Eur J Cancer Clin Oncol, Vol. 24, No. 7, pp. 1225–1231, 1988.
Printed in Great Britain

0277-5379/88$3.00+0.00

The Permanent Effect of Reproductive Events on Blood Prolactin Levels and its Relation to Breast Cancer Risk: a Population Study of Postmenopausal Women

D.Y. WANG,* B.L. DE STAVOLA,* R.D. BULBROOK,* D.S. ALLEN,* H.G. KWA,† A.A. VERSTRAETEN,† J.W. MOORE,* I.S. FENTIMAN,‡ J.L. HAYWARD‡ and I.H. GRAVELLE§

**Imperial Cancer Research Fund, P.O. Box 123, Lincoln's Inn Fields, London WC2A 3PX, U.K., †Antoni van Leeuwenhoekhuis, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands, ‡Clinical Oncology Unit, Guy's Hospital, London SE1 9RT, U.K. and §Department of Radiology, University Hospital of Wales, Cardiff, U.K.*

Abstract—*In each of two population-based studies conducted on the Island of Guernsey between* 1967–1976 *and* 1977–1984, *respectively, single specimens of blood were taken from over* 5000 *normal women. From these two studies there were* 1173 *and* 946 *postmenopausal women in whom blood prolactin was determined and multivariate analysis was used to establish the association between blood prolactin concentration and possible determinants of risk of breast cancer. Since prolactin levels were log-normally distributed these analyses were done on log-transformed data.*

The age at menarche or menopause, age at first or last childbirth, length of reproductive life (i.e. time from menarche to menopause) or post-menopausal life (i.e. time from menopause to time of blood sampling), contraceptive use and history of breast cancer were not significantly associated with blood prolactin concentration. Of significance were age, parity, time of blood sampling and assay drift. Ponderosity (Quetelet's Index) was positively associated with prolactin concentration and this was significant using a one-tail criterion. Women with a mammographic pattern designated DY by Wolfe had significantly higher prolactin levels than those with N1 patterns.

However, the main finding to emerge was that after standardizing for all the other variables increasing parity was related to a step-wise reduction in blood prolactin levels. Since this had occurred in women who had had their last child up to 35 *years previously it implies this effect is permanent. It could therefore be that the protective effect on breast cancer risk of multiparity and early first pregnancy could be mediated by such a life-long reduction in blood prolactin levels.*

INTRODUCTION

IT IS difficult to relate breast cancer risk factors with blood prolactin concentration because of the number of confounding factors which also influence prolactin levels [1]. However, this difficulty can be overcome by the use of multivariate analysis provided that prolactin measurements have been made on a sufficiently large population of women. An opportunity for such an analysis has been presented by two prospective studies on the Island of Guernsey in which over 5000 women enrolled in each study and donated a sample of blood.

A multivariate analysis on premenopausal women from these studies showed that the breast cancer risk factors of parity, ponderosity and DY mammographic pattern were significantly associated with changes in prolactin levels giving support to the hypothesis that prolactin levels are positively associated with risk [1]. In particular, multiparity, which is claimed to be protective [2–4], was significantly related with low levels of blood prolactin [1].

The intention of this paper is to apply multivariate analysis to the prolactin data from the postmenopausal women who participated in the two Guernsey studies in order to explore further the association between determinants of breast cancer risk and prolactin levels and to establish whether the relationships found previously in premenopausal women [1] are of long duration and persist in postmenopausal women.

Accepted 29 February 1988.

MATERIALS AND METHODS

Subjects

In the study of the role of blood hormones and subsequent breast cancer, two investigations have been conducted on the Island of Guernsey in which ostensibly healthy normal women volunteers living on the island have been invited to participate. Recruitment to these studies was by personal contact, appeals to local women's groups and through the local media (press, radio and television). As a result of this about 50% of the eligible women in the population volunteered for each study. These studies were carried out between 1967–1976 and 1977–1984 and, because they were the second and third experiments conducted on the Island, will be referred to as G2 and G3, respectively.

Of a total of 3901 and 3035 women who had prolactin measured in these two studies there were 1310 and 1078 postmenopausal women, respectively. Of these 1173 and 946 had had a natural menopause and their data are those used in this study.

Blood and prolactin assay

On entry to the study the women were asked to answer a questionnaire and donate blood. Blood was processed for plasma (G2) and serum (G3) and 1 ml aliquots taken for prolactin assay. These aliquots were stored at −20°C until being air-freighted, packed in solid CO_2, to Amsterdam. All samples arrived frozen at the laboratory and prolactin measurements were done within 1 year of blood being collected.

Prolactin was determined on blood specimens at five dilutions using a double-antibody radioimmunoassay method. Details of this method have already been described [5].

Mammography

The parenchymal pattern of the mammograms were categorized as being either N1, P1, P2 or DY according to the criteria of Wolfe [6, 7]. These were defined as follows; N1 (lowest risk) parenchyma consisting mainly of adipose and glandular tissue without prominent ducts; P1 (low risk) mainly adipose tissue but with prominent ducts; P2 (high risk) breast tissue consisting of prominent ducts or DY (highest risk) structure of the breast characterized by dense dysplasia.

RESULTS

Distribution of blood prolactin levels in G2 and G3 studies

The levels of prolactin were log-normally distributed in both studies (Fig. 1). The analysis was therefore performed using log-transformed data.

The average level of prolactin was higher in G3 than in G2 (Table 1) and could be attributed to a drift in the assay methods (see Section 1h).

Univariate analysis

In both G2 and G3 postmenopausal women there was no linear trend between prolactin levels and

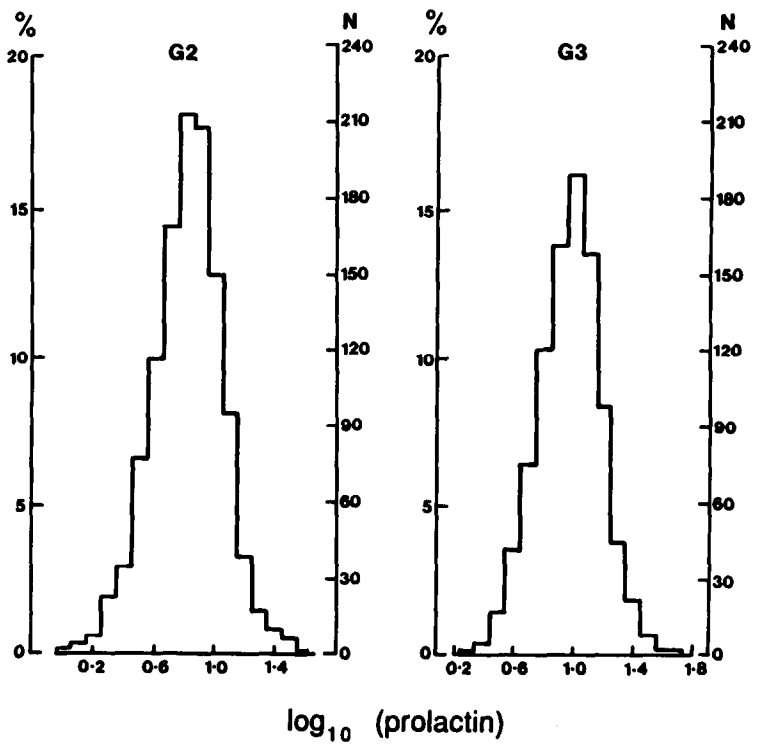

Fig. 1. Distribution of blood prolactin levels in the G2 and G3 studies. The distributions are based on 1173 and 946 postmenopausal women in the G2 and G3 studies, respectively. The vertical axes refer to the percentage (%) and the actual number (N) of the total populations.

Table 1. Univariate analysis: effect of variables on mean log prolactin levels

	G2	G3
Total	0.82 ± 0.23 (1173)*	0.97 ± 0.21 (946)
Parity		
0	0.85 ± 0.24 (303)	1.00 ± 0.20 (184)
1	0.81 ± 0.22 (232)	0.99 ± 0.22 (166)
2	0.82 ± 0.24 (302)	0.97 ± 0.21 (280)
3	0.78 ± 0.23 (170)	0.97 ± 0.22 (152)
4+	0.79 ± 0.24 (166)	0.94 ± 0.22 (164)
Test for trend	2.98	2.70
P- value†	<0.005	<0.005
Sampling time		
<18.00 h	0.81 ± 0.23 (960)	0.97 ± 0.22 (780)‡
>18.00 h	0.85 ± 0.25 (213)	1.01 ± 0.20 (159)
t-test	2.14	2.28
P-value	<0.05	<0.05
Age		
<45	0.88 ± 0.32 (31)	1.06 ± 0.27 (22)
46–55	0.82 ± 0.24 (472)	0.99 ± 0.21 (357)
56–65	0.81 ± 0.23 (542)	0.96 ± 0.21 (417)
>66	0.83 ± 0.22 (128)	0.98 ± 0.21 (150)
Test for trend	0.18	1.35
P-value†	>0.40	0.09
Family history		
No	0.81 ± 0.24 (1012)	0.98 ± 0.21 (721)‡
Yes	0.85 ± 0.21 (161)	0.96 ± 0.21 (212)
t-test	2.20	1.25
P-value	<0.05	>0.10
Ever on pill		
No	—	0.97 ± 0.21 (825)
Yes	—	1.00 ± 0.22 (121)
t-Test	—	1.42
P-value	—	>0.10
Wolfe grade		
N1	—	0.95 ± 0.24 (74)‡
P1	—	0.97 ± 0.22 (365)
P2	—	0.98 ± 0.21 (417)
DY	—	1.01 ± 0.18 (88)
Test for trend	—	1.97
P-value†	—	0.03

Only women who had a natural menopause have been included.
*All results have been expressed as $\log_{10}$ ng/ml ± S.D. The number of observations is in parentheses.
†The significance of tests for trends has been based on a one-tail criterion.
‡The number of observations does not equal 946 because of missing data.

age, or ages at menarche, menopause, first baby and last baby. In the last instance this information was available only in G3.

Increasing parity was significantly associated with decreasing blood concentrations of prolactin (test for trend: G2, 2.98, $P < 0.005$; G3, 2.70, $P < 0.005$; Table 1). Blood samples taken after 18:00 h had a significantly higher prolactin concentration than those taken before this time for both G2 and G3 (t-test = 2.14 and 2.28, both with $P < 0.05$). The effect of a family history of breast cancer on prolactin levels was inconsistent; women with a positive history had a higher mean level in the G2 but lower mean concentration in the G3 study. Only in the G2 study did this difference reach formal significance. The history of oral contracep-

tive medication was known only for G3 and the prolactin levels were similar in those who had ever-taken compared to those who had never-taken contraceptives (t-test = 1.42; $P > 0.10$). Wolfe's classification of breast parenchymal patterns was known only for G3 volunteers. In these volunteers there was a significant increase in prolactin concentration with increasing risk (i.e. N1, P1, P2 to DY) and the test for trend was 1.97 ($P = 0.03$).

MULTIVARIATE ANALYSIS

1. *Cross-sectional analysis of the G2 and G3 series*

A multivariate normal model for log prolactin levels was separately fitted to the two data sets. The variables included in the model were time of blood sampling, age, age at first and last baby, parity, ponderosity, Wolfe grade parenchymal patterns (G3 only) and assay variation to control for method drift. The lengths of reproductive and postmenopausal life were also included and were defined as the time from menarche to menopause and time from menopause to age at blood sampling, respectively. Age, parity, time from menarche to menopause and time from menopause to age at blood sampling as well as age at first and last baby were all treated as continuous variables. Ponderosity was expressed as Quetelet's Index [weight (kg)/height^2 (m^2)] and also treated as a continuous variable. Time of blood sampling, family history of breast cancer and Wolfe pattern were treated as categorical variables.

An additional analysis was performed on the combined data from the G2 and G3 postmenopausal

Table 2. Multivariate analysis: effect of variables on blood prolactin levels

Variable	Series	Changes in prolactin ($\log_{10}$ ng/ml)	t	P-value
Time (h)				
Effect of >18:0	G2	0.053	3.00	<0.01
	G3	0.062	3.46	<0.001
	G2 + G3	0.047	3.06	<0.01
Age (years)				
Linear term	G2	−0.038	3.36	<0.001
	G3	−0.035	2.64	<0.01
	G2 + G3	−0.034	3.82	<0.001
Quadratic term	G2	0.034	3.62	<0.001
	G3	0.029	2.66	<0.01
	G2 + G3	0.029	3.98	<0.001
Parity				
Effect per child	G2	−0.012	2.95	<0.01
	G3	−0.011	2.72	<0.01
	G2 + G3	−0.009	3.04	<0.01
Ponderosity				
Effect per unit	G2	0.003	1.69	0.08
	G3	0.002	0.91	>0.10
	G2 + G3	0.002	1.86	0.06
Family history				
Effect of 'yes'	G2	0.032	1.60	0.10
	G3	−0.010	0.64	>0.10
	G2 + G3	0.022	1.86	0.06
Menarche to menopause				
Effect per year	G2	0.001	0.48	>0.10
	G3	0.004	0.94	>0.10
	G2 + G3	0.001	0.63	>0.10
Menopause to present age				
Effect per year	G2	−0.003	1.22	>0.10
	G3	−0.001	0.12	>0.10
	G2 + G3	−0.002	0.81	>0.10
Wolfe grade				
N1 vs. P1	G3	0.034	1.31	>0.010
N1 vs. P2	G3	0.042	1.58	>0.10
N1 vs. DY	G3	0.082	2.43	<0.05
(N1,P1) vs. (P2,DY)	G3	0.019	1.33	>0.10

The results are based on 1173 and 946 observations from women who had undergone a natural menopause in the G2 and G3 studies, respectively.

volunteers. There were 238 postmenopausal women who volunteered for both studies and in these cases only the data for G3 were used. Because of the assay drift a continuous correction for this effect was applied before merging the data.

1(a). *Time of blood sampling.* The estimated differences, on a log scale, between prolactin levels in blood collected in the evening (18–2100 h) compared with that collected during the day (13–1800 h) was 0.053 and 0.062 for G2 and G3, respectively (Table 2). These differences were both significant ($t = 3.00$, $P < 0.01$ and $t = 3.46$, $P < 0.001$, respectively). This was confirmed by the analysis of the merged data which also showed a significant difference ($t = 3.06$, $P = 0.01$).

1(b). *Age.* The effect of age on log prolactin levels after the menopause was found to be non-linear and satisfactorily represented by a quadratic function. The coefficients for this function estimated in the multivariate analysis were −0.038 and 0.034 for G2, −0.035 and 0.029 for G3 and −0.034 and 0.029 for the merged data. All these coefficients were significant (Table 2).

1(c). *Parity.* In the analyses of the two separate studies and in the merged data the effect of parity on log prolactin was found to be essentially constant for each child. Hence parity was treated as a continuous variable. The estimated coefficients from the three analyses which represent the effect of each child on log prolactin were −0.012, −0.011 and −0.009, respectively. All these values were significant (Table 2).

1(d). *Ponderosity.* The analyses of the G2 series and of the merged data (G2 + G3) showed that there was a tendency for the amount of blood prolactin to increase with increasing ponderosity (Table 2). Neither reached formal two-tail significance although a one-tail test would have 0.04 and 0.03 significance levels, respectively.

1(e). *Family history of breast cancer.* There was an inconsistent and non-significant effect of family history of breast cancer on blood prolactin concentration (results not shown).

1(f). *Time invervals between menarche, menopause and age at blood collection.* The length of reproductive life (i.e. time interval between menarche and menopause) had no significant effect on prolactin levels. The period of time elapsing between age at menopause and age at blood sampling had a negative but non-significant effect on blood prolactin concentration. Log prolactin levels were not affected by age at first or last baby.

Table 3. Prolactin levels in women who volunteered for both G2 and G3 studies

Parity	G2 study Premenopausal	G3 study Postmenopausal	*N*
0	1.13 ± 0.24*	1.07 ± 0.23	46
1	1.08 ± 0.24	0.99 ± 0.23	42
2	1.07 ± 0.22	1.02 ± 0.19	89
≥3	1.05 ± 0.28	0.98 ± 0.24	102
	Postmenopausal	Postmenopausal	
0	0.88± 0.26†	0.96 ± 0.21†	63
1	0.85 ± 0.21	0.94 ± 0.30	49
2	0.82 ± 0.24	0.92 ± 0.22	64
≥3	0.75 ± 0.22	0.87 ± 0.24	62

*All results expressed as $\log_{10}$ mean ± I.S.D.
†Significantly different: $t = 2.34$, $P < 0.05$.
All results have been adjusted for assay drift.

1(g). *Wolfe grade mammographic pattern.* The only significant difference in blood prolactin levels was between women with an N1 compared with a DY mammographic pattern, DY being higher than N1 ($t = 2.43$, $P < 0.05$).

1(h). *Assay variation.* There was a shift in assay values with time totalling 6% in G2 and 21% in G3. Corrections were made for these variations as previously described [1].

2. *Longitudinal analysis of the G2 and G3 series*

There were 517 women who had volunteered for both studies and for whom blood prolactin had been measured. Of these 517 women 279 were premenopausal in the G2 study having become postmenopausal in the G3 investigation. The remaining 238 were postmenopausal in both studies. The data collected on these subsets of women are particularly interesting since they are essentially internally controlled, once the effect of assay drift in the two series is taken into account. On average the group of women who became postmenopausal during the interval between the two studies experienced a non-significant decrease in prolactin levels from the first to the second sampling (Table 3). It is evident that the effect of parity was still generally present in these sub-groups since in both series the highest average prolactin concentrations were found in nulliparous and the least in multiparous women.

In the group of women who were postmenopausal in both studies there was an increase in prolactin levels which were generally non-significant except for nulliparous women for whom the change in prolactin became formally significant ($t = 2.34$; $P < 0.05$). Again the inverse relationship between parity and prolactin levels was evident (Table 3).

DISCUSSION

Blood prolactin levels are clearly influenced by a variety of factors and that an accurate assessment of the relationship between any one of these variables and blood prolactin concentration can be obtained only by using multivariate-type analysis when sufficiently large numbers are available. The two prospective studies on the Island of Guernsey provide such large numbers. Furthermore, most of the conclusions found in one study can be verified with the other.

Of the reproductive factors considered, age at menarche and age at menopause were not significantly associated with changes in blood prolactin levels. The analysis on the interval between these two events showed that women who had experienced an early age at menarche and a late age at menopause (high breast cancer risk) had similar prolactin levels compared with those having a late age at menarche and early age at menopause (low risk). The ages at which a woman had her first, or last, baby were also not related to blood prolactin levels. However, increasing parity was significantly associated with decreasing concentrations of prolactin in both the G2 and G3 studies. Furthermore, each additional child appeared to have a similar inhibitory effect on the amount of blood prolactin in both study groups. This effect of parity not only confirms previous results from this and other laboratories [1, 8–11] but also extends them by showing that the reduction in blood prolactin associated with parity is of long duration, as it occurs in both premenopausal and postmenopausal women. It is of interest that the stress of mastectomy increases the amounts of blood prolactin to the same concentration irrespective of parity [12]. Thus, surgical stress induces a greater increase in prolactin levels in multiparous than in nulliparous women.

In premenopausal women ponderosity and blood prolactin levels were found to be positively and significantly associated [1]. In this study of postmenopausal women there was also a positive relationship, although this only reached formal statistical significance if the criterion of a one-tail test is applied. Such a weak association between ponderosity and prolactin concentration is probably due to the increased oestrogen production arising from aromatase activity. Thus the increased risk of breast cancer in overweight postmenopausal women [13] might be due not only to an increased oestrogen production but also to raised prolactin levels. These increased levels would commence premenopausally and continue into the postmenopausal period of a woman's life.

Over the later portion of the adult age range there is a significant reduction in prolactin concentration following the menopause which persists some 20 years. However, as is seen with volunteers who were

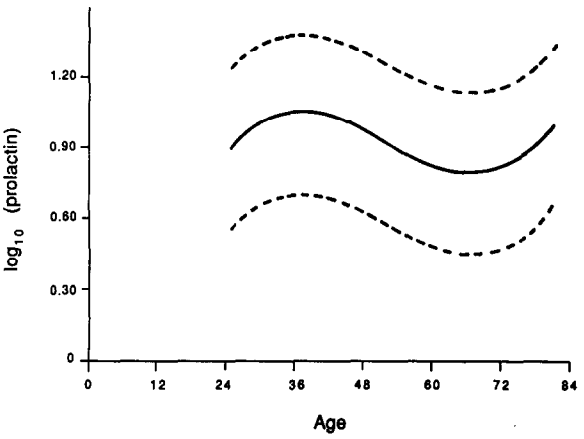

Fig. 2. The change of log prolactin levels, with age in pre- and postmenopausal volunteers of the G2 study. The cubic equation was $y = 0.00002\ (x-\bar{x})^3 - 0.0004\ (x-\bar{x})^2 - 0.010x + 1.46$, *where* y *is* $\log_{10}$ *prolactin* (ng/ml), x *is age* (*years*) *and* $\bar{x}$ *is the mean population age* (45 *years*). *The goodness of fit was* $\chi^2_4 = 30$; $P << 0.001$; $n = 3901$. *The dotted lines not only include* ± 1 *S.D. due to between-person variation, but also the effect of assay drift.*

postmenopausal in both studies, there was an, albeit non-significant, increase in prolactin concentration. There then appears to be an increase in elderly women which is best seen if the relationship between prolactin levels and age is calculated for the whole of the Guernsey cohorts. Using all the available data on prolactin for the G2 study ($n = 3901$) the association between prolactin for pre- and postmenopausal women and age is better described by a cubic relationship than either a linear, quadratic or quartic equation. The maximum of this cubic equation (see Fig. 2) is found to occur at about 37 years and the minimum at about 67 years. A similar relationship for pre- and postmenopausal women was found for the G3 data.

Women with a mammographic pattern designated DY or P2 by Wolfe have been found to have a significantly increased risk of developing breast cancer than those with N1 or P1 patterns [6, 7, 14]. Women with a DY pattern had significantly higher blood prolactin than those with an N1 pattern; which is similar to the findings in premenopausal women [1].

The most significant result of the multivariate analysis of the prolactin data for the G2 and G3 studies concerns the inverse relationship between parity and prolactin levels. The fact that this is observed for both pre- and postmenopausal women indicates that childbearing brings about a permanent reduction in prolactin secretion. Furthermore, the magnitude of this inhibition is associated with the number of births. Thus the early establishment of a lowered prolactin secretion could be a mechanism whereby early age of childbearing and multiparity lead to a diminution in breast cancer risk.

REFERENCES

1. Wang DY, De Stavola BL, Bulbrook RD *et al.* The relationship between blood prolactin levels and risk of breast cancer in premenopausal women. *Eur J Cancer Clin Oncol* 1987, **23**, 1541–1548.
2. Thein H, Thein MM. Risk factors of breast cancer in Burma. *Int J Cancer* 1978, **21**, 432–437.
3. Tulinius H, Day N, Johannesson G. Reproductive risk factors and risk of breast cancer in Iceland. *Int J Cancer* 1978, **21**, 724–730.
4. MacMahon B, Prude M, Cramer D, 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* 1982, **69**, 1035–1038.
5. Kwa HG, Wang DY. An abnormal luteal-phase evening peak of plasma prolactin in women with a family history of breast cancer. *Int J Cancer* 1977, **20**, 12–14.
6. Wolfe JN. Risk for breast cancer development determined by mammographic parenchymal pattern. *Cancer* 1976, **37**, 2486–2492.
7. Wolfe JN. Risk of developing breast cancer determined by mammography. In: Montague ACW, Stonesifer GL, Lewison EF, eds. *Breast Cancer*. New York, AR Liss, 1977, 223–238.
8. Kwa HG, Cleton F, Bulbrook RD, Wang DY, Hayward JL. Plasma prolactin levels and breast cancer: relation to parity, weight and height, and age at first birth. *Int J Cancer* 1981, **28**, 31–34.
9. Wang DY, Sturzaker HE, Kwa HG, Verhofstad F, Hayward JL, Bulbrook RD. Nyctohemeral changes in plasma prolactin levels and their relationship to breast cancer risk. *Int J Cancer* 1984, **33**, 629–632.
10. Bruning PF, Bonfrer JMG, Hart AAM, de Jong Bakker M, Kwa HG. Parity and age influence hormonal risk factors of breast cancer. *Prog Cancer Res Ther* 1984, **31**, 335–342.
11. Yu MC, Gerkins VR, Henderson BE, Brown JB, Pike MC. Elevated levels of prolactin in nulliparous women. *Br J Cancer* 1981, **43**, 826–831.
12. Wang DY, Hampson S, Kwa HG *et al.* Serum prolactin levels in women with breast cancer and their relationship to survival. *Eur J Cancer Clin Oncol* 1986, **22**, 487–492.
13. De Waard F. Body size and breast cancer risk. In: Pike MC, Siiteri PK, Welsch CW, eds. *Banbury Report* 8: *Hormones and Breast Cancer*. Cold Spring Harbor Lab, 1981, 21–30.
14. Bulbrook RD, Hayward JL, Wang DY et al. Identification of women at high risk of breast cancer. *Breast Cancer Res Treat* 1986, **7**(Suppl 5), 5–10.