Eur J Cancer Clin Oncol, Vol. 24, No. 12, pp. 1855–1860, 1988.
Printed in Great Britain

0277-5379/88$3.00 + 0.00

The Distribution of Estradiol in the Sera of Normal Caucasian, Chinese, Filipina, Hawaiian and Japanese Women Living in Hawaii

MADELEINE J. GOODMAN,* RICHARD D. BULBROOK† and JOHN W. MOORE‡

**Office of Academic Affairs, University of Hawaii, Honolulu, Hawaii 96822, U.S.A., †Imperial Cancer Research Fund, University of Glasgow, Departments of Biochemistry and Surgery, Western Infirmary, Glasgow G11 6NT, U.K. and ‡Department of Clinical Endocrinology, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, U.K.*

Abstract—*Mean concentrations of sex-hormone-binding-globulin (SHBG) and the percentage distribution of estradiol between the non-protein-bound, albumin-bound and SHBG-bound fractions were not different in Caucasian, Hawaiian, Chinese, Japanese and Filipinas living in Hawaii. The widely varying incidence rates for breast cancer are therefore not explained by variations in the availability of estradiol.*

INTRODUCTION

SINCE Pardridge *et al.* [1] demonstrated that non-protein-bound and albumin-bound estradiol can cross the blood–brain barrier in the rat it has been held that these sex hormone fractions are 'biologically available'. Several recent studies, representing different epidemiological approaches, have implicated biologically available estradiol as a determinant of breast cancer risk. A series of case-control studies reported significantly higher percentages of non-protein-bound estradiol in the blood of breast cancer patients than in matched controls [2–6]. In a prospective study, Bulbrook *et al.* found that women from Guernsey who subsequently developed breast cancer had higher proportions of serum estradiol in the non-protein-bound and albumin-bound fractions than normal controls [7, 8]. In a geographical comparison, Moore *et al.* [9] found that proportionately more serum estradiol was bound to SHBG in healthy Japanese women than in British women and they suggested that this could account for the difference in observed breast cancer incidence rates between the two groups.

A further opportunity to investigate the relationship of biologically available blood estradiol to breast cancer risk exists in the ethnically diverse population of Hawaii. Average annual age-adjusted incidence rates for breast cancer vary widely among women of different ethnic groups who live in Hawaii. The SEER data for Hawaii show incidence rates of 29.2 per 100,000 population among Filipinas, 51.3 for Japanese, 64.1 for Chinese, 104.3 for Hawaiian and 105.6 for Caucasian women [10]. If the distribution of estradiol in the blood is a critical factor in the development of breast cancer, then this may be reflected in the observed variations in incidence rates in Hawaii.

The present investigation analyzed ethnic differences among populations of normal women from five major ethnic groups who have resided all or most of their lives in Honolulu with a view to ascertaining the presence or absence of variations in the distribution of blood estrogens or the concentration of SHBG which would correspond to the observed differences in breast cancer incidence. The classification of ethnic groups followed the SEER specifications with the understanding that the 'Hawaiian' category represents an admixture of Hawaiian, Caucasian and some oriental ancestry, with at least a 25% Hawaiian component.

SUBJECTS AND METHODS

The study population was drawn from women of Caucasian, Chinese, Japanese, Hawaiian and Filipina ethnic backgrounds living in Honolulu in Spring 1984. The women who volunteered to participate in the study were invited to do so by the Straub Clinic Health Appraisal Center in Honolulu, by community and civic groups, and through a newspaper feature story accompanied by a response coupon.

In each ethnic group, a study population of

Accepted 6 July 1988.

women ages 35–70 was selected with the goal of sampling at least 10 women per quinquennium across the age range. Excluded were those who had undergone hysterectomy, were pregnant or had been diagnosed with serious illness, such as cardiovascular disease or cancer. Also excluded were those who had used steroidal contraceptives or hormone replacement therapy within the last 2 years or were currently taking drugs likely to affect the endocrine balance (e.g. thyroid hormones and anticonvulsants).

The resultant population comprised 85 Caucasian, 47 Chinese, 52 Filipina, 67 Hawaiian and 106 Japanese women. The sample was smallest in the Chinese ethnic group where cultural traditions deter blood sampling.

Reproductive and medical histories were gathered from participants by medically trained personnel using a standardized questionnaire. Measures were made of height and weight. Data on smoking habits were obtained. Body mass index was computed as weight in kg over the square of height in meters.

A 10 ml blood specimen was collected by venipuncture between 11.00 am and 1.30 pm from each volunteer. The blood was allowed to clot at room temperature for 1 h, centrifuged at 800 ***g*** and the serum aliquoted into vials in 2 ml amounts. The vials were coded in random order, stored at −20°C and shipped on dry ice to the Imperial Cancer Research Fund Laboratories in London for hormone assay. On arrival, sera were stored at −20°C until analyzed. Although long-term storage (>5 years) at −20°C is thought to decrease the affinity of binding of sex steroids to SHBG [5], recent studies of dissociation rates of 5α-dihydrotestosterone from SHBG carried out in our laboratory suggest that changes do not occur in the time our samples were in store before analysis (12–18 months). Since estradiol and 5α-dihydrotestosterone interact with SHBG at the same binding site [11], the stability of estradiol binding would not be expected to change over this time span.

Sex-hormone-binding-globulin (SHBG) concentrations were measured by immunoradiometric assay [12]. The percentage of non-protein-bound (free) E2 was measured by centrifugal-ultrafiltration-dialysis in undiluted serum at 37°C [13] using Whatman GF/D glass fiber discs to support the ultrafiltration capsules [14]. Percentages of E2 which were bound to SHBG and albumin were calculated from the percentages of free E2 in native serum and in serum which had been heat-treated at 60°C for 1 h to denature SHBG [15, 16]. To avoid bias, each batch of samples contained equal numbers of women of the same menopausal status from each of the ethnic groups under investigation.

Statistical analyses were performed at the University of Hawaii on an IBM 3081 computer using the SPSS package.

Women were classified as premenopausal if they had regular menstrual periods every 21–35 days and postmenopausal if they had not menstruated for the last 2 years. Those who had irregular periods but whose last menses was within 2 years were defined as transitionally menopausal.

The distribution of premenopausal and postmenopausal women by ethnic group is shown in Table 1. Some 136 women (38.6%) met our classification of postmenopausal and the proportion of postmenopausal women did not vary significantly across ethnic group ($\chi^2_4 = 7.52$, NS). Fifty-nine women (who were not included in the study) were classified as transitionally menopausal. Again, there was no significant variation according to ethnic group ($\chi^2_4 = 2.16$, NS)..

RESULTS

Mean values and standard deviations for study variables by ethnic group for premenopausal and postmenopausal women are given in Table 2. No ethnic differences were found in age distribution by ethnicity within the pre- and postmenopausal groups. However, significant ethnic differences were noted for weight and height. Hawaiian women were found to be the heaviest and tallest among premenopausal and the heaviest among postmenopausal women. These anthropometric differences are in accord with previous observations of other samples of adult women living in Honolulu [17–19]. By contrast, the mean SHBG concentrations and the mean percentages of non-protein-bound, albumin-bound and SHBG-bound estradiol are remarkable in their lack of variation among ethnic groups. This conclusion is emphasized by an example of the data in Fig. 1 in which SHBG concentrations and incidence rates are shown for each ethnic group.

A further description of the relationship between ethnicity and the biochemical parameters measured in this study is given in Table 3. A series of binary design variables are constructed to measure deviations from the Caucasian mean, which serves as a control. The independent effects of current age, age at menarche and first pregnancy, smoking, menopausal status and body mass are measured concurrently in this series of linear equations. No evidence of significant deviation from the Caucasian norms for SHBG concentration or estradiol distribution was found in any of the ethnic groups under study. The variation due to all ethnic group variables as a cluster did not approach significance in any of the four equations, when standard analysis of variance partition techniques were applied.

Postmenopausal status and increased body mass, independently of ethnic group background, appear to be significantly related to decreased SHBG con-

Table 1. *The distribution of pre- and postmenopausal women by ethnic group*

		Caucasian	Chinese	Filipina	Hawaiian	Japanese	Total
Premenopausal	*n*	57	29	27	47	56	216
	%	(67.1)	(61.7)	(52.9)	(71.2)	(54.4)	(61.4)
Postmenopausal	*n*	28	18	24	19	47	136
	%	(32.9)	(38.3)	(47.1)	(28.8)	(45.6)	(38.6)
Total	*n*	85	47	51	66	103	352

$\chi_4^2 = 7.52$ (NS).

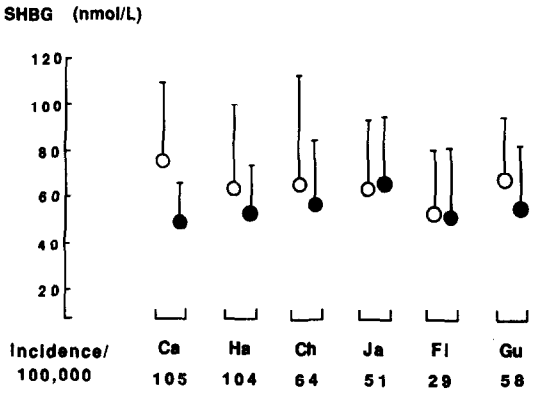

Fig. 1. *SHBG concentrations (mean S.D.) in premenopausal* (○) *and postmenopausal* (●) *Caucasian (Ca), Hawaiian (Ha), Chinese (Ch), Japanese (Ja), Filipina (Fi) women and a group of normal British women living in Guernsey (Gu). Incidence rates for British women taken from the South Thames Registry* (Cancer Incidence in Five Continents, *Vol.* 4, *Waterhouse J, Shanmugaratnam K, Muir C, Powell J, International Agency for Research on Cancer, Lyon*, 1982).

centrations. This change is reflected in the linear model for the percentage of circulating free estradiol in blood serum. The percentage of free estradiol is significantly and positively related to increased body mass and to postmenopausal status. There is also a marginally significant negative and independent counter-effect with increasing age. While the remaining two linear equations did not approach significance, it is still worth pointing out that the percentage of estradiol bound to albumin increases significantly in relation to body mass, while the percentage of estradiol bound to SHBG conversely decreases by nearly the same amount. No significant interactions between menopausal status and body mass were found in relation to any of the dependent variables and no significant ethnic differences were found when regression equations were computed separately for pre- and postmenopausal groups.

DISCUSSION

It is clear that the variation in risk of breast cancer in the five ethnic groups in Hawaii is unrelated to blood distribution of E2 between the free, albumin-bound or SHBG-bound compartments or to SHBG concentrations in our sample. This finding is entirely in keeping with the prediction from Moolgavkar's [20] mathematical model that overall incidence rates are not endocrine-related. He postulated that hormones control breast kinetics and, hence, *the shape* of the age/incidence curves. Since the latter are very similar in a variety of ethnic groups when corrected for cohort effects, the hormonal environment is also likely to be similar. It could be argued that the numbers of subjects in each population group are not large enough to support our conclusions. However, incidence rates for breast cancer vary so widely between racial groups that we would expect that some trends would have been apparent if estradiol distribution was important in the determination of risk. None of the ethnic groups studied had percentages of free, albumin-bound or SHBG-bound estradiol with confidence intervals that deviated more than 15% from the Caucasian mean.

Our current results are not in agreement with those of Moore *et al.* [9] who reported that normal Japanese women living in Japan had significantly higher percentages of serum estradiol bound to SHBG and lower percentages of albumin-bound estradiol than did Caucasian women living in Britain. The present study compares second generation Japanese immigrant women (nisei) with women from Caucasian, Chinese, Filipina and native Hawaiian background, most of whom had lived in Hawaii all or nearly all of their lives. In this single island environment of Oahu, the ethnic differences detected in geographically distinct Japanese and Caucasian populations are not observed so a possible explanation for the lack of agreement in the results is that it is environment (mainland Japan vs. Guernsey) that matters, rather than race. This explanation is unlikely, however, since in a recent study (Bulbrook and Moore, unpublished) in which Japanese women living in a semi-rural area of Tokyo were compared with British women living in Guernsey, there were no differences in the distribution of E2 between the free, albumin-bound and SHBG-bound fractions in serum. We do not have an immediate explanation for our inability to confirm our earlier findings. This could be related to recently identified defects in the method of centrifugal ultrafiltration dialysis as originally described [21].

Table 2. Anthropometic and hormonal variables by ethnic group

	Caucasian Mean ± S.D.	*n*	Chinese Mean ± S.D.	*n*	Filipina Mean ± S.D.	*n*	Hawaiian/Pacific Mean ± S.D.	*n*	Japanese Mean ± S.D.	*n*	
Premenopausal											
1 Weight (kg)	60.13 ± 11.12	43	57.85 ± 9.39	19	58.18 ± 13.65	21	71.48 ± 13.64	33	53.34 ± 6.7	40	$F = 13.00^{***}$ (4,151 d.f.)
2 Height (m)	1.63 ± 0.08	43	1.59 ± 0.05	19	1.53 ± 0.07	21	1.65 ± 0.06	33	1.56 ± 0.05	40	$F = 16.78^{***}$ (4,151 d.f.)
3 Age	39.98 ± 4.94	43	42.05 ± 4.96	20	40.10 ± 4.74	21	41.82 ± 5.72	33	42.38 ± 6.28	40	NS
4 SHBG	76.19 ± 31.61	31	66.31 ± 46.78	13	53.50 ± 25.99	12	64.48 ± 35.68	25	64.08 ± 28.94	25	NS
5 Percentage free E2	1.32 ± 0.27	31	1.52 ± 0.37	13	1.58 ± 0.26	11	1.48 ± 0.36	25	1.40 ± 0.31	25	NS
6 Percentage SHBG E2	44.81 ± 12.99	31	36.34 ± 20.29	13	38.26 ± 10.00	11	42.08 ± 14.46	25	40.84 ± 13.99	25	NS
7 Percentage ALB E2	53.87 ± 12.78	31	62.15 ± 19.96	11	60.15 ± 9.89	11	56.43 ± 14.16	25	57.76 ± 13.75	25	NS
8 Age at menarche	12.35 ± 2.50	43	12.60 ± 1.47	20	13.33 ± 1.50	21	12.97 ± 1.45	33	12.25 ± 1.48	40	NS
9 Age at first pregnancy	21.58 ± 4.17	26	26.31 ± 4.59	16	25.13 ± 6.47	15	21.73 ± 3.19	26	25.09 ± 3.25	32	$F = 6.03^{**}$ (4,110 d.f.)
Postmenopausal											
1 Weight (kg)	59.97 ± 9.34	28	55.63 ± 6.74	18	56.28 ± 12.26	24	63.78 ± 15.94	19	51.45 ± 6.28	47	$F = 6.57^{***}$ (4,131 d.f.)
2 Height (m)	1.63 ± 0.08	18	1.58 ± 0.04	18	1.50 ± 0.10	24	1.62 ± 0.05	19	1.52 ± 0.05	47	$F = 20.82^{***}$ (4,131 d.f.)
3 Age	61.68 ± 4.76	28	62.56 ± 5.24	18	64.63 ± 6.84	24	63.00 ± 6.75	19	60.57 ± 5.14	46	NS
4 SHBG	49.04 ± 17.88	23	56.80 ± 28.09	15	52.53 ± 28.74	19	53.00 ± 20.91	17	66.47 ± 29.33	34	NS
5 Percentage free E2	1.53 ± 0.29	21	1.42 ± 0.25	13	1.63 ± 0.32	16	1.60 ± 0.32	15	1.46 ± 0.28	32	NS
6 Percentage SHBG E2	35.08 ± 9.49	20	35.69 ± 8.31	13	35.62 ± 10.79	15	35.32 ± 9.95	15	37.38 ± 12.39	32	NS
7 Percentage ALB E2	63.45 ± 9.40	20	62.89 ± 8.23	13	62.78 ± 10.57	15	63.09 ± 9.76	15	61.16 ± 12.24	32	NS
8 Age at menarche	12.75 ± 1.43	28	12.39 ± 3.36	18	14.12 ± 2.35	24	13.21 ± 1.96	19	13.30 ± 1.64	47	NS
9 Age at first pregnancy	25.05 ± 4.44	21	25.27 ± 4.06	15	21.43 ± 3.87	21	23.00 ± 3.82	14	26.60 ± 4.60	44	$F = 5.84^{***}$ (4,110 d.f.)

$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Table 3. The relationship of sex hormone binding globulin and estradiol distribution to study variables

	Y = Sex hormone binding globulin (SHBG)	Y = Percentage free E2	Y = Percentage E2 bound to SHBG	Y = Percentage E2 bound to albumin
Age	0.5708 ± 0.4149	−0.0081 ± 0.0040*	0.0212 ± 0.1171	−0.0131 ± 0.1745
Age at menarche	1.5696 ± 1.4969	−0.0082 ± 0.0145	0.6749 ± 0.6391	−0.6667 ± 0.6297
Age at first pregnancy	−0.2616 ± 0.5549	−0.0013 ± 0.0054	−0.1313 ± 0.2369	0.1328 ± 0.2334
Body mass index	−2.4968 ± 0.6598***	0.0212 ± 0.0064***	−0.6601 ± 0.2817*	0.6387 ± 0.2776*
Post menopausal (1 = yes; 0 = pre)	−21.5967 ± 9.4116*	0.2146 ± 0.0909*	−6.3092 ± 4.0183	6.0971 ± 3.9590
Smokes (1 = yes; 0 = no)	8.9519 ± 6.9034	−0.1240 ± 0.6668	2.5766 ± 2.9474	−2.4532 ± 2.9039
Chinese (1 = yes; 0 = no)	1.3619 ± 8.5470	0.0743 ± 0.0826	−3.0315 ± 3.6492	2.9543 ± 3.5953
Filipina (1 = yes; 0 = no)	−7.5317 ± 8.7342	0.1600 ± 0.0844	−0.6615 ± 3.7291	0.4993 ± 3.6741
Hawaiian (1 = yes; 0 = no)	1.0899 ± 8.0306	0.0784 ± 0.0776	−0.4735 ± 3.4287	0.3941 ± 3.3781
Japanese (1 = yes; 0 = no)	1.0804 ± 7.1160	0.0503 ± 0.0687	−1.5659 ± 3.0382	1.5143 ± 2.9934
	$R^2 = 0.16$ $F_{10,144} = 2.73$** Intercept = 85.5593	$R^2 = 0.16$ $F_{10,144} = 2.80$** Intercept = 1.3920	$R^2 = 0.10$ $F_{10,144} = 1.67$ Intercept = 51.1344	$R^2 = 0.10$ $F_{10,144} = 1.65$ Intercept = 47.4725

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

No attempt was made to control for the stage of the cycle in premenopausal women since, at the time the study was set up, there was no convincing evidence that SHBG changed significantly during the cycle. Recently it has been shown that small increases do occur in the luteal phase, which would very marginally affect the distribution of E2 [22, 23]. This would not affect our findings since in this study the distribution of cycle stages in each racial grouping was approximately the same.

The positive relationship between the percentage of unbound estradiol and body weight and the progressive decline in SHBG levels with increasing body weight which our data reveal have also been reported in several other Caucasian populations (see for example Refs. [4, 8, 24]). These trends were observed in our data for each ethnic group when premenopausal and postmenopausal groups were analyzed separately.

The influence of reproductive history now appears not to have a strong impact on breast cancer risk and age at diagnosis [25]. It has recently been shown, in a large study, that early age at menarche is associated with diminished SHBG concentrations in premenopausal women [26]. In our data, age at menarche and age at first pregnancy were not found to be significantly associated with estradiol binding or with SHBG concentration but the numbers are too small to pick up a weak relationship.

The anti-estrogenic effects of smoking described by Michnovicz *et al.* [27] were not observed in the present study. Smoking was found to have no significant effect on the percentage of free or bound estradiol in any ethnic group nor among pre- or postmenopausal women.

In conclusion, therefore, variation in estrogenic status does not account for geographical differences in incidence rates. The distribution of estradiol in the blood of five ethnic groups in Hawaii did not differ and was within the range of that found for British women living in Guernsey. The values were similar to those found in Egyptian women living in Cairo or in the Nile delta [28].

Acknowledgements—We wish to thank Susan Hoare and Graham Clark for carrying out the serum assays.

REFERENCES

1. Pardridge WM, Mietus LJ, Frumar AJ, Davidson BJ, Judd HL. Effects of human serum on transport of testosterone and estradiol into rat brain. *Am J Physiol* 1980, **239**, 103–108.
2. Moore JW, Clark GMG, Bulbrook RD *et al.* Serum concentrations of total and non-protein bound oestradiol in patients with breast cancer and in normal controls. *Int J Cancer* 1982, **29**, 17–21.
3. Reed MJ, Cheng RW, Noel CT, Dudley HAF, James VHT. Plasma level of estrone, estrone sulphate, and estriol and the percentage of unbound estradiol in post-menopausal women with and without breast disease. *Cancer Res* 1983, **43**, 3940–3943.
4. Ota DM, Jones LA, Jackson GL, Jackson PM, Kemp K, Bauman D. Obesity, non-protein-bound estradiol levels, and distribution of estradiol in the sera of breast cancer patients. *Cancer* 1986, **57**, 558–562.
5. Langley MS, Hammond GL, Bardsley A, Sellwood RA, Anderson DC. Serum steroid binding proteins and the bioavailability of estradiol in relation to breast diseases. *J Natl Cancer Inst* 1985, **75**, 823–829.
6. Siiteri PK, Hammond GL, Nisker JA. Increased availability of serum estrogens in breast

cancer: a new hypothesis. In: Pike MC, Siiteri PK, Welsch CW, eds. *Banbury Report: Hormones and Cancer.* Cold Spring Harbor Laboratory, New York, 1981.

7. Bulbrook RD, Moore JW, Clark GMG, Wang DY. Relation between risk of breast cancer and biological availability of estradiol in the blood: prospective study in Guernsey. *Ann NY Acad Science* 1986, **464**, 378–388.
8. Moore JW, Clark GMG, Hoare SA *et al.* The binding of oestradiol to blood proteins and aetiology of breast cancer. *Int J Cancer* 1986, **38**, 625–630.
9. Moore JW, Clark GMG, Takatani O, Wakabayashi Y, Hayward JL, Bulbrook RD. Distribution of 17β-estradiol in the sera of normal British and Japanese women. *J Natl Cancer Inst* 1983, **71**, 749–754.
10. Kolonel LN. Cancer incidence and mortality in Hawaii 1973–1977. In: NCI Monograph No. 57, *Surveillance, Epidemiology, and End Results Program (SEER)*, 1981, 791–945.
11. Anderson DC. Sex-hormone-binding-globulin. *Clin Endocrinol* 1974, **3**, 69–96.
12. Hammond GL, Langley MS, Robinson PA. A liquid phase immunoradiometric assay for sex-hormone-binding-globulin. *J Steroid Biochem* 1985, **23**, 451–460.
13. Hammond GL, Nisker JA, Jones JA, Siiteri PK. Estimation of the percentage of free steroid in undiluted serum by centrifugal ultrafiltration-dialysis. *J Biol Chem* 1980, **255**, 5023–5026.
14. Dowsett M, Mansfield MD, Griggs DJ, Jeffcoate SL. Validation and use of centrifugal ultrafiltration–dialysis in the measurement of percent non-protein-bound oestradiol in serum. *J Steroid Biochem* 1984, **21**, 343–345.
15. Hammond GL, Lahteenmaki PL, Lahteenmaki P, Luukkainen T. Distribution and percentages of non-protein bound contraceptive steroids in human serum. *J Steroid Biochem* 1982, **17**, 375–380.
16. Siiteri PK, Murai JT, Hammond GL, Nisker JA, Raymoure WJ, Kuhn RW. The serum transport of steroid hormones. *Recent Prog Horm Res* 1982, **38**, 457–510.
17. Grove JS, Goodman MJ, Gilbert F, Clyde D. Factors associated with breast structure in breast cancer patients. *Cancer* 1979, **43**, 1895–1899.
18. Goodman MJ, Grove JS, Gilbert FI. Age at menarche and year of birth in relation to adult height and weight among Caucasian, Japanese and Chinese women living in Hawaii. In: Golub S, ed. *Menarche: the Transition from Girl to Woman.* Lexington Books, 1983.
19. Goodman MJ, Grove JS, Gilbert FI. Recalled characteristics of menstruation in relation to reproductive history among Caucasian, Japanese and Chinese women living in Hawaii. *Ann Human Biol* 1984, **11**, 235–242.
20. Moolgavkar SH. Hormones and multistage carcinogenesis. *Cancer Surv* 1986, **5**, 635–648.
21. Moore JW, Hoare SA, Quinlan MK, Clark GMG, Wang DY. Centrifugal ultrafiltration dialysis for non-protein-bound oestradiol in blood: importance of the support. *J Steroid Biochem* 1987, **28**, 677–681.
22. Dowsett M, Attree SL, Virdee SS, Jeffcoate SL. Oestrogen-related changes in sex hormone binding globulin levels during normal and gonadotropin stimulated menstrual cycles. *Clin Endocrinol* 1985, **23**, 303–312.
23. Plymate SR, Moore DE, Cheng CY, Bardin CW, Southworth MB, Levinski MJ. Sex hormone binding globulin changes during the menstrual cycle. *J Clin Endocrinol Metab* 1985, **61**, 993–996.
24. Kirschner MA, Schneider G, Ertel NH, Worton E. Obesity, androgens, estrogens and cancer risk. *Cancer Res (Suppl)* 1982, **42**, 3281–3285.
25. Wang DY, Rubens RD, Allen DS *et al.* Influence of reproductive history on age at diagnosis of breast cancer and prognosis. *Int J Cancer* 1985, **36**, 427–432.
26. Moore JW, Key TGA, Bulbrook RD *et al.* Sex hormone binding globulin and risk factors for breast cancer in a population of normal women who had never used exogenous hormones. *Br J Cancer* 1987, **56**, 661–666.
27. Michnovicz JJ, Hershcopf RJ, Naganuma H, Bradlow HL, Fishman J. Increased 2-hydroxylation of estradiol as a possible mechanism for the anti-estrogenic effect of cigarette smoking. *New Engl J Med* 1986, **315**, 1305–1309.
28. The Anglo-Egyptian Collaborative Study. Serum hormone levels in breast cancer patients and controls in Egypt and Great Britain. *Eur J Cancer Clin Oncol* 1988, **24**, 1329–1335.