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Reproductive factors and family history of breast cancer in relation to plasma estrogen and prolactin levels in postmenopausal women in the Nurses' Health Study (United States)

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Parity, age at first birth, age at menarche, and a family history of breast cancer have each been associated consistently with breast cancer risk. Whether this increase in risk is mediated, at least in part, through changes in endogenous hormone levels is unclear. We conducted a cross-sectional study of the relationships between these factors and plasma hormone levels in 216 healthy postmenopausal women in the Nurses' Health Study (United States). The hormones evaluated were estradiol, percent and total free estradiol, percent and total bioavailable estradiol, estrone, estrone sulfate, and prolactin. After controlling for age, body mass index (weight/height^2), and alcohol use, we observed inverse associations between estrone sulfate and parity ($r = -0.15$, $P = 0.03$) and between percent bioavailable estradiol and age at first birth ($r = -0.17$, $P = 0.02$). Although women with a family history of breast cancer tended to have higher estrogen levels compared with women without such history, the differences were not statistically significant. Age at menarche was not related significantly to any of the hormones. These data provide some additional evidence that the inverse relationship observed between parity and breast cancer risk may be mediated, at least in part, through decreased estrogen levels. Our data do not support a substantial influence of either family history of breast cancer or age at menarche on postmenopausal estrogen or prolactin levels. *Cancer Causes and Control* 1995, 6, 217-224

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Introduction

Endogenous hormones are considered to play a central role in the development of breast cancer in women,[1] primarily because of the consistent associations observed between reproductive factors, such as late age at menarche, parity, and early age at first birth, and subsequent breast-cancer risk. These factors may reduce cancer risk by increasing the differentiation of breast tissue, thus making it more resistent to neoplastic transformation.[2] However, higher levels of plasma hormones, including both estrogens and prolactin, also have been hypothesized to increase breast cancer risk.[3] Whether the association between these reproductive events and breast cancer may be mediated, at least in part, through a lasting influence on plasma hormone levels is unclear. In addition, family history of breast cancer is associated with an increase in breast cancer risk.[1,4] Whether postmenopausal women with a family history of breast cancer have higher estrogen and prolactin levels is unknown.

The relationships between reproductive factors and hormone levels in postmenopausal women have been assessed in just two previous studies.[5,6] Parous postmenopausal women were reported to have lower plasma prolactin[6] and urinary estrogen levels[5] compared with nulliparous women, although the latter association was not statistically significant. A nonsignificant inverse association also was noted between age at menarche and urinary estrogens.[5] Begg *et al*[7] have observed higher plasma estrone levels in postmenopausal siblings of breast cancer patients compared with control women. In addition, adolescent and adult premenopausal daughters of women with breast cancer have been reported to have higher plasma estrogen and prolactin levels[8,9] and higher urinary-estrogen levels[10] than daughters of healthy women. However, the determinants of endogenous estrogen levels in pre- and postmenopausal women may differ given the differences in their primary source (*i.e.*, ovarian *cf* extra-ovarian).

In this study, we assess age at menarche, age at first birth, parity, and family history of breast cancer in relation to plasma hormone levels in a cross-sectional study of 216 postmenopausal women. Estradiol, free estradiol (*i.e.*, non-protein-bound estradiol), percent free estradiol, bioavailable estradiol (*i.e.*, non-protein-bound estradiol plus albumin-bound estradiol), percent bioavailable estradiol, estrone, estrone sulfate (an estrogen conjugate and the most abundant estrogen in plasma), and prolactin levels are each evaluated, as all have been hypothesized to play a role in breast cancer etiology.

Materials and methods

Blood sample collection

The Nurses' Health Study began in 1976 among 121,700 female registered nurses in the United States who were 30 to 55 years of age at that time.[4] The women have been followed biennially by mailed questionnaire since 1976. In 1989 and 1990, we collected blood samples from over 32,000 Nurses' Health Study participants. Participants were sent a blood collection kit containing all supplies needed to have a blood sample drawn. Each woman arranged to have her blood sample drawn and then returned to us, as whole blood, via overnight courier. An icepack was included with the blood sample to keep it cool during transport. Ninety-seven percent of the samples were received in our laboratory within 26 hours of being drawn. The stability of estrogens and prolactin in whole blood for 24 to 48 hours has been previously documented.[11] Upon arrival in our laboratory, the blood was centrifuged and aliquotted into plasma, and red blood-cell and white blood-cell components. The samples have been archived in continuously monitored, liquid nitrogen freezers since collection. This project was approved by the institutional review board of the Brigham and Women's Hospital.

Study populations

Two datasets from this cohort were available for the present analysis. In each, the women were postmenopausal (last menstrual period at least 12 months before the blood sample was collected), had not used postmenopausal hormones for at least three months prior to blood collection, and were free from diagnosed cancer (except nonmelanoma skin cancer).

The first dataset consisted of 116 control subjects from a nested case-control study of plasma hormone levels and subsequent breast-cancer risk. In this dataset, we also had included 20 randomly selected participants from the Nurses' Health Study who reported consuming at least 15 grams of alcohol per day and 10 women who reported drinking at least four cups of coffee per day, based on their responses to a 1990 food frequency questionnaire (these associations are the subject of another report). Results of the current analyses were unchanged when repeated after excluding these 30 women; thus, these women are included in the results shown here. Laboratory analyses were performed in May and June, 1993.

The second dataset consisted of 80 women who were participants in a study of the reproducibility of hormone levels over time. After the initial blood collection, each woman had sent us two additional

samples over the following two years. Results from the first blood sample are used in the present analyses. Laboratory analyses were conducted in February 1993 (estrone sulfate and prolactin) and September 1993 (all other hormones); all analyses were performed by the same two laboratories and using the same methods as in the first dataset.

Exposure data

Age at menarche, age at first birth, and parity (defined as a pregnancy lasting for six months or more) were first asked on the 1976 study questionnaire. Parity was queried on every biennial questionnaire until 1984; as of 1984, the women ranged in age from 38 to 63 and thus we would have missed few subsequent pregnancies. History of breast cancer in the participant's mother or in her sisters was asked in 1976 and updated in 1982 and 1988.

Differences in obesity and alcohol use may distort the relationships between reproductive factors and hormones, thus they were included as covariates in this analysis. We collected data on height on the 1976 questionnaire. Data on current weight was obtained at the time of the blood collection. (For one woman who did not complete this question on the questionnaire, the weight reported on her 1990 study questionnaire was used.) Body mass index (BMI) (weight [kg]/ height $[m]^2$) was used as the measure of obesity. Alcohol intake was ascertained by use of a previously validated, semi-quantitative food-frequency questionnaire sent to participants in June 1990.[12-14]

Laboratory methods

With the exception of estrone sulfate and prolactin, all hormone analyses were conducted by Nichols Institute (San Juan Capistrano, CA, USA). Estradiol and estrone were assayed by radioimmunoassay (RIA) preceded by organic extraction and celite chromatography.[15-17] Estrone sulfate was assayed, after extraction of estrone, by RIA (of estrone) following enzyme hydrolysis, organic extraction and separation by column chromatography.[18] Percent free estradiol (*i.e.*, percent non-protein bound) was assayed using equilibrium dialysis;[19] the percent dialyzable estradiol was calculated as per Vermuelen.[19] Absolute levels of free estradiol were calculated using the total estradiol and percent free estradiol. The percent bioavailable estradiol (*i.e.*, percent free plus percent albumin-bound estradiol) was assayed using an ammonium sulfate precipitation.[19-20] Absolute levels of bioavailable estradiol were calculated using the total estradiol and percent bioavailable estradiol. Prolactin was assayed by use of a kit (Ciba-Corning, East Walpole, MA, USA).

In each batch of samples, we interspersed replicate plasma samples (one per 10 study samples) which were labeled to preclude their identification by the hormone laboratory; these samples were used to assess laboratory precision. All inter- and intra-assay laboratory coefficients of variation (CV) were 15 percent or less with the exception of estrone sulfate which had an intra-assay CV of 15 percent but an inter-assay CV of 28 percent. The higher inter-assay CV was due to a single run with high replicate values. Our results did not change when the 12 study samples in this run were excluded, thus all samples are included in the results presented here.

With the exception of percent bioavailable estradiol and estrone levels, the mean and standard deviation of the plasma hormone levels in each of the two datasets were similar. Mean percent, bioavailable estradiol levels were lower and estrone levels were higher in the case-control dataset compared with the reproducibility dataset. The mean value of the replicate samples also varied in the same manner between datasets, indicating that most (if not all) of the difference was due to laboratory drift rather than true differences in hormone levels between the two populations. Therefore, in all analyses, we controlled for dataset.

Statistical analysis

We excluded from the analysis women with missing data on age at menarche ($n = 1$), no 1990 questionnaire ($n = 4$), insufficient plasma for at least one hormone analysis ($n = 2$), or plasma hormone values more than twice the normal postmenopausal range of the assaying laboratory ($n = 2$). In addition, one woman was included in both datasets; only values from the case-control dataset were used. These exclusions left 139 women in the case-control dataset and 77 in the reproducibility dataset (total $n = 216$).

Several women did not have all eight hormones assayed because of an insufficient plasma volume. The following number of women were missing plasma hormone values: prolactin ($n = 1$); estrone ($n = 2$); estrone sulfate ($n = 2$); estradiol ($n = 4$); free estradiol ($n = 4$); percent bioavailable estradiol ($n = 7$); and bioavailable estradiol ($n = 10$).

First-order, Spearman rank correlation-coefficients, controlling for dataset, were calculated to assess the linear association between the reproductive factors and plasma hormone levels. To account for possible confounding, we separately regressed the hormone value and the reproductive factors on the potential confounders. Spearman correlation coefficients between these two sets of residuals then were calculated. To calculate mean hormone levels within each category of parity or age at first birth, we first regressed the hormone values on the potential confounders and then added a constant value (the overall mean of the log

Table 1. Descriptive characteristics and plasma hormone levels in 216 postmenopausal women, Nurses' Health Study

Characteristic	Mean	(SD)[a]
Age (yrs)	61.5	(5.0)
Years menopausal	13.3	(7.0)
Age at menarche (yrs)	12.6	(1.4)
Parity	3.3	(1.9)
Age at first birth[b] (yrs)	25.4	(3.5)
Family history of breast cancer (%)	13.0	—
Plasma hormone	**Geometric mean**	**Range**
Estradiol (pg/ml)	6.9	2-46
Free estradiol (pg/ml)	0.11	0.02-0.62
Percent free estradiol (%)	1.5	0.6-2.1
Bio estradiol[c] (pg/ml)	1.8	0.2-20.8
Percent bio estradiol[c]	26.1	6.5-73.6
Estrone (pg/ml)	31.6	9-131
Estrone sulfate (pg/ml)	175	34-668
Prolactin (ng/ml)	9.4	1.9-31.5

[a] SD = standard deviation.
[b] Among parous women only ($n = 197$).
[c] bio = bioavailable, *i.e.*, non-protein bound estradiol plus albumin-bound estradiol.

hormone level) to the residuals; the averaged values then were exponentiated. To assess differences in hormone levels according to family history of breast cancer, we regressed plasma hormone levels on family history. Continuous variables were used for all exposures and covariates and, because of increased normality, all plasma hormone levels were natural log-transformed in these analyses. We used the SAS statistical package.[21]

Results

The women ranged in age from 45 to 69 years (mean age = 61.5 years) and had been menopausal for at least one year and up to 35 years (mean = 13.3 years) (Table 1). The average parity (including nulliparous women as 0) was 3.3; among parous women only, average parity was 3.7. Nineteen women (nine percent) were nulliparous. Twenty-eight women (13 percent) reported a family history of breast cancer. The participants' average BMI was 26.1 kg/m^2 (standard deviation [SD] = 5.1 kg/m^2) and their average alcohol intake was 8.0 g/day (SD = 12.8 g/day). The geometric means and range of values for each plasma hormone are provided in Table 1.

Shown in Table 2 are the Spearman correlations between the reproductive factors and plasma hormone levels controlling only for dataset, and secondly, controlling for dataset, age, BMI, and alcohol use. In the analysis controlled for dataset, only the inverse relations between age at menarche and estrone sulfate and between age at first birth and percent bioavailable estradiol were statistically significant. After controlling for the other covariates, the relationship between estrone sulfate and age at menarche was attenuated and no longer significant ($r = -0.11$, P-value = 0.12). Age at menarche was unassociated with any other plasma hormone. Parity (defined as 0 through $\geqslant 8$) tended to be associated inversely with each of the plasma estrogens, although only the correlation with estrone sulfate was significant ($r = -0.15$, P-value = 0.03); the increase in the correlations was due primarily to control for BMI. Age at first birth remained associated significantly with percent bioavailable estradiol

Table 2. Spearman correlation coefficients between characteristics of interest and plasma hormone levels in 216 postmenopausal women, Nurses' Health Study

Plasma hormone	Age at menarche		Parity		Age at 1st birth[c]	
	r^a	r^b	r^a	r^b	r^a	r^b
Estradiol (pg/ml)	−0.04	0.04	0.10	−0.01	−0.01	0.02
Free estradiol (pg/ml)	−0.04	0.06	0.11	−0.01	−0.01	0.01
Percent free estradiol (%)	0.04	0.12	−0.01	−0.07	0.00	−0.02
Bio estradiol[d] (pg/ml)	−0.09	0.02	0.07	−0.10	−0.06	−0.05
Percent bio estradiol[d] (%)	−0.04	0.08	0.00	−0.11	−0.15[e]	−0.17[e]
Estrone (pg/ml)	−0.03	0.03	0.03	−0.05	0.00	0.03
Estrone sulfate (pg/ml)	−0.16[e]	−0.11	−0.05	−0.15[e]	0.07	0.09
Prolactin (ng/ml)	0.05	0.05	0.03	0.03	0.01	0.02

[a] Controlling for dataset only.
[b] Controlling for age (years), dataset, body mass index (kg/m^2), and alcohol use (g/day).
[c] Among parous women only ($n = 197$).
[d] Bio = bioavailable.
[e] $P \leq 0.05$.

Table 3. Spearman correlation coefficients between reproductive factors and plasma hormone levels among 197 parous, postmenopausal women, Nurses' Health Study

Plasma hormone	Parity r^a	Age at first birth r^a
Estradiol (pg/ml)	−0.06	0.03
Free estradiol (pg/ml)	−0.05	0.02
Percent free estradiol (%)	−0.08	−0.07
Bio[b] estradiol (pg/ml)	−0.13	−0.07
Percent bio[b] estradiol (%)	−0.13	−0.22[c]
Estrone (pg/ml)	−0.13	0.01
Estrone sulfate (pg/ml)	−0.20[c]	0.06
Prolactin (ng/ml)	0.04	0.03

[a] Controlling for age (yrs), dataset, BMI (kg/m^2), alcohol use (g/day), and each other.
[b] Bio = bioavailable.
[c] $P \leq 0.01$.

($r = -0.17$, P-value = 0.02). Prolactin levels were unassociated with any of the reproductive variables. When analyses were repeated controlling for time since menopause, instead of age, findings were unchanged.

When we assessed the correlations between plasma hormone levels and both parity and age at first birth among parous women only and controlling for each other, findings were similar (Table 3). Because of the positive correlations between parity and a number of the plasma estrogens in this analysis, we also assessed these associations controlling for one other plasma estrogen at a time. The correlation of parity with plasma estrone sulfate and percent bioavailable estradiol changed only slightly after controlling for either estrone or bioavailable estradiol ($r = -0.19$, $P = 0.01$ for estrone sulfate; and $r = -0.12$, $P = 0.08$ for percent bioavailable estradiol) while the relationships with estrone and total bioavailable estradiol were attenuated ($r = -0.05$ for each).

We calculated mean plasma-hormone levels within each category of parity or age at first birth for the hormones which had a significant association with these factors (Table 4). Women in the highest parity category had estrone sulfate levels that were 21 percent lower than those among women of parity one (195 pg/ml *cf* 154 pg/ml); estrone sulfate decreased in a stepwise manner with increasing parity. Nulliparous women, however, had levels which were similar to women of parity four or five (164 pg/ml). Among parous women, the difference in percent bioavailable estradiol between extreme categories of age at first birth was just eight percent (26.3 percent for age at first birth $=\leq 22$ yrs *cf* 24.1 percent for age at first birth $=\geq 30$ yrs).

Finally, we assessed hormone levels in women with and without a family history of breast cancer. Most plasma estrogens were higher in the group of women with a family history; the greatest difference was in bioavailable estradiol levels (Table 5). After accounting for age, BMI, dataset, parity, age at menarche, and alcohol use, women with a family history of breast cancer had 25 percent higher bioavailable-estradiol levels compared with women without a family history (95 percent confidence interval [CI] = −5-66%). None of the differences in plasma hormone levels between women with and without a family history was statistically significant however.

Table 4. Geometric mean plasma hormone levels in 216 postmenopausal women within each category of parity and age at first birth, Nurses' Health Study

Hormone[a]	Parity					
	0 ($n = 18$)	1-2 ($n = 51$)	3 ($n = 46$)	4 ($n = 43$)	5 ($n = 32$)	≥ 6 ($n = 24$)
Estrone sulfate (pg/ml)	164	195	184	179	151	154
Hormone[c]	**Age at first birth (yrs)[b]**					
	≤ 22 ($n = 35$)	23 ($n = 30$)	24 ($n = 33$)	25-26 ($n = 42$)	27-29 ($n = 26$)	≥ 30 ($n = 25$)
Percent bio estradiol (%)	26.3	29.9	27.4	25.8	21.9	24.1

[a] Controlling for dataset, age (yrs), body mass index (kg/m^2), and alcohol use (g/day).
[b] Among parous women only.
[c] Controlling for dataset, age (yrs), body mass index (kg/m^2), parity, and alcohol use (g/day).

Table 5. Comparison of geometric mean plasma hormone levels in postmenopausal women with a family history of breast cancer ($n = 28$) and with no family history ($n = 188$), Nurses' Health Study

Hormone	Family history		Regression coefficient[a]	*P*-value[a]
	No	Yes		
Estradiol (pg/ml)	6.8[b]	7.5	0.104	0.35
Free estradiol (pg/ml)	0.11	0.12	0.123	0.30
Percent free estradiol (%)	1.5	1.6	0.021	0.49
Bio estradiol[c] (pg/ml)	1.7	2.3	0.226	0.11
Percent bio estradiol[c] (%)	25.5	30.0	0.119	0.17
Estrone (pg/ml)	31.7	31.1	−0.022	0.79
Estrone sulfate (pg/ml)	172	196	0.078	0.43
Prolactin	9.4	9.6	0.012	0.91

[a]Regression coefficient (and *P*-value) for family history from model controlling for age, body mass index, dataset, parity, age at menarche, and alcohol use.
[b]Geometric mean.

Prolactin levels have a pronounced circadian variation and tend to vary in response to food intake; this variation might serve to obscure an association with our exposures of interest. However, when analyses were repeated among the 141 women who had their blood drawn between 6 a.m. and 10 a.m. after at least an eight-hour fast, findings were unchanged.

Discussion

We observed inverse associations between estrone sulfate and parity and between percent bioavailable estradiol and age at first birth among these healthy postmenopausal women. Although women with a family history of breast cancer tended to have higher estrogen levels, particularly for bioavailable estradiol, none of these differences was statistically significant. Age at menarche also was not related significantly to any of the plasma hormones.

It is unlikely that we have missed any substantial correlations with plasma estrogen levels due to having only one blood sample per woman. In this same population, we have found correlations over two to three years between replicate measures of plasma estrogen levels to be quite high, ranging from 0.68 for estradiol to 0.86 for percent bioavailable estradiol.[22] In addition, assay precision was high as assessed by the replicate samples which were interspersed among our study samples. The reproducibility of plasma prolactin levels over a several-year period was somewhat lower in our dataset ($r = 0.53$), indicating that any modest correlation with prolactin levels might have been missed. Although each of the reproductive factors predated the measurement of plasma hormone levels, we cannot exclude the possibility that premenopausal hormone levels influenced the reproductive events (*e.g.*, parity) and that the relations we observe are due to a correlation between premenopausal and postmenopausal hormone levels. The likelihood of this relationship is unknown as we are unaware of any reports in which the correlations between pre- and postmenopausal hormone levels were assessed.

An inverse association between parity and breast cancer risk has been observed quite consistently in epidemiologic studies.[1] Nonsignificantly higher urinary-estrogen levels were observed previously in postmenopausal nulliparous women relative to postmenopausal parous women.[5] A significant inverse association between parity and both plasma estrogens and prolactin has been reported among premenopausal women.[23] We also observed a trend towards lower estrogen levels with increasing parity; the inverse correlation with estrone sulfate was statistically significant. When comparing mean hormone levels by category of parity, we observed that women with parity of six or more had estrone sulfate levels that were 21 percent lower than levels among women of parity one or two. Rather than having the highest estrone sulfate levels as would be hypothesized, nulliparous women had levels similar to multiparous women, although these estimates were based on fewer measurements ($n = 18$) and thus would be less precise. Estrone sulfate is the most abundant plasma estrogen in postmenopausal women[24] and has been hypothesized to be an important source of estradiol in breast cancer cells[25] and normal breast tissue.[26] We were unable to confirm a previously reported, inverse relationship between parity and postmenopausal prolactin levels,[6] although our

smaller sample size ($n = 216$ *cf* 1,881) would have prevented our detection of a very modest relationship.

In the only other assessment among postmenopausal women, Trichopoulos *et al*[5] reported a nonsignificant decrease in urinary estrogen levels with increasing age at menarche. In two reports of premenopausal women, plasma or urinary estrogens were significantly higher in subjects with an earlier age at menarche;[27,28] however, in a third study,[29] no consistent relationship with plasma or urinary estrogens was noted among either US or Chinese women. We did not observe any significant association between age at menarche and either the plasma estrogens or prolactin.

Later ages at first birth are associated with increased breast cancer risk.[1] However, the only correlation we observed between hormone levels and age at first birth was an inverse one with percent bioavailable estradiol, a relationship opposite to what one would expect. We have no explanation for this finding although perhaps it occurred simply by chance. To our knowledge, this is the first report of this relationship among postmenopausal women. In a study among premenopausal women, age at first birth was unrelated to either estrone, estradiol, or free estradiol.[23] Age at first birth was unrelated to postmenopausal prolactin levels in one previous study;[6] our findings support this observation.

A relationship between family history of breast cancer and postmenopausal hormone levels is plausible as higher estrogen levels are thought to increase breast cancer risk[3] and endogenous hormone levels, in part, may be genetically determined. Postmenopausal siblings of breast cancer patients were observed[7] to have higher plasma-estrone levels compared with control women. The only other estrogen assessed in that study was estradiol which did not vary significantly between the two groups. In our data, plasma estrogens also tended to be higher in women with a family history of breast cancer compared with those with no such family history, but none of the comparisons was statistically significant. Of note, only 28 women had a family history of breast cancer in this dataset. Examination of these relationships in a larger dataset would be worthwhile. In previous studies, both adolescent and adult premenopausal daughters of women with breast cancer also have had higher plasma or urinary estrogens relative to control women of similar ages.[8-10]

These data provide some additional evidence that the relationship between parity and subsequent breast-cancer risk in postmenopausal women may be mediated, at least in part, through alterations in hormone levels, which are observable many years after the reproductive events. Our data do not support any substantial influence of age at menarche or family history of breast cancer on postmenopausal estrogen or prolactin levels, although estrogen levels did tend to be higher in women with a family history. Given the sparse literature on these relationships and the potential for these studies to lend insight into the biologic mechanisms underlying breast cancer risk, additional assessments are warranted.

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