Europ. J. Cancer Vol. 15, pp. 1269–1274.
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0014-2964/79/1001-1269 $02.00/0

Plasma and Urinary Androgens in Women with Varying Degrees of Risk of Breast Cancer

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Abstract—*Using as indices of risk the age at menarche, age at first child, family history of breast cancer and the amount of urinary aetiocholanolone excreted, 386 normal women aged 30–69 yr were divided into 4 groups with a mean calculated risk of breast cancer of 0.016, 0.023, 0.051 and 0.111.*

Plasma levels of dehydroepiandrosterone and its sulphate, androstenedione and androst-5-en-3β, 17β-diol have now been determined on blood specimens from the same women, taken approximately 5 yr after urine collection.

The levels of plasma androst-5-en-3β, 17β-diol, dehydroepiandrosterone and its sulphate correlated with the excretion of urinary aetiocholanolone and were at the lower end of the normal range in the highest risk group. The significant correlation between plasma androgen levels and the amount of urinary aetiocholanolone measured 5 yr previously indicates that abnormalities in androgen production are not transient.

INTRODUCTION

A STUDY by Farewell [1] of a population of over 5000 normal women who had been followed-up for 17 yr showed that 4 risk factors can be used to calculate the probabilty of a particular woman developing breast cancer. The factors are (i) age at first child (favourable if <24 yr), (ii) age at menarche (favourable if >14 yr, (iii) family history of breast cancer and, (iv) the amount of aetiocholanolone excreted in the urine (favourable if >1 mg/24 hr).

It so happened that many of these women for whom an estimate of risk was available provided blood specimens in a second study conducted 5 yr later. It was therefore possible to determine the amounts of a variety of hormones in the plasma to see whether they correlated with estimated risk.

We have already shown that the calculated probability of developing breast cancer in normal pre-menopausal women in Guernsey is correlated with plasma progesterone levels in the luteal phase of the menstrual cycle [2]. Estimated risk is inversely correlated with progesterone concentration. What is more, low levels of progesterone are generally found in women who excrete low amounts of urinary aetiocholanolone.

The relationship between plasma progesterone and urinary aetiocholanolone has led to us to examine more thoroughly the androgenic status of women with varying estimates of risk of breast cancer. Plasma levels of dehydroepiandrosterone‡ and its sulphate, Δ^4-androstenedione‡, and Δ^5- androstenediol‡ have now been measured in women hose expectations of developing breast cancer ranged from 1 in 100 to 1 in 8 calculated from previously defined factors [1].

MATERIALS AND METHODS

Subjects

The normal women who took part in this prospective experiment have already been described in detail [2]. Briefly, plasma prepared from blood samples taken between 2 and 8 p.m., was available from 386 normal women aged from 30 to 69 yr, for each of whom an estimate of risk of breast cancer was available. This estimate was based on age at menarche, age at first child, family history of breast cancer and the amount of aetiocholanolone

Accepted 22 March 1979.

‡The following abbreviations are used: D for dehydroepiandrosterone, DS for dehydroepiansterone sulphate, Δ^4 for androstenedoine and Δ^5 for androst-5-en-3β, 17β-diol.

excreted in the urine [1]. The amount of aetiocholanolone and androsterone was measured in 24 hr-volume urine collected on average 5 yr before the blood specimens described above [3]. Four risk groups were defined (0–3) with calculated mean risks of 0.016, 0.023, 0.051 and 0.111 respectively.

Analytical methods

Dehydroepiandrosterone and its sulphate, androstenedione and androstenediol were all assayed using radioimmunoassay methods. Unless otherwise stated, the buffer used in all assays was isotonic phosphate–saline (pH 7.1) containing 0.1% of both sodium azide and gelatine. Androstenedione (1, 2, 6, 7-tritiated, specific activity 86.8 Ci/mmole), dehydroepiandrosterone and androstenediol (both 1,2-tritiated, specific activity 58.6 Ci/mmole), were all obtained from New England Nuclear Corporation, Boston, U.S.A.

Dehydroepiandrosterone

Plasma (200 μl) was added to 1 ml of buffer containing 1000 counts/min of tritiated D (for recovery purposes) and extracted with 7 ml of ether.

The dried extract was dissolved in buffer (1 ml) and aliquots taken for assay and recovery. The antibody used was raised against D-7-(*o*-carboxymethyl)imino-bovine serum albumin in rabbit, and used at a final dilution of 1:20,000.

The specificity of the method was considered to be satisfactory since cross-reaction with steroids likely to interfere was less than 4% (see Table 1) and results obtained before and after column chromatography using LH20 and alumina were not significantly different. Precision calculated from duplicate estimations showed that the coefficient of variation was less than 8% over the range estimated.

Dehydroepiandrosterone sulphate

Plasma (50 μl) was added to acetate buffer (2ml, pH4.5 1.M,) containing about 1000 counts/min of tritiated DS (for recovery). This mixture was extracted with dichloromethane (5 ml) to remove non-conjugated steroids and lipids. The aqueous phase was then heated to 120°C for $1\frac{1}{2}$ hr to hydrolyse DS [4]. After cooling on ice the hydrolysate was extracted with iso-pentane (4ml). The residue after evaporation of the iso-pentane was dissolved in buffer (1 ml) and aliquots were assayed as previously described for the estimation of D.

The specificity of the assay was satisfactory and the same antibody was used as for the estimation of D. Paper chromatography purification of the plasma extract made no difference to the assay results. Precision calculated from duplicate determinations showed a coefficient of variation of less than 10% over the range used in the assay.

Table 1. Specificity of antiserum to dehydroepiandrosterone-7-cmo-BSA

Steroid	Cross-reaction (%)
Dehydroepiandrosterone (D)	100
Dehydroepiandrosterone sulphate (DS)	6.325
Androstenedione (Δ^4)	1.900
Androstenediol (Δ^5)	2.200
Testosterone	0.060
5α-Dihydrotestosterone	0.100
5α-Androstane-3β, 17β-diol	0.200
5α-Androstane-3α, 17β-diol	0.010
Androsterone	0.114
Androsterone sulphate	0.024
Aetiocholanolone	0.026
Cortisol	0.001
Progesterone	0.036
Pregnenolone	0.008
Cholesterol	0.006
Oestrone	0.330
Oestradiol-17β	0.020

Percentage of cross-reaction is expressed as the ratio of $A/B \times 100$, where A = the amount of dehydropiandrosterone and B = the amount of competitor required to reduce binding of (^{3}H) dehydroepiandrosterone by 50%. Where, due to limited solubility of a steroid, this inhibition of binding was not possible, the ratio at the maximum inhibition of binding was used.

Androst-5-en-3β, 17β-diol

Plasma (1–2 ml) containing tritiated Δ^5 (for recovery) was extracted with ether (7 ml). The dried extract was chromatographed on a column of LH20 using benzene/methanol (85:15 v/v). The fraction containing Δ^5 was dried down and dissolved in buffer, aliquots being taken for assay and recovery. The antibody used was raised against Δ^5-7-(*o*-carboxymethyl) imino-bovine serum albumin in rabbit. The specificity and precision were satisfactory and have been described fully by Moore [5].

Androst-4-en-3, 17-dione

Except for the source of the antibody the levels of plasma androstenedione were measured essentially by the method already described [5]. The antibody was raised against androstenedione-6β-hemisuccinate coupled to bovine serum albumin and used at a final concentration of 1 in 30,000. The precision and specificity of these determinations were similar to those already described and were, therefore, satisfactory [6].

RESULTS

1. *Dehydroepiandrosterone sulphate*

The mean plasma levels of DS in the 4 risk groups are shown in Table 2. As there were no significant differences between DS levels in the follicular and luteal phases of the menstrual cycles the phase of the cycle has been ignored for statistical analysis. The plasma DS in the group with the highest estimated risk is significantly lower than in the remaining 3 risk groups (Table 2).

There were no significant differences in DS levels in the 4 postmenopausal risk groups.

Since plasma DS levels are inversely related to age [7], the relation between the amount of this plasma steroid and age for both pre- and post-menopausal women in each of the 4 risk groups has been examined. The results parallel those in Table 2; the elevation of the regression of DS on age for the group with the highest estimated risk is significantly lower than that for risk group 0, 1 and 2 ($F=4.7$, $P<0.05$; $F=9.5$, $P<0.005$; $F=5.5$, $P<0.025$) respectively (fig. 1).

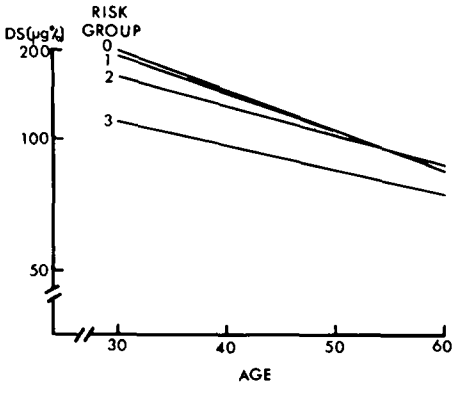

Fig. 1. Comparison of the linear regression of plasma DS on age for risk groups 0, 1, 2 and 3. The elevation of the highest risk group 3 is significantly lower than risk groups 0 ($F=4.7$, $P<0.05$, $d.f.=90$), 1 ($F=9.5$, $P<0.005$, $d.f.=175$) and 2 ($F=5.5$, $P<0.025$, $d.f.=194$). There was no significant difference between the elevations of any other pair pair of lines.

2. $\triangle^5$-*androstenediol*

There were no differences between follicular and luteal phase levels of $\triangle^5$ and the phase of the cycle has therefore been ignored.

The plasma $\triangle^5$ in the highest risk group is significantly lower than that in risk groups 0, 1 or 2 (Table 3).

There is no significant difference between the risk groups in the post-menopausal women.

Because of the significant decline in $\triangle^5$ levels with age the elevations of the regression lines for the combined pre- and post-menopausal women in the various risk groups were compared and essentially agreed with the differences already shown for DS. Thus the highest risk group was significantly lower than risk groups 0 or 2 ($F=8.7$, $P=0.005$; $F=10.9$, $P<0.005$ respectively). Paradoxically risk group 1 was significantly lower than risk group 2 ($F=4.8$, $P<0.05$), although the difference just reached formal significance.

3. *Dehydroepiandrosterone and* $\triangle^4$-*androstenedione*

In pre-menopausal women no relation between estimated risk and the levels of D or $\triangle^4$ was found (Tables 4 and 5). In post-menopausal women, the highest risk group had significantly lower plasma D than risk group 0; similarly the $\triangle^4$ levels in risk group 1 were lower than those in risk group 0, but in both instances the numbers of women in the lowest risk group were small (6 and 7) and little reliance can be placed upon these results. Within the remaining three risk groups there was no correlation between the steroid levels and risk.

Table 2. Relation between plasma DS *levels and risk of breast cancer*

	Risk group			
Pre-menopausal	0	1	2	3
Follicular phase	2.13±0.10 (10)	2.08±0.17 (39)	2.07±0.21 (44)	1.96±0.21 (16)
Luteal phase	2.10±0.12 (12)	2.14±0.17 (39)	2.09±0.16 (41)	2.03±0.20 (15)
Combined phases	2.11±0.11 (22)	2.11±0.17 (78)	2.08±0.19 (85)	1.99±0.21 (31)
Post-menopausal	2.04±0.08 (7)	1.97±0.24 (37)	1.95±0.20 (43)	1.88±0.21 (33)

All results are expressed as logarithms of the plasma DS levels (µg/100ml). Standard deviations are shown. Figures in parentheses refer to the number of subjects in each group.

Significance tests. Pre-menopausal, combined phases.

Group 0 vs 3 $t=2.473$, $P<0.02$

Group 1 vs 3 $t=2.910$, $P<0.01$

Group 2 vs 3 $t=2.177$, $P<0.05$

Table 3. Relation between plasma Δ^5 levels and risk of breast cancer

	Risk group			
Pre-menopausal	0	1	2	3
Follicular phase	2.67 ± 0.18 (10)	2.62 ± 0.20 (26)	2.62 ± 0.17 (30)	2.51 ± 0.21 (16)
Luteal phase	2.70 ± 0.21 (8)	2.66 ± 0.23 (29)	2.76 ± 0.23 (38)	2.56 ± 0.17 (13)
Combined phases	2.69 ± 0.19 (18)	2.64 ± 0.22 (55)	2.70 ± 0.22 (68)	2.53 ± 0.19 (29)
Post-menopausal	2.70 ± 0.10 (3)	2.37 ± 0.27 (8)	2.54 ± 0.19 (5)	2.52 ± 0.29 (5)

All results are expressed as logarithms of the plasma Δ^5 levels (pg/ml).
Significance tests. Pre-menopausal, combined phase.
Group 0 vs 3 $t = 2{,}736$, $P < 0.01$.
Group 1 vs 3 $t = 2{,}244$, $P < 0.05$.
Group 2 vs 3 $t = 3{,}585$, $P < 0.001$.

Table 4. Relation between plasma D and risk of breast cancer

	Risk group			
Pre-menopausal	0	1	2	3
Follicular phase	3.94 ± 0.13 (8)	3.93 ± 0.14 (35)	3.94 ± 0.17 (43)	3.80 ± 0.21 (17)
Luteal phase	3.95 ± 0.17 (12)	3.96 ± 0.17 (40)	3.96 ± 0.16 (41)	3.93 ± 0.20 (15)
Combined phases	3.95 ± 0.15 (20)	3.95 ± 0.16 (75)	3.95 ± 0.16 (84)	3.86 ± 0.21 (32)
Post-menopausal	3.96 ± 0.14 (6)	3.82 ± 0.21 (29)	3.81 ± 0.16 (36)	3.71 ± 0.15 (28)

All results are expressed as logarithms of the plasma D levels (pg/ml).
Significance tests. Post-menopausal.
Group 0 vs 2 $t = 2.12$, $P < 0.05$.
Group 0 vs 3 $t = 2.88$, $P < 0.01$.

Table 5. Relation between plasma Δ^4 and risk of breast cancer

	Risk group			
Pre-menopausal	0	1	2	3
Follicular phase	3.02 ± 0.18 (11)	3.01 ± 0.17 (30)	3.03 ± 0.13 (39)	3.03 ± 0.15 (16)
Luteal phase	3.14 ± 0.14 (10)	3.06 ± 0.21 (37)	3.06 ± 0.18 (37)	3.05 ± 0.17 (16)
Combined phase	3.08 ± 0.17 (21)	3.04 ± 0.19 (67)	3.05 ± 0.15 (76)	3.04 ± 0.16 (32)
Post-menopausal	3.04 ± 0.07 (7)	2.92 ± 0.14 (27)	2.93 ± 0.17 (28)	2.91 ± 0.19 (24)

All results are expressed as the logarithms of the plasma Δ^4 levels (pg/ml).
Significance tests. Post-menopausal.
Group 0 vs 1 $t = 2.111$, $P < 0.05$.

However, since both Δ^4 and D are significantly correlated with age [6, 8] the elevations of the regression lines have been compared for the pre- and post-menopausal women in 4 risk groups. In the case of Δ^4 there was no significant difference between any of the risk groups. However, the group with the highest estimated risk (category 3) had significantly lower levels of D than the other three risk groups (0 vs 3 $F=7.5$, $P<0.01$; 1 vs 3 $F=5.8$, $P<0.025$; 2 vs 3 $F=5.6$, $P<0.025$).

4. *Relation between plasma and urinary steroid levels*

The correlations between the levels of the four plasma steroids are shown in Fig. 2. All the correlations are significant except between Δ^4 and D.

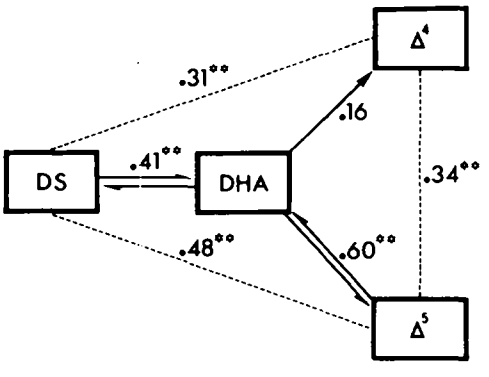

*Fig. 2. The correlation coefficients between the levels of plasma DS, D, Δ^4 and Δ^5. Solid lines represent metabolic pathways. The regression coefficients are shown and those marked with ** indicate the correlation is significant at $P<0.001$. Other correlations are non-significant.*

The relationships between these four plasma steroids and the urinary androgen metabolites, aetiocholanolone and androsterone, are shown in Fig. 3. All the correlations are significant except for those involving Δ^4.

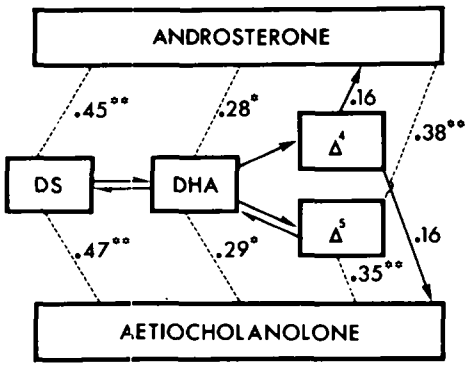

*Fig. 3. The correlation coefficients between the plasma steroid levels and urinary excretion of aetiocholanolone and androsterone. The regression coefficients are shown and those marked with * or ** indicate that the correlation is significant at $P<0.01$ or $P<0.001$, respectively. Other correlations are statistically non-significant.*

5. *Relation between plasma androgens and single risk factors*

Four determinants of risk were used to calculate the probability of developing breast cancer: these were age at menarche, age at first child, family history and the amount of aetiocholanolone excreted in the urine [1, 2]. Although the present investigation was not designed to see whether plasma androgens varied within each of these risk factors, an approximation may be obtained by taking each determinant separately and ignoring the presence or absence of any other risk factor. When this is done, no correlation is found between plasma androgen levels and any risk factor except a low excretion of aetiocholanolone or androsterone.

DISCUSSION

The major findings in this investigation are that the levels of D, Δ^5 and DS are low in the group of women with the highest estimated risk and are significantly correlated with urinary aetiocholanolone levels. Plasma Δ^4 does not vary with risk nor was a significant association found between the concentrations of this steroid and urinary aetiocholanolone.

Aetiocholanolone is a urinary metabolite derived from a wide variety of precursors [9] and the amount of this urinary compound may not give an accurate reflection of the plasma levels of these precursors: for example, plasma progesterone or cortisol levels are not correlated with the urinary excretion of pregnanediol or corticosteroids [10, 11]. However, DS, D and Δ^5 were found to be highly correlated with aetiocholanolone.

Since these plasma steroids are overwhelmingly of adrenal origin [12, 13] this study serves to emphasise that the diminished urinary aetiocholanolone levels, previously observed in women at risk of breast cancer [1], truly reflect subnormal adrenal androgen production and also shows that D, DS, Δ^5 and aetiocholanolone are probably interchangeable as a measure of risk.

Another finding is the constancy in the temporal relation between plasma and urinary steroids. The latter were measured in urine obtained approximately 5 yr before blood was taken for plasma androgen assays. This shows that the androgen–risk relationship is relatively constant with time.

We have already shown that luteal phase insufficiency in the production of progesterone is related to estimated risk [2]. Since as stated above all the blood androgens, except for Δ^4, are predominantly adrenal in origin, it is now apparent that high risk is also associated with an adrenal androgen component.

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