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The Relationship Between Blood Prolactin Levels and Risk of Breast Cancer in Premenopausal Women

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Abstract—*Single specimens of blood have been taken from over 5000 normal volunteer women in each of two sequential (1967–1976, 1977–1984) population-based studied on the Island of Guernsey.*

Multivariate analysis was used to determine the relationship between prolactin levels and risk factors in breast cancer in 2591 and 1959 premenopausal women in whom blood prolactin had been measured. In both populations the prolactin concentrations appeared to be log-normally distributed and therefore all analyses have been done on log-transformed data. Initially the variables in the statistical model were age at menarche, ages at first and last baby, parity, ponderosity (Quetelet Index), mammographic pattern (as graded by Wolfe), family history of breast cancer, age, menstrual cycle status, time of day of blood sampling, oral contraceptive use, history of breast feeding and methodological changes in the laboratory measurement of prolactin. Of these variables age at menarche, ages at first and last child and family history of breast cancer were found not to be significant and were excluded from the final model.

The main finding to emerge was that after standardizing for all the other variables, prolactin levels in the follicular phase were significantly lower than those found at midcycle or during the luteal phase of the menstrual cycle. A peak level of prolactin was found at day 12 of the cycle.

Increasing parity was related to a steady decrease in prolactin concentration. Increasing ponderosity was associated with an increased prolactin level as was a DY compared to an N1 mammographic pattern. Women with a history of oral contraceptive use had lowered prolactin concentration. All these effects occurred evenly over the menstrual cycle and were generally found for both data sets. Thus body weight, parity and, indirectly, age at first baby might influence breast cancer risk by being associated with changes in blood prolactin concentration.

INTRODUCTION

THE POSSIBILITY that risk factors for breast cancer, especially those related to reproductive history, are mediated by a hormonal mechanism has been the underlying basis of many investigations. One of the more important hormones which has been considered is prolactin. However, difficulties in relating prolactin levels to risk variables arise because of the number of confounding factors which can influence blood levels of prolactin. Indeed, the amount of prolactin is associated not only with risk factors such as parity and age [1–4] but also, for example, with the time of day at which blood is sampled [5]. Furthermore, in premenopausal women an additional confounding factor might be the stage of the menstrual cycle. As a consequence, a large number of observations are required to establish with a satisfactory degree of statistical accuracy how these, and other, factors interact.

The opportunity to investigate results from a large number of blood samples has been provided by two prospective studies based on the Island of Guernsey. In each of these two studies single blood samples have been collected from over 5000 normal women volunteers. Prolactin has been measured in 2591 and 1959 premenopausal women in these two studies, respectively. In addition, each volunteer has answered a detailed questionnaire which included menstrual cycle status and reproductive history.

The purpose of this paper is to apply multivariate

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analysis methods to the two sets of data in order to establish the association between recognized determinants of breast cancer risk and blood prolactin levels. Since these analyses were performed on each data set separately an assessment has also been made of the concordance of the conclusions.

MATERIALS AND METHODS

Subjects

In a sequence of experiments conducted on the Island of Guernsey women have been asked to volunteer for three prospective studies, the purpose of the last two being to assess the importance of blood-borne hormones to the subsequent risk of developing breast cancer. These last two studies were carried out between 1967 and 1976 and between 1977 and 1984, respectively. They will be referred to as Guernsey 2 (G2) and Guernsey 3 (G3).

Volunteers were recruited by advertizing in the local media (press, radio, television), personal contact and appeals to local womens' groups. An accrual target of 5000 women was set for each study, this being approx. 50% of eligible women living on the island.

Blood

In the G2 study blood was taken into heparinized tubes and plasma separated whilst in the G3 study the blood was allowed to clot and serum was separated. An aliquot of approx. 1 ml was taken from each blood specimen for prolactin assay. All plasma or serum samples were stored at −20°C. Every 6–9 months blood aliquots were packed with solid CO_2 and air-freighted to Amsterdam for prolactin assays. On arrival at the laboratory all samples were still frozen.

Prolactin

Assays were performed using a double-antibody radioimmunoassay method on blood specimens at five dilutions. The details of this method are as described [6]. Estimation of prolactin was done within 1 year of the blood being collected.

Statistical analysis

There were 3283 and 3014 premenopausal or menopausal women in G2 and G3, respectively. After excluding menopausal women (i.e. aged greater than 45 years and who had had regular cycles but were experiencing irregular cycles at the time of volunteering) there were 2591 and 1957 premenopausal women for whom blood prolactin levels were known. Of these subsets of premenopausal women there were 700 who had volunteered for both G2 and G3. A further 54 and 21 women from G2 and G3 had cycle lengths in excess of 40 days and these were omitted in calculations involving menstrual cycle.

Table 1. Information available in the Guernsey 2 and Guernsey 3 studies

Variables	Series G2	Series G3
Age	Y	Y
Height	Y	Y
Weight	Y	Y
Age at first baby	Y	Y
Age at last baby	N	Y
Parity	Y	Y
Age at menarche	Y	Y
Day of cycle	Y	Y
Cycle length	N	Y
Family history of breast cancer:		
(a) first degree	Y*	Y
(b) second degree	Y*	
Time of blood sampling†	Y	Y
Oral contraceptive use:		
(a) past use	N	Y
(b) present use	Y	Y
Mammographic pattern	N	Y

'Y' and 'N' denote that the data are available and not available, respectively.
*First and second degree family history combined.
†Blood was taken from volunteers between 14.00 h and 20.00 h.

The questionnaire used in the G3 study covered a broader spectrum of information than the G2 study. Table 1 shows the list of all the risk variables and potentially confounding factors examined in this paper and compares their availability in the two studies.

Logarithmic transformation of the prolactin levels observed in the two studies shows distributions very close to the normal (Fig. 1), in accord with the findings of Lenton *et al.* [7]. It is then plausible to assume that the observed log-prolactin levels are derived from a normally distributed variable. In addition a combination of factors, such as time of blood sampling and parity, may have some influence on these observed values and should be examined. The assumption of a multivariate normal distribution for log-prolactin levels and the selection of significantly influential variables are discussed in the following section.

RESULTS

1. *Descriptive analysis*

Initially the data were examined on a univariate basis to establish the relationship between each variable listed in Table 1 and prolactin. The geometric mean prolactin level in G3 women (11.48 ng/ml) is slightly higher than in G2 (10.96 ng/ml) but the same relationships between the observed variables and prolactin emerge from the two data sets. Table 2 shows the average prolactin levels

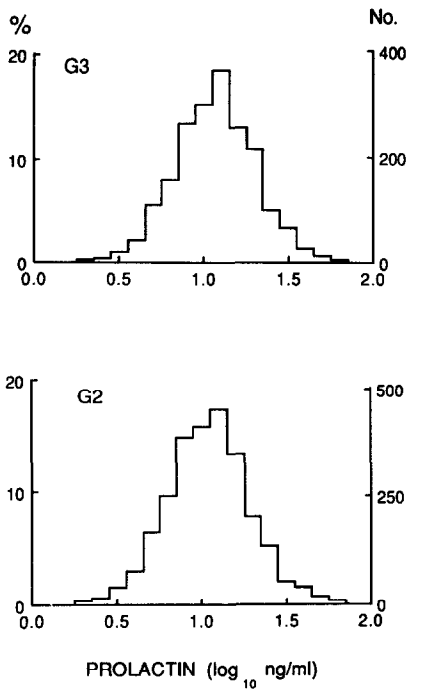

Fig. 1. Distribution of blood prolactin levels in the G2 and G3 studies. The distributions are based on 2591 *and* 1957 *premenopausal women in the G2 and G3 studies, respectively. The geometric mean levels of G2 and G3 were* 10.96 ng/ml *and* 11.48 ng/ml. *The vertical axes refer to percentage* (%) *and the actual number* (*No.*) *of the total population.*

observed in the two studies for different values of these variables. It appears that time of blood sampling, parity and age (G2 only) are all significantly related to prolactin levels; the tests computed for differences, or trend when appropriate, among the categories of these variables all reached formal 2-tail significance.

Changes over the menstrual cycle were complex but broadly similar features were found in both G2 and G3. Thus the lowest levels of prolactin were found during days 1–8 of the cycle. A general increase followed reaching a peak level at day 12 for both G2 and G3. After day 12 the amounts of prolactin were still significantly higher than those found in days 1–8. After about day 30 the pattern of prolactin levels was erratic for G2 and G3, probably reflecting the small numbers of women observed on these days.

2. *Multivariate analysis of the data*

A multivariate normal model for log-prolactin levels was separately fitted to the two data sets. Initially all the available variables listed in Table 1 were included in its specification. Age was treated as a 3 category factor, taking value 1 if the woman was less than 36, 2 if her age was between 36 and 40, 3 if greater than or equal to 41. Body weight and height were combined and expressed as Quetelet's Index [weight (kg)]/[height (m)]^2. Menstrual cycle was expressed as the proportion of cycle elapsed at the time of blood sampling. Since in the G2 study the information on the cycle length was not collected, cycle length was assumed to be equal to 29 days, the mean cycle length in the G3 study being 28.8 days. There were 139 women in the G2 study (5.4%) for whom the day of cycle was greater than 29 at the time of blood sampling and their proportion of cycle was considered to be equal to 100%. The inclusion of this group did not substantially alter the final conclusions.

As a first assessment of the effects of all the variables, likelihood ratio tests of their individual significance were computed. In both studies age at menarche, age at first or last baby, and family history of breast cancer were not statistically significant so that they were omitted from the final model. Interactions between the variables were also examined. None was significant so that the full specifications of the model included: time of blood sampling, percentage of cycle, age, parity, breast feeding, history of oral contraception, ponderosity and, for the G3 study only, Wolfe grading. The goodness-of-fit of this model was examined using graphical procedures and found to be satisfactory.

The estimated effect of all the variables included in the final model are given in Table 3. They were expressed as the resulting variation in log-prolactin levels due to a unit change in one of these variables (e.g. a change from age category 1 to age category 2) when all the others were held constant. As an overall summary of these results, Figs. 2–5 show the log-prolactin levels predicted by the model for a selection of values of these variables over the whole menstrual cycle; they are based on the G3 data only, since the estimates from the two studies were in substantial agreement and the G3 data are more detailed.

2(a). *Menstrual cycle*

The coefficients associated with the menstrual cycle represent the differences in log-prolactin levels that would be expected between observations at different points of the cycle and the observation at the beginning of the cycle, with other variables being held constant. Initially the menstrual cycle was divided into 20 intervals, each corresponding to 5% of the cycle, and the average prolactin level within each interval was estimated. On the basis of these results the intervals were grouped according to their similarity in prolactin levels. This procedure led to four intervals, defined by the 30, 70 and 85 percentage points. The average level of log-prolactin during the first interval (approx. day 1 to day 9) was significantly lower than that found either in the second (day 10 to day 20) or in the fourth phase (26th day onwards), while no significant difference was detected with respect to the third (day 21 to day 25) phase of the cycle in the G3 data. The *t*-tests for the differences between the average levels

Table 2. Univariate analysis: effect of variables on mean prolactin levels

	Series G2	Series G3
Total population	1.04 ± 0.25 (2591)*	1.06 ± 0.24 (1957)*
Sampling time		
<18.00 h	1.01 ± 0.25 (1762)	1.05 ± 0.24 (1517)
≥18.00 h	1.10 ± 0.23 (829)	1.15 ± 0.24 (437)
Test for difference between means†	9.03	7.68
P-value	<0.001	<0.001
Parity		
0	1.11 ± 0.25 (368)	1.13 ± 0.23 (213)
1	1.06 ± 0.24 (371)	1.11 ± 0.25 (236)
2	1.03 ± 0.24 (939)	1.06 ± 0.25 (843)
3	1.01 ± 0.26 (562)	1.03 ± 0.23 (435)
≥4	1.00 ± 0.24 (351)	1.03 ± 0.23 (230)
Test for trend among means‡	-6.90	-6.03
P-value	<0.001	<0.001
Age at first baby		
<24	1.02 ± 0.25 (897)	1.05 ± 0.25 (771)
≥24	1.03 ± 0.24 (1326)	1.06 ± 0.24 (973)
Test for difference between means†	0.94	0.63
P-value	>0.10	>0.10
Age		
≤30	1.00 ± 0.26 (255)	1.03 ± 0.19 (2)
31–35	1.02 ± 0.25 (779)	1.07 ± 0.25 (439)
36–40	1.04 ± 0.24 (647)	1.04 ± 0.25 (742)
40+	1.06 ± 0.24 (910)	1.08 ± 0.23 (774)
Test for trend among means‡	4.29	1.51
P-value	<0.01	0.07

*All results expressed as $\log_{10}$ ng/ml ± S.D.; numbers in parentheses are number of volunteers.
†Distributed as a standardized normal [29].
‡Distributed as a standardized normal [30].

of the second, third and fourth phases with respect to the first phase had, in both studies, *P*-values respectively $<< 0.001$, > 0.10 and $<< 0.001$. The highest levels of prolactin occurred in the mid phase of the cycle, precisely within the 50–55 percentile of the cycle.

2(b). *Time of sampling*

The estimated effect of sampling in the evening (18–21 h) compared with during the day (13–18 h) is 0.112 in the G2 study and 0.110 in G3; the associated *t*-tests for both these parameters being highly significant ($P << 0.001$). Figure 2 summarizes these findings.

2(c). *Age*

In the G2 study the expected log-prolactin level of women in the 36–40 age category was significantly higher than in younger women, while it was not in the G3 study. In both studies however, the 40+ age category showed expected levels respectively 0.065 and 0.038 higher than the less than 36 age group. The associated *t*-test statistics are significant in the G2 study but only approach significance in G3 when the oldest and youngest groups are compared. Figure 3 shows the result for the G3 study.

2(d). *Parity*

As parity increases the expected level of blood prolactin decreases. The effect of each additional child was separately included in the model and found to be essentially constant with an estimated effect per child of −0.022 and −0.024, respectively, in G2 and G3. Figure 4 shows this decreasing effect of parity on log-prolactin over the whole menstrual cycle.

2(e). *Oral contraceptive use*

The current use of oral contraceptives was not associated with changes in blood prolactin levels in either series of women; its coefficients were 0.019 in G2 and 0.019 in G3 and are not significant. However, in G3 the estimated effect of ever having taken oral contraceptives was −0.041 with the associated *t*-test indicating a significant inhibitory effect on blood prolactin concentration. Figure 5 shows the result for the G3 study.

*Table 3. Multivariate analysis: effect of variables on blood prolactin levels**

Variables	Series	Changes in prolactin (log_{10} ng/ml)	*t*	*P*-values
Time				
<18.00 h vs. ≥18.00 h	G2	0.112	11.20	<<0.001
	G3	0.110	6.69	<<0.001
Age				
≤35 vs. 36–40	G2	0.051	4.25	<<0.001
	G3	−0.007	0.49	>0.10
≤35 vs. ≥41	G2	0.065	5.80	<<0.001
	G3	0.038	2.49	<0.01
Children				
Effect per child	G2	−0.022	6.05	<<0.001
	G3	−0.024	5.17	<<0.001
Oral contraceptive				
Current vs.	G2	0.019	1.21	>0.10
non-current use	G3	0.019	1.02	>0.10
Ever vs. never	G2	—	—	—
	G3	−0.041	3.33	<0.001
Quetelet Index				
Effect per unit	G2	0.003	2.24	<0.05
	G3	0.004	2.89	<0.01
Breast feeding				
Ever vs. never	G2	−0.031	2.88	<0.01
	G3	−0.019	1.68	<0.10
Wolfe grade				
N1 vs. P1	G3	0.034	1.70	<0.10
N1 vs. P2	G3	0.018	1.05	>0.10
N1 vs. DY	G3	0.054	2.78	<0.01
P1 vs. DY	G3	0.021	0.74	>0.10
P2 vs. DY	G3	0.036	1.42	>0.10
Cycle				
1st vs. 2nd phase	G2	0.060	5.38	<<0.001
	G3	0.094	7.48	<<0.001
1st vs. 3rd phase	G2	0.085	5.62	<<0.001
	G3	0.026	1.48	>0.10
1st vs. 4th phase	G2	0.064	4.66	<<0.001
	G3	0.066	4.27	<<0.001

*The results are based upon 2537 and 1936 observations from, respectively, the G2 and G3 series.

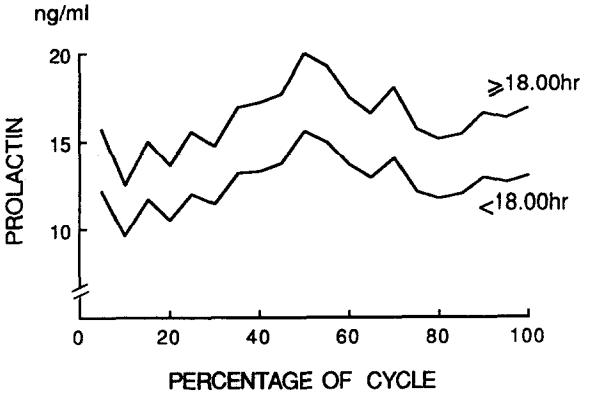

Fig. 2. Effect of blood sampling time on blood levels of prolactin over the menstrual cycle. Multivariate analysis showed that the prolactin concentration in blood taken between 18.00 h and 21.00 h was 29% higher than in blood taken between 13.00 h and 18.00 h. This elevation was significant (t = 6.69; P << 0.001) and this difference was constant over the whole of the menstrual cycle.

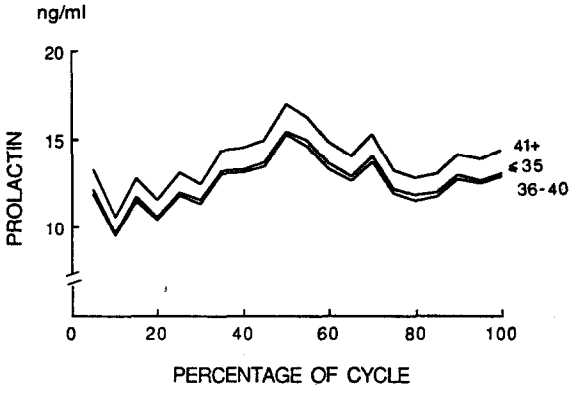

Fig. 3. Effect of age on blood prolactin levels over the menstrual cycle. Multivariate analysis showed that the blood prolactin concentrations were 9% higher between women aged over 40 years and women aged less than 36 years (t = 2.49; P < 0.01). The blood prolactin concentration was 11% higher in women aged over 40 years compared with women aged 36–40 years (t = 2.17, P < 0.05).

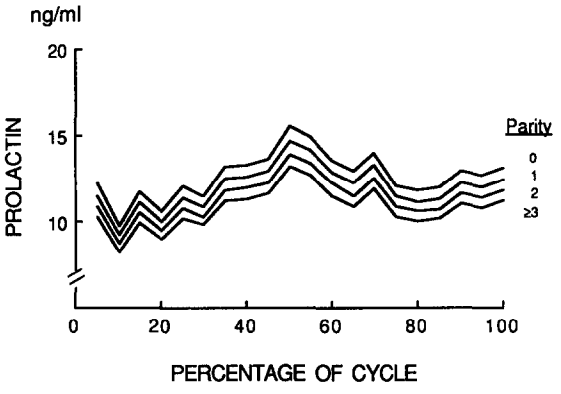

Fig. 4. Effect of parity on blood prolactin levels over the menstrual cycle. Multivariate analysis showed that the blood prolactin concentration decreased with increasing parity. The effect of each child was almost constant giving a decrease of 5.4% *in prolactin concentration* (t = 5.17; P << 0.001).

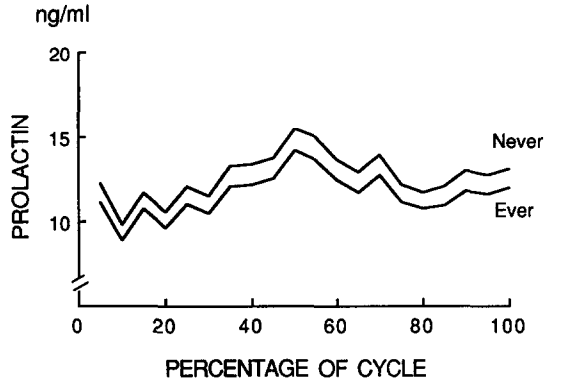

Fig. 5. Effect of ever having used oral contraceptives on blood prolactin concentration over the menstrual cycle. Multivariate analysis showed that the use of oral contraceptives was associated with a decrease of 9% *in prolactin levels* (t = 3.33; P < 0.001).

2(f). *Ponderosity*

There was a significant increase in blood prolactin levels with increasing ponderosity of the order of 0.003 and 0.004 per unit Quetelet Index.

2(g). *Breast feeding*

Breast feeding was associated with a decrease in prolactin concentration which attained formal significance only in G2.

2(h). *Mammographic pattern*

Women with a mammographic pattern designated DY by Wolfe had significantly raised prolactin levels compared with those with a N1 pattern. Comparison of the high risk group (DY + P2) with respect to the low risk group (N1 + P1) showed no difference in prolactin concentrations. Only 15% of women had DY grades compared with 50% with P2 patterns.

2(i). *Assay variation*

There was a slight shift in assay values with time totalling about 6% in G2. In G3 this was greater there being about 16% difference in assay values from the beginning to the end of the series. Corrections for these variations were applied during the analyses.

DISCUSSION

Changes in prolactin levels in women are commonly associated with a variety of factors such as time of sampling and parity. In this paper we have collected information from two large population-based studies on a variety of potentially related variables, some of which deal with reproductive history. These data bases are of sufficient size to evaluate the relationship between single reproductive events and blood prolactin concentration controlling at the same time for the effects of confounding factors using multivariate analysis.

In our analysis one of the important factors affecting prolactin levels was menstrual cycle. This study has shown that after standardizing for confounding factors the level of prolactin during the follicular phase is significantly lower than those in the part of the menstrual cycle encompassing the periovulatory or luteal phases. The highest levels occur during midcycle in both G2 and G3 women. This confirms the results of the majority of workers who have found the mid-cycle or luteal levels to be higher than those in the follicular phase [7–14]. However, others have not [15–17], possibly because of the large between-person variations commented on by others [11, 18]. Our results show that the variables which affect prolactin levels do so uniformly over the menstrual cycle.

Of the four reproductive factors considered here, age at menarche, age at first or last birth and parity, only parity was significantly associated with changes in prolactin concentration. In particular each additional child appeared to have a similar inhibitory effect on prolactin concentration. It thus seems that the protective effect of parity could be mediated by a reduction in circulating prolactin levels. This finding confirms previous results from this and other laboratories [1–4]. Although age at first baby does not appear to be a significant variable when parity is also included in the model, it becomes fairly significant once parity is excluded beause of the strong negative correlation between the two (see also Ref. [19]). It follows that the sooner a woman commences childbearing the sooner will be the establishment of a lowered blood prolactin concentration.

The influence of oral contraceptives on breast cancer risk is uncertain. Indeed, there is a debate whether oral contraceptives increase the breast cancer risk in young women [20–22]. It has been suggested that some of the inconsistencies could be related to the latent period between pill usage and

detection of the tumour [23, 24]. The present results show that women who have ever taken oral contraceptives have a lowered prolactin concentration compared with those who have never taken such medication. It is difficult to know what relevance these data have on present day usage of oral contraceptives since in our studies the formulations of the preparations used are unknown and may well have been prescribed at a time when their composition differed greatly from those presently available.

A positive association was found between increased ponderosity and prolactin levels. Since oestrogens can increase prolactin secretion a reason for the higher prolactin levels might be the increased oestrogen production due to adipocytic aromatase activity. Increased weight is associated with an enhanced risk of breast cancer in postmenopausal women [25] and it is conceivable that a long exposure, starting during the premenopausal years, not only to raised oestradiol levels but prolactin could be a contributory cause.

There was no significant effect of a family history, whether first or second degree, of breast cancer on prolactin concentration in either G2 or G3. This is at variance with an earlier report whcih claimed that women with a family history of breast cancer have a raised evening prolactin level during the luteal phase of the menstrual cycle [6]. This may have arisen because the statistical analysis did not standardize for the effects of confounding factors such as parity and age.

It is widely recognized that women with mammographic patterns designated DY or P2 by Wolfe have a significantly increased risk of breast cancer compared with those with N1 or P1 patterns [26–28]. However, in our analysis there was no difference between prolactin levels in women with a low risk (N1 and P1) and high risk (P2 and DY) although women with a DY pattern had significantly raised prolactin levels compared with those having a N1 pattern.

This study has attempted to standardize for hopefully most of the confounding factors which influence prolactin levels in pre-menopausal women. Time of blood sampling and menstrual cycle status were some of the more important confounding factors. Standardizing for these, and other, variables we have shown that parity, and in turn age at first baby, and ponderosity might influence breast cancer risk by alterations in prolactin secretion. Furthermore, these results are strengthened by the agreement found in the two populations.

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