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CIGARETTE SMOKING AND STEROID HORMONES IN WOMEN

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Summary—Epidemiological evidence has suggested that cigarette smoking has an anti-oestrogenic effect in women, but the effects of smoking on steroid hormone metabolism are not fully understood. We compared serum concentrations of oestradiol, progesterone (luteal phase) and dehydroepiandrosterone sulphate (DHEA-S), and urinary excretion rates of six steroids of predominantly adrenal origin, in healthy premenopausal and postmenopausal female smokers and non-smokers. Serum concentrations of oestradiol, progesterone and DHEA-S did not differ between smokers and non-smokers by >5%, and none of these differences was statistically significant. Mean urinary excretion rates of androsterone, aetiocholanolone, DHEA, 11-keto-aetiocholanolone, 11-hydroxyandrosterone and 11-hydroxyaetiocholanolone were very similar in smokers and non-smokers in premenopausal women, but were from 2–44% higher in smokers than non-smokers in postmenopausal women. The difference was statistically significant only for 11-hydroxyandrosterone. These results confirm previous reports that cigarette smoking does not affect serum oestradiol in premenopausal or postmenopausal women, but provide only weak evidence to support previous findings of increased levels of some adrenal steroids in postmenopausal women smokers. The mechanism for the apparent anti-oestrogenic effect of cigarette smoking remains unclear.

INTRODUCTION

The apparent anti-oestrogenic effect of cigarette smoking [1], particularly the evidence that smoking reduces the risk of endometrial cancer [2–8], has stimulated considerable interest in the possible effects of smoking on steroid hormones. MacMahon *et al.* [9] reported in 1982 that, in comparison with premenopausal non-smokers and ex-smokers, premenopausal smokers had substantially lower urinary levels of oestrone (E1), oestradiol (E2) and oestriol (E3) in the luteal phase of the cycle, but not in the follicular phase. MacMahon *et al.* [9] suggested that smoking might reduce luteal phase oestrogen production. Jensen *et al.* [10] subsequently reported that serum concentrations of E1 and E2 in postmenopausal women receiving oral oestrogen replacement therapy were 50% lower in smokers than in non-smokers, but they found no such difference between smokers and non-smokers among women receiving percutaneous oestrogen replacement therapy, suggesting that smoking increased catabolism of oral oestrogens during their first pass through the liver. Michnovicz *et al.* [11] reported that the proportion of E2 metabolized by 2-hydroxylation was significantly higher in premenopausal women who smoked than in non-smokers; they subsequently measured all five major urinary oestrogens (E1, E2, E3, 16α-hydroxyoestrone and 2-hydroxyoestrone), and found that, in follicular phase urine samples, smokers had increased excretion of 2-hydroxyoestrone and E2, and decreased excretion of E3, with no difference in total oestrogen excretion [12]. Several recent studies in postmenopausal women have found that serum concentrations of endogenous E1 and E2 are similar in smokers and non-smokers [10, 13–16], and a single study of premenopausal women has also failed to find any differences

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between smokers and non-smokers in serum E1 or E2 [13]. Because of these negative results for serum oestrogens, it has been suggested that the protective effect of smoking against endometrial cancer in postmenopausal women might be related to high serum concentrations of progesterone (Pg) or of adrenal androgens in smokers [14, 15].

To help clarify the effects of cigarette smoking on steroid hormones, the current study reports mean serum concentrations of E2, Pg and dehydroepiandrosterone sulphate (DHEA-S), and urinary excretion rates of six steroids of predominantly adrenal origin, in premenopausal and postmenopausal smokers and non-smokers.

METHODS

Subjects and data collection

Serum and urine samples were drawn from a bank of samples from 5000 women participating in a prospective study of breast cancer on the Island of Guernsey [17]. Women aged 35 and above were invited to attend for breast cancer screening, during which a questionnaire (covering risk factors for breast cancer) was completed, height and weight were measured, and a blood sample was taken. 24-h urine samples were collected approximately two weeks before the blood samples. Aliquots of serum and urine were stored at −20°C. The dates of the last menstrual periods before collection of blood and urine were recorded on the questionnaire, and women were given postcards to report the dates of the menstrual periods following sample collection. The standard questionnaire did not include a question on smoking, but an additional short questionnaire which did cover smoking at the time of screening was collected for approximately the first 1000 women recruited during 1977 and 1978. This permitted categorization of these women as current non-smokers or as current smokers of 1–10, 11–20 or 21 + cigarettes per day.

For the analyses presented here women were excluded if they were using exogenous sex hormones around the time of specimen collection, or if we did not have information on their smoking habits. Further choice of samples was made in two ways for measurements of serum E2 and Pg and for measurements of serum DHEA-S and urinary steroids.

E2 and Pg were measured in all serum samples from premenopausal current smokers with cycle lengths of 21–35 days, and E2 was measured in all serum samples from current smokers who were naturally postmenopausal (no ovariectomy or hysterectomy). E2 and (in premenopausal women) Pg were also measured in a similar number of non-smokers, matched as closely as possible firstly (in premenopausal women) for day of cycle at blood collection, then for age, and then for body mass index (BMI: kg/m^2). The mean ages and BMIs of these subjects are given in Table 1 (i).

As part of a study of androgens and risk factors for breast cancer [18], DHEA-S was measured soon after the beginning of the

Table 1. Subject characteristics

	Non-smokers			Smokers			
	Mean	SD	*N*	Mean	SD	*N*	*P*[a]
			(i) Subjects assayed for E2 and Pg				
Premenopausal							
Age (years)	40.7	4.5	78	40.6	4.6	69	0.88
BMI (kg/m^2)	24.6	3.6		24.2	3.6		0.60
Postmenopausal							
Age (years)	57.1	5.4	40	56.6	5.7	36	0.71
BMI (kg/m^2)	24.0	2.4		24.4	3.7		0.62
			(ii) Subjects assayed for DHEA-S				
Premenopausal							
Age (years)	41.9	4.3	314	41.0	4.7	97	0.10
BMI (kg/m^2)	24.9	3.7		24.1	3.7		0.09
Postmenopausal							
Age (years)	57.3	6.6	213	55.8	6.1	48	0.15
BMI (kg/m^2)	25.5	3.4		24.5	3.6		0.06
			(iii) Subjects assayed for urinary steroids				
Premenopausal							
Age (years)	42.0	4.8	85	41.4	5.7	20	0.62
BMI (kg/m^2)	24.9	3.6		23.5	3.7		0.11
Postmenopausal							
Age (years)	59.3	6.0	51	56.1	3.3	10	0.11
BMI (kg/m^2)	25.4	2.8		24.5	3.7		0.43

[a]Two-sided test for difference between means.

prospective study in serum samples from a substantial number of women among the first 1000 recruited. These results were used in the present study irrespective of the length of the menstrual cycle in premenopausal women or of the type of menopause ("natural" or ovariectomy) in postmenopausal women. The mean ages and BMIs of these subjects are given in Table 1 (ii).

The measurements of urinary steroids (androsterone, aetiocholanolone, DHEA, 11-keto-aetiocholanolone, 11-hydroxyandrosterone and 11-hydroxyaetiocholanolone) were made in samples from a subset of the subjects in whom the serum DHEA-S measurements were made [18]. The characteristics of these subjects are given in Table 1 (iii).

Steroid hormone assays

E2 and Pg were assayed in 1987. Aliquots of pools of serum from premenopausal and postmenopausal women were included in each assay run to measure assay variation.

E2 was measured in duplicate with a non-extraction double-antibody radioimmunoassay kit (Diagnostic Products Corp., Los Angeles, CA, U.S.A.). The intra- and inter-assay coefficients of variation were both 5% at an E2 concentration of 373 pmol/l (premenopausal level) and were 11 and 15%, respectively at an E2 concentration of 23 pmol/l (postmenopausal level). Twenty percent of the results from the postmenopausal women were at or below the detection limit of the assay (5.14 pmol/l) and were assigned this value in the analyses.

Pg was measured in duplicate with a solid-phase coated-tube radioimmunoassay kit (Diagnostic). The intra- and inter-assay coefficients of variation were 7 and 6%, respectively at a Pg concentration of 10.8 nmol/l (early luteal phase level).

The assay methods for serum DHEA-S and for the urinary steroids were as described previously [18]. These assays were completed within 2 years of sample collection.

Statistical analyses

Hormone values were logarithmically transformed to achieve approximate normality. The mean values presented are geometric means, adjusted where appropriate for other variables.

Differences between the means of the logarithmically transformed values were tested, and adjustments made, using analysis of covariance. All mean values were adjusted for age and BMI as linear variables. The mean values for DHEA-S and urinary steroids in premenopausal women were also adjusted for parity (0, 1 +) because of the substantial effect of first pregnancy on DHEA-S in premenopausal women [19, 20]. For comparisons of E2 and Pg in smokers vs non-smokers among premenopausal women, adjustments were made for stage of cycle as well as for age and BMI: for E2, values from all 147 premenopausal subjects were used, and adjustments were made using six indicator variables to define seven cycle stage categories (0–3, 4–7, 8–11, 12–15, 16–19, 20–23 and 24 + days before the end of the cycle); for Pg, the results from the 55 subjects sampled between 11 and 2 days before the end of their menstrual cycle were used, and adjustments were made using four indicator variables to specify the five two-day intervals in this ten day section of the menstrual cycle. Neither DHEA-S nor the urinary steroids were significantly related to the stage of the menstrual cycle, therefore no adjustments for this variable were made. None of the adjusted mean values differed greatly from the unadjusted mean values, except for urinary steroids in postmenopausal women as noted below. Two-sided *P*-values are quoted.

RESULTS

Serum E2 and Pg

In both premenopausal smokers and non-smokers E2 and Pg fluctuated in the normal pattern during the menstrual cycle [Table 2 (i)], with no suggestion that either E2 or Pg was related to cigarette smoking [Table 2(ii)]. In comparison with non-smokers, smokers had 4% higher mean E2 and virtually identical mean Pg, with no evidence of a dose-response relationship for either hormone.

In postmenopausal women, mean E2 in smokers was 5% higher than in non-smokers, with no suggestion of any dose-response effect (Table 3).

Serum DHEA-S

There was no substantial difference between smokers and non-smokers in mean DHEA-S, nor was there a significant linear trend in DHEA-S with increasing cigarette consumption, among either premenopausal or postmenopausal women (Table 4).

Table 2. Serum E2 and Pg in premenopausal smokers and non-smokers
(i) By stage of cycle

Days before end of cycle	E2 (pmol/l)		Pg (nmol/l)		N	
	Non-smokers	Smokers	Non-smokers	Smokers	Non-smokers	Smokers
24+	74	73	2.8	3.0	9	9
20–23	149	167	1.8	3.1	13	10
16–19	345	409	1.8	2.0	10	9
12–15	507	383	3.8	3.0	9	10
8–11	262	289	26.8	30.4	15	12
4–7	310	299	41.1	42.0	9	10
0–3	190	216	14.1	18.7	13	9

(ii) By number of cigarettes smoked

Smoking category	E2 (pmol/l)	N	Pg (nmol/l)[a]	N
Non-smokers	224 (2.351 ± 0.024)[b]	78	31.4 (1.497 ± 0.055)	29
Smokers, all amounts	233 (2.376 ± 0.026)	69	31.3 (1.496 ± 0.058)	26
1–10 Cigarettes/day	238 (2.377 ± 0.047)	21	27.1 (1.433 ± 0.086)	12
11–20 Cigarettes/day	217 (2.336 ± 0.041)	28	39.3 (1.594 ± 0.113)	7
21 + Cigarettes/day	252 (2.401 ± 0.048)	20	32.4 (1.510 ± 0.113)	7
All subjects	228 (2.359 ± 0.018)	147	31.4 (1.497 ± 0.040)	55

Values are geometric means, adjusted for age, BMI, and in (ii), stage of cycle. [a]Subjects sampled between 11 and 2 days before the end of their menstrual cycle. [b](Log 10 ± SEM). Two-sided test for difference between non-smokers and smokers, adjusting for age, BMI and stage of cycle, E2, $P = 0.66$; Pg, $P = 0.99$.

Urinary steroids

Mean excretion rates of the six urinary steroids were almost identical in premenopausal smokers and non-smokers [Table 5(i)]. Among postmenopausal women, mean excretion rates of the urinary steroids were from 2–44% higher in smokers than in non-smokers. This difference was statistically significant for 11-hydroxyandrosterone. The differences between smokers and non-smokers were a little larger before adjustment for age, because the smokers were on average 3.2 years younger than the non-smokers and the steroid excretion rates were inversely related to age (data not shown).

DISCUSSION

We used a direct assay method for E2. This has good precision, but high sex hormone-binding globulin (SHBG) concentrations cause underestimation of the E2 concentration [21]. This effect will only bias the comparisons made if the mean SHBG concentration differs substantially between the two groups. Previous analyses in this population of women have not shown any consistent relationship between SHBG and smoking [22, 23]. In the subjects for whom E2 was measured in the current study, geometric mean SHBG concentrations measured by immunoradiometric assay [24] were 71.5 and 63.7 nmol/l in smokers and non-smokers, respectively among premenopausal women and 64.3 and 61.1 nmol/l in smokers and non-smokers, respectively among postmenopausal women. These differences would cause a slight underestimation of the mean E2 of smokers relative to that of non-smokers, and thus could not invalidate our conclusion that E2 is not lower in smokers than in non-smokers.

As a check of the validity of the assay in postmenopausal women, we examined the relationship between the current results and E2 measured several years earlier in 34 of the postmenopausal samples using a radioimmunoassay kit method with ether extraction (Steranti,

Table 3. Serum E2 in postmenopausal smokers and non-smokers

Smoking category	E2 (pmol/l)	N
Non-smokers	13.8 (1.141 ± 0.057)	40
Smokers, all amounts	14.5 (1.160 ± 0.060)	36
1–10 Cigarettes/day	12.9 (1.110 ± 0.083)	19
11–20 Cigarettes/day	17.3 (1.238 ± 0.109)	11
21 + Cigarettes/day	15.0 (1.177 ± 0.147)	6
All subjects	14.1 (1.150 ± 0.041)	76

Values are geometric means (log 10 ± SEM), adjusted for age and BMI. Two-sided test for difference between non-smokers and smokers, adjusting for age and BMI, $P = 0.81$.

Table 4. Serum DHEA-S in smokers and non-smokers

Smoking category	DHEA-S (μmol/l)	N
(i) Premenopausal		
Non-smokers	2.79 (0.446 ± 0.010)	314
Smokers, all amounts	2.83 (0.451 ± 0.019)	97
1–10 Cigarettes/day	2.67 (0.427 ± 0.031)	35
11–20 Cigarettes/day	2.88 (0.459 ± 0.030)	38
21 + Cigarettes/day	2.98 (0.475 ± 0.038)	24
All subjects	2.80 (0.447 ± 0.009)	411
(ii) Postmenopausal		
Non-smokers	1.95 (0.290 ± 0.016)	213
Smokers, all amounts	1.85 (0.268 ± 0.034)	48
1–10 Cigarettes/day	1.86 (0.269 ± 0.052)	20
11–20 Cigarettes/day	2.11 (0.325 ± 0.057)	17
21 + Cigarettes/day	1.51 (0.180 ± 0.070)	11
All subjects	1.93 (0.286 ± 0.014)	261

Values are geometric means (log 10 ± SEM), adjusted for age, BMI, and in (i), parity. Two-sided test for difference between non-smokers and smokers, adjusting for covariates, premenopausal, $P = 0.82$; postmenopausal, $P = 0.56$.

Table 5. Excretion rates of urinary steroids in smokers and non-smokers

Steroid	Non-smokers Mean	N	Smokers Mean	N	P[a]
	(i) Premenopausal				
Androsterone	2.50 (0.398 ± 0.028)	85	2.66 (0.424 ± 0.058)	20	0.69
Aetiocholanolone	3.10 (0.491 ± 0.027)	85	3.43 (0.535 ± 0.056)	20	0.48
DHEA	1.33 (0.123 ± 0.040)	68	1.42 (0.153 ± 0.083)	16	0.75
11-Keto-aetiocholanolone	1.49 (0.175 ± 0.044)	55	1.39 (0.144 ± 0.087)	14	0.75
11-Hydroxyandrosterone	2.17 (0.337 ± 0.024)	83	2.14 (0.330 ± 0.052)	18	0.91
11-Hydroxyaetiocholanolone	1.10 (0.040 ± 0.037)	80	1.08 (0.035 ± 0.078)	18	0.95
	(ii) Postmenopausal				
Androsterone	1.50 (0.176 ± 0.037)	51	2.08 (0.318 ± 0.083)	10	0.13
Aetiocholanolone	2.24 (0.350 ± 0.037)	51	3.22 (0.508 ± 0.085)	10	0.10
DHEA	0.57 (−0.241 ± 0.046)	41	0.58 (−0.234 ± 0.099)	9	0.95
11-Keto-aetiocholanolone	2.00 (0.301 ± 0.028)	51	2.14 (0.329 ± 0.066)	9	0.70
11-Hydroxyandrosterone	2.02 (0.306 ± 0.026)	51	2.83 (0.452 ± 0.063)	9	0.04
11-Hydroxyaetiocholanolone	1.23 (0.090 ± 0.035)	50	1.31 (0.117 ± 0.078)	10	0.52

Values are geometric means, μmol/24 h (log 10 ± SEM), adjusted for age, BMI, and in (i), parity. [a]Two-sided test for difference between non-smokers and smokers, adjusted for age, BMI, and in (i), parity.

St Albans, England). The regression equation was: current E2 measure = 7.7 + 0.45 * extraction E2 measure, and the Pearson correlation coefficient between the results from the two methods was 0.83. These results suggest that the current assay may underestimate the concentration of E2 in postmenopausal women, but this should not cause any bias in the comparison of smokers with non-smokers.

We found no difference in endogenous serum E2 between premenopausal or postmenopausal smokers and non-smokers matched for BMI, in agreement with previous investigations [10, 13–16]. The consistency of these results makes it now very unlikely that endogenous serum E2 differs between smokers and non-smokers. Our results also suggest that cigarette smoking has no effect on serum Pg in premenopausal women.

For serum DHEA-S, however, our results do not agree with the previous reports; Friedman *et al.* [14] reported that mean serum DHEA-S was 88% higher in postmenopausal smokers than in non-smokers and Khaw *et al.* [15] reported that mean serum DHEA-S was 43% higher in postmenopausal smokers than in non-smokers, although only in the latter study was the difference statistically significant. We found that age and BMI adjusted mean DHEA-S was 5% lower in postmenopausal smokers, though our results are compatible (with 95% confidence) with a 33% increase in DHEA-S in smokers. We also found that mean serum DHEA-S was almost identical in smokers and non-smokers among premenopausal women. We have no explanation for the differences between our results and the previous investigations.

Our results did not suggest any differences in the six urinary steroids according to smoking habit among premenopausal women, but the mean excretion rates for all six steroids were at least slightly higher in smokers than non-smokers among postmenopausal women, and this difference was just statistically significant for 11-hydroxyandrosterone. Urinary androsterone and aetiocholanolone are largely metabolites of serum DHEA and DHEA-S, therefore the increases in excretion rates of these steroids in postmenopausal smokers weakly corroborate the findings of Friedman *et al.* [14] and Khaw *et al.* [15], but are not consistent with our own results for serum DHEA-S. Urinary 11-keto-aetiocholanolone, 11-hydroxyandrosterone and 11-hydroxyaetiocholanolone are largely metabolites of cortisol rather than of adrenal androgens.

The mechanism for the apparent anti-oestrogenic effect of cigarette smoking remains unclear. There is now substantial evidence that smoking does not decrease endogenous serum E2 in either premenopausal or postmenopausal women. Michnovicz *et al.* [11, 12] have shown that smoking alters the metabolism of E2 in a way that could be anti-oestrogenic, but the physiological significance of this effect is not firmly established. Friedman *et al.* [14] found higher Pg in postmenopausal smokers than non-smokers and suggested that this might provide partial protection against endometrial cancer, but serum Pg concentrations are very low in postmenopausal women and thus unlikely to be important [25], and we found no evidence for any effect of smoking on Pg in premenopausal women.

As noted above, some studies have found that smoking is associated with higher serum con-

centrations of DHEA-S [14, 15], and of androstenedione [13–16]. These hormones do not have very potent effects on the endometrium, but they may be metabolized to the more potent androgen testosterone. Investigations of the relationship of smoking with this hormone in women have produced mixed results, with various studies showing no association [15, 16], a small increase in smokers [13], and a large increase in smokers [14].

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