions. Although midcycle estradiol tended to increase with age in nulliparous women but tended to decrease with age in parous women, the interaction was weaker and no longer significant. During the luteal phase, age remained associated inversely with estrone sulfate (percentage difference/year = −2.7; 95% CI = −5.3–0.0). Additionally, the interaction between parity and age remained marginally significant ($P = 0.07$) for total estradiol and significant ($P = 0.01$) for estrone. The interaction was significant for free and albumin-bound but not SHBG-bound estradiol.

Discussion

Results of this cross-sectional study suggest that there are age-related changes in plasma estrogen and androgen levels in adult premenopausal women, and for the estrogens, these changes vary by parity. Age at menarche, however, was not associated with any of the hormones measured.

The occurrence of ovulatory cyles in women with regular menses increases with age during the teens and 20s, plateaus during the 30s, and falls off again in the 40s (40, 41). The women in our cross-sectional study were 20–40 years of age, and the positive association that we observed between age and luteal phase progesterone rise is consistent with age-related changes in ovulation. Musey *et al.* (42) also reported higher levels of estradiol but not estrone during the follicular phase of the menstrual cycle in older (29–40 years) compared to

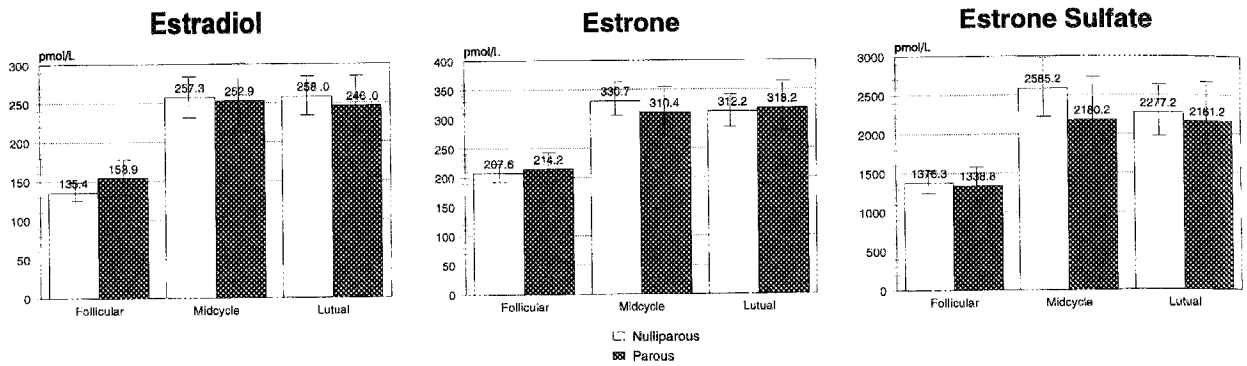

Fig. 1. Geometric mean plasma estrogen levels in nulliparous and parous women by menstrual cycle phase. Geometric means adjusted for hormone analysis batch, age, and for follicular phase estradiol, height. *Columns*, mean; *bars*, 95% CI.

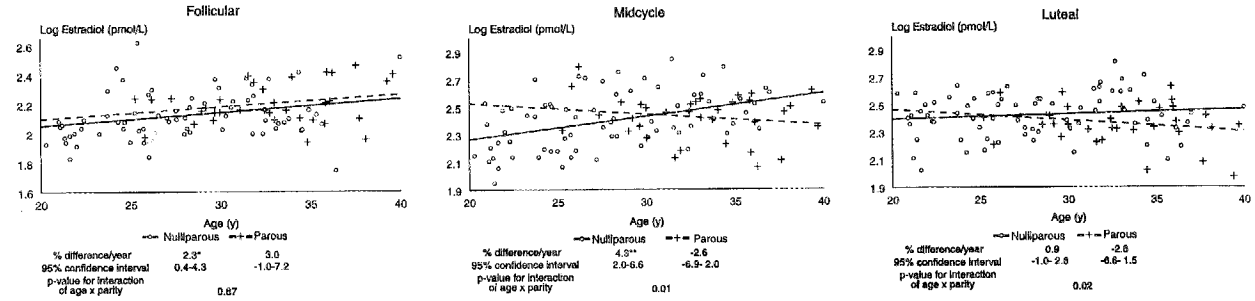

Fig. 2. Relations of plasma estradiol levels to age for nulliparous and parous women by menstrual cycle phase. Scatter plots of residuals from regressions of $\log_{10}$ (estradiol) on hormone analysis batch, and for follicular phase only height, centered on the mean $\log_{10}$ (estradiol) concentration for the menstrual cycle phase. *, $P \leq 0.05$; **, $P \leq 0.001$.

Table 3 Odds ratio (95% confidence interval) for progesterone rise during the luteal phase of the menstrual cycle related to age and reproductive history[a]

	OR[b]	95% CI
Age (yr)	1.3[c]	1.1–1.5
Age at menarche (yr)	0.8	0.5–1.3
Parous (*vs.* nulliparous)	3.0	0.3–28.3

[a] Estimates from a logistic regression model including all variables listed in table and hormone analysis batch.
[b] ORs are per year except for parity.
[c] $P \leq 0.001$.

younger (18–23 years) women and suggested that decreased hepatic metabolism may account for the elevated estradiol levels, but increased ovarian secretion could not be ruled out. The increases in follicular phase plasma estradiol and estrone levels that we observed with age in all participants were diminished when we restricted analysis to women who experienced a luteal phase progesterone rise, which suggests that the estradiol and estrone level increase might, at least partly, be due to increased ovarian secretion.

We did not detect any significant marginal associations between parity and plasma hormones. There were, however, significant interactions between parity and age in relation to plasma estradiol midcycle and during the luteal phase and to plasma estrone during the luteal phase of the menstrual cycle. Among nulliparous women, hormone levels changed only slightly or increased with increasing age, but among parous women levels decreased with age. The incidence of ovulatory cycles is higher in parous compared to nulliparous women (43). In our data, however, age-related differences in estrogen levels by parity could not be explained totally by differences in the frequency of ovulatory cycles as determined by a luteal phase progesterone rise. When we included in the analysis only women who experienced a progesterone rise, the directionality of relationships still differed by parity. Although interactions were no longer significant for estradiol midcycle, interactions were marginally significant for estradiol and significant for estrone during the luteal phase of the menstrual cycle.

Age was significantly ($P = 0.02$) and positively correlated (Spearman $\rho = 0.40$) with years since last live birth. We therefore were concerned that higher midcycle and luteal phase estradiol levels in younger parous women might reflect the shorter interval since last pregnancy. Adjustment for years since last birth, however, did not affect or strengthened the inverse associations that we observed between plasma estradiol and age in parous women during these phases of the menstrual cycle.

Because our data were cross-sectional, we could not directly assess whether differences in age associations by parity were a cause or effect of pregnancy. Seventeen of the nulliparous women had at least one pregnancy that terminated in a spontaneous or induced abortion. Age-related differences in estrogens among these women were in the direction we observed for nulliparous women, suggesting that a term pregnancy may induce long-term changes in the secretion or metabolism of estrogens.

In a prospective study, Musey *et al.* (31) did not detect changes in serum estradiol or estrone measured before and 7–19 months after a pregnancy during the early follicular phase of the menstrual cycle. We also did not detect an association between parity and plasma estrogens or an in-

teraction between parity and age in relation to plasma estrogens during the follicular phase. Musey *et al.* (31) did not report midcycle or luteal phase results, and we are not aware of any other studies that prospectively evaluated the effect of pregnancy on plasma estrogens.

In a cross-sectional study, Bernstein *et al.* (30) reported significantly higher mean levels of estradiol and marginally significantly higher levels of estrone in nulliparous compared to parous women on day 11 of their menstrual cycles. This late follicular blood collection was timed more closely to our midcycle collection on days 12–15 than our follicular collection on days 5–7. Plasma estradiol levels were not related to parity in either our follicular or midcycle specimens, but midcycle estradiol increased with age among nulliparous women, whereas it decreased with age among parous women. The average age of the women in the study by Bernstein *et al.* (30) was 33.3 years, which is older than the women in our study and may have contributed to the discrepancy in findings. Similar to us, Ingram *et al.* (29) did not find an association between parity and luteal phase estradiol.

We did not observe any differences in plasma hormone levels by age at menarche. In the only prospective study, Apter *et al.* (25) reported an inverse association. In this study, age at menarche ($\leq$11, 12, or $\geq$13 years) recalled during adulthood was misclassified 25% of the time when compared to recorded age at menarche. Misclassification of reported age at menarche could be biasing results to the null in our and other (28, 29) studies.

Both androgens that we measured, androstenedione and DHEAS, decreased significantly as age increased. DHEAS is produced solely by the adrenals, and as a result of changes in secretion, blood levels peak during the 20s and decline thereafter (44). Although both the ovaries and adrenals secrete androstenedione, because of diurnal variation in adrenal output, their relative contributions differ throughout the day. We collected blood in the morning when the majority of circulating androstenedione is adrenal in origin (44), and the lower levels that we observed in older women may have been due to decreased adrenal secretion.

In conclusion, results of this study suggest that pregnancy may induce long-term changes in secretion or metabolism of estrogens. Since estrogens are believed to be important in the etiology of breast cancer (32), if confirmed, this finding would suggest a mechanism by which pregnancy could modify breast cancer risk. A prospective study of women during their reproductive years is needed to rigorously evaluate this possibility.

References

1. Kvale, G., and Heuch, I. Menstrual factors and breast cancer risk. Cancer (Phila.), *62:* 1625–1631, 1988.

2. Hsieh, C. C., Trichopoulos, D., Katsouyanni, K., and Yuasa, S. Age at menarche, age at menopause, height and obesity as risk factors for breast cancer: associations and interactions in an international case-control study. Int. J. Cancer, *46:* 796–800, 1990.

3. Brinton, L. A., Schairer, C., Hoover, R. N., and Fraumeni, J. F. Menstrual factors and risk of breast cancer. Cancer Invest., *6:* 245–254, 1988.

4. Paffenbarger, R. S., Kampert, J. B., and Chang, H. G. Characteristics that predict risk of breast cancer before and after the menopause. Am. J. Epidemiol., *112:* 258–268, 1980.

5. Kampert, J. B., Whittemore, A. S., and Paffenbarger, R. S. Combined effect of childbearing, menstrual events, and body size on age-specific breast cancer risk. Am. J. Epidemiol., *128:* 962–979, 1988.

6. Helmrich, S. P., Shapiro, S., Rosenberg, L., Kaufman, D. W., Slone, D., Bain, C., Miettinen, O. S, Stolley, P. D., Rosenshein, N. B., Knapp, R. C., Leavitt, T., Schottenfeld, D., Engle, R. L., and Levy, M. Risk factors for breast cancer. Am. J. Epidemiol., *117:* 35–45, 1986.

7. Lipnick, R., Speizer, F. E., Bain, C., Willett, W., Rosner, B., Stampfer, M. J., Belanger, C., and Hennekens, C. H. A case-control study of risk indicators among women with premenopausal and early postmenopausal breast cancer. Cancer (Phila.), *53:* 1020–1024, 1984.

8. Yuan, J. M., Yu, M. C., Ross, R. K., Gao, Y. T., and Henderson, B. E. Risk factors for breast cancer in Chinese women in Shanghai. Cancer Res., *48:* 1949–1953, 1988.

9. Ewertz, M., and Duffy, S. W. Risk of breast cancer in relation to reproductive factors in Denmark. Br. J. Cancer, *58:* 99–104, 1988.

10. Lubin, J. H., Burns, P. E., Blot, W. J., Lees, A. W., May, C., Morris, L. E., and Fraumeni, J. F. Risk factors for breast cancer in women in Northern Alberta, Canada, as related to age at diagnosis. J. Natl. Cancer Inst., *68:* 211–217, 1982.

11. Kvale, G., Heuch, I., and Eide, G. E. A prospective study of reproductive factors and breast cancer. I. Parity. Am. J. Epidemiol., *126:* 831–841, 1987.

12. Leon, D. A. A prospective study of the independent effect of parity and age at first birth on breast cancer incidence in England and Wales. Int. J. Cancer, *43:* 986–991, 1989.

13. Tulinius, H., Sigvaldason, H., Hrafnkelsson, J., Olafsdottir, G., Tryggvadottir, L., and Sigurdsson, K. Reproductive factors and breast cancer risk in Iceland. A second cohort study. Int. J. Cancer, *46:* 972–975, 1990.

14. La Vecchia, C., Decarli, A., Parazzini, F., Gentile, A., Negri, E., Cecchetti, G., and Franceschi, S. General epidemiology of breast cancer in Northern Italy. Int. J. Epidemiol., *16:* 347–355, 1987.

15. Bain, C., Willett, W., Rosner, B., Speizer, F. E., Belanger, C., and Hennekens, C. H. Early age at first birth and decreased risk of breast cancer. Am. J. Epidemiol., *114:* 705–709, 1981.

16. Layde, P. M., Webster, L. A., Banghwan, A. L., Wingo, P. A., Rubin, G. L., and Ory, H. W. The independent associations of parity, age at first full-term pregnancy, and duration of breastfeeding with the risk of breast cancer. J. Clin. Epidemiol., *42:* 963–973, 1989.

17. Ewertz, M., Duffy, S. W., Adami, H. O., Kvale, G., Lund, E., Meirik, O., Mellemgaard, A., Soini, I., and Tulinius, H. Age at first birth, parity and risk of breast cancer: a meta-analysis of 8 studies from the Nordic countries. Int. J. Cancer, *46:* 597–603, 1990.

18. Trapido, E. J. Age at first birth, parity and breast cancer risk. Cancer (Phila.), *51:* 946–948, 1983.

19. Brinton, L. A., Hoover, R., and Fraumeni, J. F. Reproductive factors in the aetiology of breast cancer. Br. J. Cancer, *47:* 757–762, 1983.

20. MacMahon, B., Purde, M., Cramer, D., and Hint, E. Association of breast cancer risk with age at first and subsequent births: a study in the population of the Estonian Republic. J. Natl. Cancer Inst., *69:* 1035–1038, 1982.

21. Talamini, R., La Vecchia, C., Franceschi, S., Colombo, F., Decarli, A., Grattoni, E., Grigoletto, E., and Tognoni, G. Reproductive and hormonal factors and breast cancer in a Northern Italy population. Int. J. Epidemiol., *14:* 70–74, 1985.

22. Brignone, G., Cusimano, R., Dardanoni, G., Gugliuzza, M., Lanzarone, F., Scibilia, V., and Dardanoni, L. A case-control study on breast cancer risk factors in a Southern European population. Int. J. Epidemiol., *16:* 356–361, 1987.

23. Pathak, D. R., Speizer, F. E., Willett, W. C., Rosner, B., and Lipnich, R. J. Parity and breast cancer risk: possible effect on age at diagnosis. Int. J. Cancer, *37:* 21–25, 1986.

24. Russo, J., Tay, L. K., and Russo, I. H. Differentiation of the mammary gland and susceptibility to carcinogenesis. Breast Cancer Res. Treat., *2:* 5–73, 1982.

25. Apter, D., Reinila, M., and Vihko, R. Some endocrine characteristics of early menarche, a risk factor for breast cancer, are preserved into adulthood. Int. J. Cancer, *44:* 783–787, 1989.

26. MacMahon, B., Trichopoulos, D., Brown, J., Andersen, A. P., Cole, P., DeWaard, F., Kauraniemi, T., Polychronopoulou, A., Ravnihar, B., Stormby, N., and Westlund, K. Age at menarche, urine estrogens and breast cancer risk. Int. J. Cancer, *30:* 427–431, 1982.

27. Moore, J. W., Key, T. J. A., Wang, D. Y., Bulbrook, R. D., Hayward, J. L., and Takatani, O. Blood concentrations of estradiol and sex hormone-binding globulin in relation to age at menarche in premenopausal British and Japanese women. Breast Cancer Res. Treat., *18:* s47–s50, 1991.

28. Bernstein, L., Pike, M. C., Ross, R. K., and Henderson, B. E. Age at menarche and estrogen concentrations of adult women. Cancer Causes Control, *2:* 221–225, 1991.

29. Ingram, D. M., Nottage, E. M., Willcox, D. L., and Roberts, A. Oestrogen binding and risk factors for breast cancer. Br. J. Cancer, *61:* 303–307, 1990.

30. Bernstein, L., Pike, M. C., Ross, R. K., Judd, H. L., Brown, J. B., and Henderson, B. E. Estrogen and sex hormone-binding globulin levels in nulliparous and parous women. J. Natl. Cancer Inst., *74:* 741–745, 1985.

31. Musey, V. C., Collins, D. C., Brogan, D. R., Santos, V. R., Musey, P. I., Martino-Saltzman, D., and Preedy, J. R. K. Long term effects of a first pregnancy

on the hormonal environment: estrogens and androgens. J. Clin. Endocrinol. Metab., *64:* 111–118, 1987.

32. Bernstein, L., and Ross, R. K. Endogenous hormones and breast cancer risk. Epidemiol. Rev., *15:* 48–65, 1993.

33. Metropolitan Life Insurance Company. 1983 Metropolitan height and weight tables. Stat. Bull. Metrop. Insur. Co., *64:* 1–9, 1983.

34. Longcope, C., Franz, C., Morello, C., Baker, R., and Johnston, C. C. Steroid and gonadotropin levels in women during the peri-menopausal years. Maturitas, *8:* 189–196, 1986.

35. Longcope, C., Hui, S. L., and Johnston, C. C. Free estradiol, free testosterone and sex hormone binding globulin in peri-menopausal women. J. Clin. Endocrinol. Metab., *64:* 513–518, 1987.

36. Kleinbaum, D. G., and Kupper, L. L. Applied Regression Analysis and Other Multivariable Methods. North Scituate, MA: Duxbury Press, 1978.

37. Dorgan, J. F., Reichman, M. E., Judd, J. T., Brown, C., Longcope, C., Schatzkin, A., Albanes, D., Campbell, W. S., Franz, C., Kahle, L., and Taylor, P. The relation of body size and physical activity to plasma levels of estrogens and androgens in premenopausal women (Maryland, United States). Cancer Causes Control, *6:* 3–8, 1995.

38. Dorgan, J. F., Reichman, M. E., Judd, J. T., Brown, C., Longcope, C., Schatzkin, A., Campbell, W. S., Franz, C., Kahle, L., and Taylor, P. The relation of reported alcohol ingestion to plasma levels of estrogens and androgens in premenopausal women (Maryland, United States). Cancer Causes Control, *5:* 53–60, 1994.

39. SAS Institute, Inc. SAS User's Guide, Ver. 6. Cary, NC: SAS Institute, Inc., 1990.

40. Metcalf, M. G., and Mackenzie, J. A. Incidence of ovulation in young women. J. Biosoc. Sci., *12:* 345–352, 1980.

41. Metcalf, M. G. Incidence of ovulation from the menarche to the menopause: observations of 622 New Zealand women. N. Zealand Med. J., *96:* 645–648, 1983.

42. Musey, V. C., Collins, D. C., Brogan, D. R., Santos, V. R., Musey, P. I., Martino-Saltzman, D., and Preedy, J. R. K. Age-related changes in the female hormonal environment during reproductive life. Am. J. Obstet. Gynecol., *157:* 312–317, 1987.

43. Trichopoulos, D., Cole, P., Brown, J. B., Goldman, M. B., and MacMahon, B. Estrogen profiles of primiparous and nulliparous women in Athens, Greece. J. Natl. Cancer Inst., *65:* 43–46, 1980.

44. Yen, S. S. C. Chronic anovulation caused by peripheral endocrine disorders. *In:* S. S. C. Yen and R. B. Jaffe (eds.), Reproductive Endocrinology, Ed. 3, pp. 576–630, Philadelphia, PA: W. B. Saunders Co., 1991.