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Predictors of Ovarian Steroid Secretion in Reproductive-Age Women

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During the baseline period (1985–1988) of a prospective study, midcycle and luteal-phase estrogens and progestins were measured in 175 healthy women aged 21–36 years with spontaneous, cyclic menses in Brooklyn, New York. Subjects contributed daily first-morning urine specimens and three blood specimens during a single menstrual cycle monitored by basal body temperature. Hormone levels were compared according to age, race, and levels of known or suspected breast cancer risk factors. Late age at menarche was associated with increased urinary and serum progestin levels. Increased body weight was associated with decreased progestin levels, even in ovulatory women. Neither weight nor age at menarche was related to estrogen levels. Cigarette smoking was associated with decreased midcycle and luteal-phase estradiol levels. No other factors were associated with differences in any of the hormones measured either midcycle or during the luteal phase, despite good statistical power to detect moderate differences. Sources of individual variability in ovarian steroid levels remain unexplained. These data do not support hypotheses that breast cancer risk factors act through an effect on ovarian hormones during the middle reproductive years. *Am J Epidemiol* 1996;144:381–8.

breast neoplasms; estrogens; menarche; obesity; ovary; progestational hormones; smoking

The risk factors for breast cancer that have been identified in epidemiologic studies indicate that ovarian hormones are critically important in the etiology of that disease. International comparisons of ovarian hormone levels that were carefully measured in populations of women at low and high risk of breast cancer, such as Japanese or Chinese women versus American or British women (1, 2), have indicated group differences that may explain much of the variation in breast cancer incidence between these populations. The observed population differences have not been well explained. Individual variation in ovarian hormone production in reproductive-age women has not been well studied with regard to known breast cancer risk factors or other factors (3). Known breast cancer risk factors such as body size and age at menarche may prove to influence cancer risk through an effect on ovarian hormones.

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Abbreviations: E2G, estradiol 17-beta-glucuronide; PdG, pregnanediol 3-alpha-glucuronide.

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The value of existing studies is limited, in part, because of the collection of single specimens for hormone measurement. The day-to-day within-woman variation in estrogen and progesterone production is enormous, and cycle day is difficult to define; thus, the single specimens typically collected from cycling women are poor indicators of integrated ovarian hormone production throughout an entire menstrual cycle or longer periods of time. Analysis of pooled specimens eliminates the possibility of evaluating individual sources of variation.

In 1985, we initiated a prospective study of the effects of contraceptive tubal ligation on menstrual function. Extensive baseline information was obtained from all women at enrollment, and included daily urine collection and several luteal-phase blood collections during a single menstrual cycle. The goal of this analysis was to quantify the associations between various baseline characteristics and ovarian hormone production. Our particular interest was whether known breast cancer risk factors are predictors of ovarian hormone production.

MATERIALS AND METHODS

Study population

Women were recruited for the study between 1985 and 1988 from the family planning clinics of the

Kings County Hospital Center and the State University Hospital in Brooklyn, New York. Women were eligible for the study if they were between 21 and 36 years of age, had experienced at least one pregnancy, were not pregnant or trying to get pregnant, and reported having menstrual cycles at intervals ranging from 23 to 38 days and lasting for 2–7 days with moderate flow. Women who had recently been 1) using intrauterine devices or hormonal medications, 2) pregnant, or 3) breastfeeding could not enroll in the study until they had experienced at least three normal, cyclic menstrual periods after discontinuation. Women with endocrinopathies or gynecologic abnormalities were excluded.

Data collection

At baseline, all subjects were interviewed regarding their menstrual history, pregnancies, past and present contraceptive use, sexual activity, and use of cigarettes, alcohol, caffeine, and medications, including vitamin supplements. Information regarding other dietary intakes and exercise habits was not obtained. A 47-item life stress instrument modified from the Life Experiences Survey (4) was self-administered. During the baseline study cycle, all women underwent a complete physical examination during which height and weight were measured. Daily basal body temperatures were measured by each subject throughout the study cycle.

Blood and urine specimens were collected during all study cycles, in the following manner: Aliquots from daily first-morning urine specimens were frozen immediately from approximately cycle day 10 until the onset of the following menses (5, 6). Urine specimens were stored in home freezers until the end of each study cycle, when they were transferred in insulated containers to the study office, where they were stored at −20°C. Blood was collected on three separate days, usually in the morning, during the estimated middle of the luteal phase. Blood specimens were centrifuged within 1 hour of collection, and serum was frozen in aliquots at −20°C immediately. All specimens were shipped in insulated containers to the Johns Hopkins University Reproductive Endocrinology Laboratory (Baltimore, Maryland) for assay. Only the baseline cycle results were considered in this analysis.

Laboratory analyses

Each unextracted urine specimen was assayed in duplicate for pregnanediol 3-alpha-glucuronide (PdG) and estradiol-17-beta-glucuronide (E2G) by radioimmunoassay. The specimens were centrifuged at 1,000 × *g* for 10 minutes at 8°C and then diluted 1 : 10 (for PdG) or 1 : 1,000 (for E2G) in 0.1 M phosphate-buffered saline (pH 7) with 0.1 percent gelatin. After addition of tritiated antigen and antibody, the samples were successively incubated at 37°C for 1 hour and at 0°C for 30 minutes. Free and antibody-bound labeled antigens were separated with dextran-coated charcoal, followed by centrifugation and counting of the supernatant. E2G, PdG, antisera to E2G and PdG, and tritiated-hydrogen E2G and PdG were obtained from P. Samarajeewa at the Courtauld Institute of Biochemistry, Middlesex Hospital Medical School, London, England. The PdG antiserum had 19.5 percent cross-reactivity with pregnanediol, but cross-reaction to other steroids or their conjugates was less than 10.1 percent. PdG assay sensitivity was 15 pg/100 μl; the intraassay coefficient of variation was 6.6 percent, with an interassay coefficient of variation of 15.7 percent. E2G assay sensitivity was 7.5 pg/100 μl; the intraassay coefficient of variation was 5.3 percent, with an interassay coefficient of variation of 13.2 percent.

Urinary creatinine concentrations were measured by the picric acid method (7). Serum progesterone concentrations were determined in duplicate by radioimmunoassay using reagents supplied by Diagnostic Products, Inc. (Los Angeles, California). Assay sensitivity was 0.03 ng/ml with an intraassay coefficient of variation of 4.7 percent and a between-assay coefficient of variation of 8 percent. All specimens from a subject were assayed in duplicate and in a single run when she had completed her participation in the study.

Data analysis

The day of ovulation for each study cycle was estimated by a single investigator using the basal body temperature record and the dates of onset of the preceding and subsequent menstrual flows. All blood and urine specimens were then assigned a cycle day corresponding to the number of days plus or minus the estimated day of ovulation. In 15 ovulatory subjects, the day of ovulation could not be determined from the basal body temperature record; for these subjects, the hormone values were used for assistance in assigning a day of ovulation. The following hormonal measures were evaluated: 1) for serum progesterone, *a*) the values from the specimens obtained closest to postovulation days 0 and 8 and *b*) the maximum serum progesterone level; 2) for urinary pregnanediol, *a*) the values from the urine specimens obtained on postovulation days 0 and 8, *b*) the peak value, *c*) the mean of the five individual values for postovulation days 6–10,

and *d*) each of the above values adjusted for urinary creatinine; and 3) for estradiol, the same measures as described for pregnanediol above.

Initial inspection demonstrated that the serum and unadjusted urinary hormone values were not normally distributed. Therefore, all of these hormone measurements were analyzed using both original and log-transformed values. For ease of interpretation, results are presented in the original units. The values for the urinary hormone levels after adjustment for creatinine followed a uniform distribution; therefore, no transformations were used in the analyses of the adjusted urinary hormones. Relations between independent continuous variables and hormonal outcomes were initially evaluated by examining Kendall rank correlations. The effect of categorical and dichotomized continuous independent variables on the hormone measurements was assessed using analysis of variance. All *p* values presented are two-sided and, where relevant, refer to the analyses performed using log-transformed hormone values. Evaluation of the statistical power to compare subgroup means was performed using the Power software package (Epicenter Software, Inc., Pasadena, California).

RESULTS

A total of 175 women were enrolled in the study; 118 were seeking tubal ligation and 57 were fertile contraceptors not seeking tubal ligation. Their characteristics are summarized in table 1. The two groups of participants were similar with regard to age, age at menarche, menstrual cycle length, weight, and religion. The women seeking tubal ligation had higher parity, were more likely to smoke, and were more likely to be Hispanic. The women not seeking tubal ligation were more likely to be employed outside the home, reported more years of education and a higher household income, and were more likely to be white.

During the baseline study cycle, 140 women (80 percent) submitted five urine specimens for cycle days +6 to +10; 21 women (12 percent) submitted four urine specimens, and nine women submitted two or three specimens. The urinary hormone values from these 170 women were analyzed. Results were similar when only those women with at least four specimens were retained in the analysis. A total of 167 women had a serum specimen collected during the same interval (postovulation day 8 ± 2 days).

TABLE 1. Baseline characteristics of 175 reproductive-age (21–36 years) women in a study of ovarian steroid levels, Brooklyn, New York, 1985–1988

Characteristic	Mean	Median	Range	IQR*
Age (years)	29.8	30	21 to 36	6.0
Age (years) at menarche	12.5	13	8 to 17	1.0
Age (years) at first full-term birth	20.8	20	14 to 34	5.0
Parity	2.0	2	0 to 6	2.0
Cycle length (days)	28.3	28.5	22.5 to 34	2.5
Oral contraceptive use (months)	25.4	12.5	0 to 159	34.0
Height (inches)	63.9	64	57 to 71	4.0
Weight (pounds)	150.6	140	90 to 270	45.0
Quetelet index†	25.8	24.3	17.4 to 52.4	6.8
Education (years)	13.3	13	7 to 20	3.0
Annual income (thousands of dollars)	25.6	22.5	2 to 98	24.0
Relationship duration (months)	78.8	66	0 to 216	84
Caffeine intake (mg/day)	184	108	0 to 1,426	238
Alcohol (no. of drinks/week)	2.2	0.7	0 to 26	2.6
Sexual activity‡	6.7	5	0 to 48	8.0
Life stress score§	−1.9	−1.5	−21 to +11	
% smokers	28.0			
No. of cigarettes/day (among smokers)	12.8	10	1 to 40	9.1
Race (%)				
White	18			
Black	60			
Hispanic	21			
% employed	61.5			
% married/cohabiting	61.5			
% vitamin users	31.2			

* IQR, interquartile range.
† Weight (kg)/height (m)^2.
‡ No. of times per month.
§ Modified from the Life Experiences Survey (4).

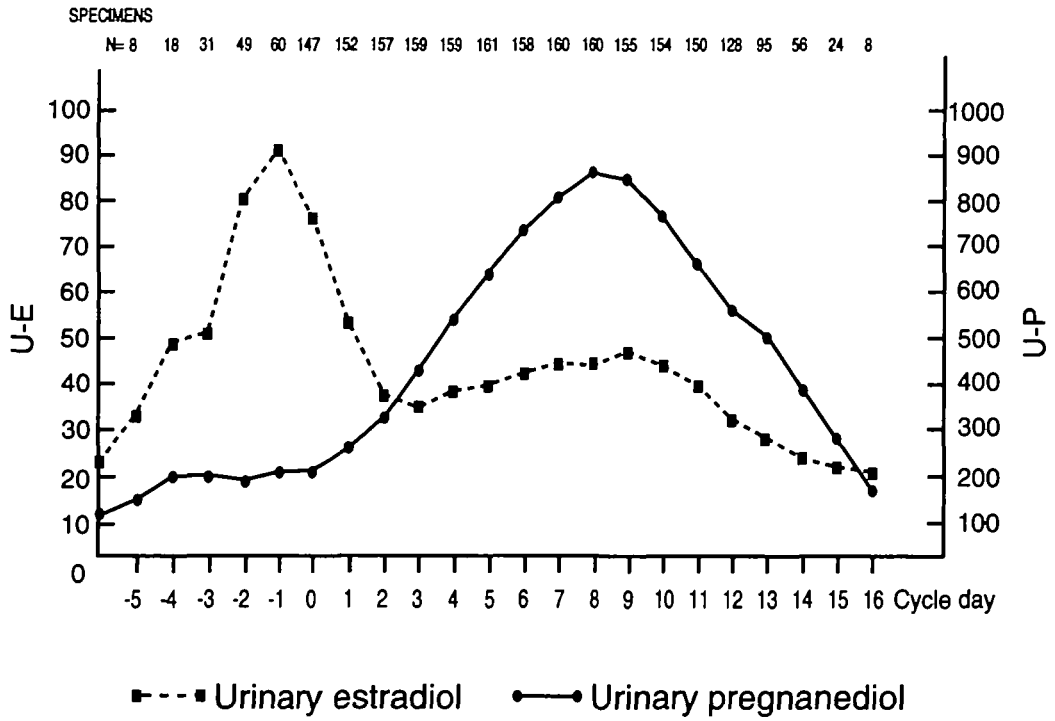

FIGURE 1. Urinary estradiol (■) and pregnanediol (●) levels in 170 ovulatory women aged 21–36 years, by menstrual cycle day. Cycle day 0 was the estimated day of ovulation, determined by basal body temperature. Each point represents the geometric mean of individual values for that cycle day. UE, urinary estradiol (pg/ml); UP, urinary pregnanediol (pg/ml); *N*, number of specimens available for that cycle day.

Four subjects were judged to be anovulatory during the study cycle because they had no serum progesterone values above 4 ng/ml; their maximum serum progesterone values were 1.3, 1.3, 1.5, and 1.8 ng/ml. All statistical analyses were performed both with and without these four subjects, with similar results. Only the results of analyses that excluded these subjects are presented here.

The geometric mean values for urinary estradiol and pregnanediol, by cycle day and the number of specimens collected and analyzed from each day, are shown in figure 1. Table 2 presents the hormone values obtained for all ovulatory subjects. The correlation between 5-day mean values and the area under the curve was high for urinary steroids (data not shown); only 5-day means are presented here, because we had

TABLE 2. Selected values for serum and urinary hormones recorded during the baseline study cycle, Brooklyn, New York, 1985–1988

Hormone	No.*	Median	Geometric mean	Standard deviation
Serum progesterone (ng/ml)				
Day 0	64	2.3	2.3	2.4
Day 8	163	14.4	13.9	6.3
Maximum	166	15.4	14.9	6.1
Urinary pregnanediol (pg/ml)				
Day 0	147	238	212	230
Day 8	160	911	864	734
Maximum	168	1,254	1,294	903
Days 6–10	166	865	863	629
Urinary estradiol (pg/ml)				
Day 0	147	70.2	76.2	73.3
Day 8	160	44.7	43.8	35.9
Maximum	168	87.6	89.2	71.1
Days 6–10	166	47.0	45.7	31.0

* No. of available specimens.

TABLE 3. Ovarian hormone values (geometric means) in selected subgroups of reproductive-age women, Brooklyn, New York, 1985–1988

Variable	Serum progesterone—day 8 (ng/ml)	Urinary pregnanediol—days 6–10 (pg/ml)	Urinary estradiol—days 6–10 (pg/ml)	Urinary estradiol—day 0 (pg/ml)
Age (years)				
21–27 (n = 43)	16.2	934	50.1	77.6
28–32 (n = 79)	13.2	833	47.3	79.0
33–36 (n = 48)	12.9	838	39.5	70.6
Two-sided p value	0.02	0.55	0.11	0.69
Race				
White (n = 33)	12.2	897	41.8	73.3
Black (n = 102)	14.6	853	45.4	77.9
Hispanic (n = 35)	13.2	857	47.0	72.8
Two-sided p value	0.12	0.94	0.71	0.89
Smoking				
No (n = 122)	13.5	856	49.5	84.9
Yes (n = 48)	14.4	867	36.9	58.6
Two-sided p value	0.42	0.90	0.003	0.002
Weight (pounds)				
90–140 (n = 86)	15.2	958	42.8	72.2
141–270 (n = 84)	12.4	771	48.8	79.8
Two-sided p value	0.003	0.02	0.16	0.38
Age (years) at menarche				
8–12 (n = 83)	12.8	775	47.3	77.1
13–17 (n = 87)	14.8	949	43.9	75.3
Two-sided p value	0.03	0.02	0.42	0.77
Parity				
0 (n = 20)	13.6	853	50.0	74.3
1 (n = 33)	13.8	865	46.3	74.7
2 (n = 66)	14.4	920	46.5	84.4
≥3 (n = 48)	13.0	782	42.3	66.8
Two-sided p value	0.52	0.57	0.38	0.64
Age (years) at first birth				
14–19 (n = 68)	15.0	906	48.1	74.3
20–24 (n = 59)	12.9	812	41.1	73.9
25–34 (n = 13)	14.6	851	45.8	86.4
Two-sided p value	0.34	0.57	0.38	0.64
Caffeine intake (mg/day)				
<60 (n = 59)	14.9	804	44.0	78.3
60–179 (n = 51)	13.0	922	46.1	75.7
≥180 (n = 59)	13.4	864	46.6	73.9
Two-sided p value	0.35	0.81	0.86	0.81
Vitamin supplements				
None (n = 117)	15.0	838	49.2	76.3
Any (n = 53)	13.3	870	44.2	76.0
Two-sided p value)	0.30	0.53	0.13	0.98
Alcohol (drinks/week)				
0 (n = 52)	12.0	796	42.8	71.2
<2 (n = 65)	15.0	856	45.3	75.0
≥2 (n = 57)	14.2	921	48.2	81.8
Two-sided p value	0.002	0.80	0.64	0.73
Life stress score*				
−21 to −5 (n = 34)	13.3	798	45.8	76.6
−4 to 0 (n = 102)	13.6	877	47.0	77.6
+1 to +11 (n = 38)	14.6	865	41.6	71.4
Two-sided p value	0.79	0.55	0.48	0.96

* Modified from the Life Experiences Survey (4).

more complete data for this variable. The creatinine-adjusted urinary hormone values and the unadjusted values had similar distributions (H. Zacur, The Johns Hopkins University School of Medicine, unpublished manuscript); therefore, only the unadjusted values are presented in table 2. However, subgroup analyses were performed using both adjusted and unadjusted urinary hormone values (see below).

The detectable differences in mean hormone values are presented below for comparisons of two equal subgroups (each with 80 subjects) based on the means and standard deviations shown in table 2. All differences would be detectable with 80 percent power and a two-sided alpha of 0.05. For serum progesterone endpoints, an increase of 46 percent or greater in day 0 values, an increase of 20 percent in day 8 values, and an increase of 18 percent in the maximum values would be detectable. For urinary pregnanediol endpoints, an increase of 48 percent or greater in day 0 values, an increase of 38 percent in day 8 values, an increase of 31 percent in the maximum values, and an increase of 32 percent in the day 6–10 mean values would be detectable. For urinary estradiol endpoints, a decrease of 30 percent or greater in day 0 values, a decrease of 27 percent in day 8 values, a decrease of 25 percent in the maximum values, and a decrease of 21 percent in the day 6–10 mean values would be detectable.

Despite sufficient power to detect moderate subgroup differences, few of the baseline characteristics showed associations with the hormone levels. Table 3 lists the geometric mean values for serum progesterone day 8, urinary pregnanediol days 6–10, urinary estradiol days 6–10, and urinary estradiol day 0 observed according to subgroups of several variables. Estradiol levels (urinary estradiol day 0 and urinary estradiol days 6–10) were lower among smokers. Mean values (days 6–10) were somewhat lower among smokers of more than 12 cigarettes per day as compared with smokers of fewer cigarettes (33.1 pg/ml vs. 40.1 pg/ml), although this finding could have arisen by chance ($p = 0.27$). Among smokers, the rank correlation coefficients for the association between number of daily cigarettes and urinary estradiol day 0 and urinary estradiol days 6–10 were not significant ($p = 0.10$ and $p = 0.14$, respectively); however, there were too few smokers in the study for us to obtain adequate statistical power to identify even a strong dose-response relation.

Pregnanediol levels and serum progesterone levels were lower in the heaviest subjects and were also lower among those with an earlier age at menarche. In pairwise correlation analyses considering the associations between weight (continuous) and age at menarche (continuous) and the hormones pregnanediol and progesterone, each correlation coefficient was significant (all p values < 0.001). When Quetelet index (weight/height^2) was used as the independent variable instead of weight, results were nearly identical (data not shown). The results of stratified analyses, shown in table 4, demonstrate the independent effects of weight and age at menarche. Adjustment for age and race did not diminish the observed associations. There was an age-related decrement in serum progesterone day 8, but age was not related to any other hormone measurements.

Unrelated to any of the measured hormone levels were age at first full term birth, gravidity, parity, reported or measured menstrual cycle length, frequency of sexual intercourse, and duration of past oral contraceptive use. In addition, vitamin supplementation, caffeine and alcohol consumption, and use of over-the-counter analgesics were unrelated to hormone levels. Other social variables, including years of education, religion, employment, household income, and current life stress scores, were also unrelated to these hormone levels. Subgroup analyses were performed using the creatinine-adjusted urinary hormone levels; these results are presented in table 5. No new

TABLE 4. Progestin values (geometric means) in 170 reproductive-age women, by weight and age at menarche, Brooklyn, New York, 1985–1988

Measure	Current weight (pounds)	Age (years) at menarche		Current age* (years)		
		8–12	13–17	21–27	28–32	33–36
Urinary pregnanediol (mean of days 6–10)† (pg/ml)	90–140	795	1,099			
	141–270	759	788			
Serum progesterone (day 8)‡ (ng/ml)	90–140	14.4	15.8	17.3	15.9	12.5
	141–270	11.7	13.5	14.6	11.1	13.2

* Two-way analysis of variance: $p = 0.005$ for weight; $p = 0.02$ for age; $p = 0.03$ for interaction.
† Two-way analysis of variance: $p = 0.03$ for weight; $p = 0.01$ for age at menarche; $p = 0.11$ for interaction.
‡ Two-way analysis of variance: $p = 0.01$ for weight; $p = 0.03$ for age at menarche; $p = 0.69$ for interaction.

TABLE 5. Ovarian hormone values (arithmetic means) adjusted for urinary creatinine levels in selected subgroups of reproductive-age women, Brooklyn, New York, 1985–1988

Variable	Adjusted urinary pregnanediol—days 6–10 (pg/ml)	Adjusted urinary estradiol—days 6–10 (pg/ml)	Adjusted urinary estradiol—day 0 (pg/ml)
Age (years)			
21–27 (n = 43)	9.3	0.52	0.82
28–32 (n = 79)	8.7	0.53	0.86
33–36 (n = 48)	10.7	0.54	0.79
Two-sided p value	0.15	0.97	0.83
Race			
White (n = 33)	11.1	0.58	0.82
Black (n = 102)	9.2	0.52	0.80
Hispanic (n = 35)	9.1	0.50	0.70
Two-sided p value	0.22	0.08	0.66
Smoking			
No (n = 122)	9.4	0.56	0.87
Yes (n = 48)	9.5	0.44	0.73
Two-sided p value	0.90	0.04	0.21
Weight (pounds)			
90–140 (n = 86)	10.4	0.52	0.75
141–270 (n = 84)	8.5	0.55	0.93
Two-sided p value	0.02	0.11	0.57
Age (years) at menarche			
8–12 (n = 83)	8.8	0.55	0.83
13–17 (n = 87)	10.0	0.50	0.74
Two-sided p value	0.16	0.08	0.33
Parity			
0 (n = 20)	10.0	0.55	0.93
1 (n = 33)	10.7	0.61	0.88
2 (n = 66)	9.7	0.53	0.82
≥3 (n = 48)	8.0	0.47	0.77
Two-sided p value	0.14	0.34	0.82
Age (years) at first birth			
14–19 (n = 68)	9.1	0.53	0.73
20–24 (n = 59)	8.8	0.50	0.85
25–34 (n = 13)	11.1	0.57	0.98
Two-sided p value	0.14	0.70	0.31
Alcohol (drinks/week)			
0 (n = 52)	9.4	0.51	0.85
<2 (n = 65)	9.8	0.58	0.87
≥2 (n = 57)	9.1	0.49	0.78
Two-sided p value	0.40	0.42	0.71
Life stress score*			
−21 to −5 (n = 34)	9.0	0.53	0.89
−4 to 0 (n = 102)	9.2	0.52	0.80
+1 to +11 (n = 38)	10.4	0.56	0.86
Two-sided p value	0.11	0.95	0.85

* Modified from the Life Experiences Survey (4).

associations emerged in the analyses of the adjusted variables.

DISCUSSION

The ovarian hormone measurements analyzed here provided several superior indicators of the hormonal milieu of reproductive-age women. The number of women studied, combined with the careful, prospective assessment of cycle day and the collection of multiple blood and urine specimens during a single cycle, was unprecedented. Failure to identify important sources of variation in progesterone and pregnanediol levels due to misclassification of true levels should have been minimized in this study. A possible uncontrolled source of random error in serum progesterone measurements may have been pulsatile and diurnal variation (8, 9); these sources of variation were identified after data collection had begun. Statistical power to detect differences in serum progesterone during the midluteal phase was good despite this potential source of random error.

Predictors of peak follicular-phase estrogen levels cannot be evaluated here with equal precision because of a lack of complete data collection during that phase of the menstrual cycle; however, estradiol levels on the day of ovulation (day 0) were close to peak levels that occurred 1–2 days earlier. Evaluation of midcycle estradiol was the best available surrogate for the identification of predictors of peak estradiol values. Collection of earlier follicular-phase specimens, as well as measurement of other analytes of widespread interest (sex hormone-binding globulin, prolactin, gonadotropins, androgens, and additional urinary estrogen metabolites), was beyond the scope of this study.

Our finding of lower urinary estradiol levels in smokers agrees in direction and magnitude with the results of MacMahon et al. (10), in which estrone, estradiol, and estriol were all reduced in 39 current smokers compared with 43 nonsmokers. In contrast, Berta et al. (11) found no difference in these three metabolites between 174 smokers and 311 nonsmokers in Italy. The disagreement may be due, in part, to a failure to measure other estrogen metabolites, particularly the hydroxyestrones. In a study of 15 heavy smokers and 14 nonsmokers, five urinary estrogen metabolites were measured during the follicular phase (12). Estriol was the only metabolite that was lower in the smokers. Thus, results of laboratory studies remain inconsistent. While our data strongly support those epidemiologic studies which have found that smoking is antiestrogenic (13), there is no consistent evidence that smoking is relevant to breast cancer risk (14).

An impact of age at menarche on estrogen levels later in life has been reported. In a Finnish cohort of 44 women, those with known early menarche had higher follicular-phase serum estradiol levels measured at ages 21–30 years; there was no difference in maximum luteal-phase serum progesterone by age at menarche (15). Those authors suggested that the increased breast cancer risk of women with early ages at menarche might be mediated through higher estrogen lev-

els. MacMahon et al. (16) and Bernstein et al. (17) found no effect of age at menarche on various estrogen measurements among women in their thirties. We found no effect of menarcheal age on either midcycle or luteal-phase estradiol levels, but our observation of increased progesterone and pregnanediol in women with a later age at menarche has not been previously reported. This association does not, however, help to clarify how age at menarche affects breast cancer risk.

The strongest association observed here was decreased urinary and serum progestins with increased weight. While previous studies have identified obesity as a cause of altered hormone levels secondary to anovulation (18), decreased progesterone levels in ovulatory obese women have not been previously described. Weight and age at menarche are related to breast cancer risk (3); however, the effect of these variables on levels of progesterone and its metabolites in this study was not consistent with respect to the direction of their effects on breast cancer risk. Other factors associated with breast cancer risk showed no association with midcycle or luteal-phase ovarian hormone levels.

Despite our carefully timed collection of multiple specimens designed to reduce random variation, we were able to identify few variables that were strongly associated with daily or integrated hormone levels, and the associations that were identified were not clearly consistent with previous studies. Although we had no information on diet and exercise, many other environmental exposures were measured during the same time interval as the hormone levels. The source of most of the variation in these hormone levels remains unexplained. In particular, the individual breast cancer risk factors considered here do not appear to modify that risk through direct effects on midcycle or luteal-phase ovarian function during the middle reproductive years.

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