Research Article

Endogenous Sex Steroids and Risk of Cervical Carcinoma: Results from the EPIC Study

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Abstract

Background: Epidemiologic data and animal models suggest that, despite the predominant role of human papillomavirus infection, sex steroid hormones are also involved in the etiology of invasive cervical carcinoma (ICC).

Methods: Ninety-nine ICC cases, 121 cervical intraepithelial neoplasia grade 3 (CIN3) cases and 2 control women matched with each case for center, age, menopausal status and blood collection–related variables, were identified in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. Circulating levels of testosterone (T) and estradiol (E_2); dehydroepiandrosterone sulfate (DHEAS); progesterone (premenopausal women); and sex hormone–binding globulin (SHBG) were measured using immunoassays. Levels of free (f) T and E_2 were calculated from absolute concentrations of T, E_2, and SHBG. Odds ratios (ORs) and 95% confidence intervals (CI) were computed using regularized conditional logistic regression.

Results: Among premenopausal women, associations with ICC were observed for fT (OR for highest vs. lowest tertile = 5.16, 95% CI, 1.50–20.1). SHBG level was associated with a significant downward trend in ICC risk. T, E_2, fE_2, and DHEAS showed nonsignificant positive association with ICC. Progesterone was uninfluential. Among postmenopausal women, associations with ICC were found for T (OR = 3.14; 95% CI, 1.21–9.37), whereas E_2 and fT showed nonsignificant positive association. SHBG level was unrelated to ICC risk in postmenopausal women. No associations between any hormone and CIN3 were detected in either pre- or postmenopausal women.

Conclusions: Our findings suggest for the first time that T and possibly E_2 may be involved in the etiology of ICC.

Impact: The responsiveness of cervical tumors to hormone modulators is worth exploring. *Cancer Epidemiol Biomarkers Prev; 20(12); 2532–40. ©2011 AACR.*

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Introduction

Persistent infections with oncogenic human papillomavirus (HPV) types are the necessary cause of invasive cervical carcinoma (ICC) and its precursor lesion (cervical intraepithelial neoplasia grade 3, CIN3; ref. 1). However, sex steroid hormones are essential for the differentiation and maturation of the human cervix and its vulnerability to HPV infection (2). The cervical epithelium remains relatively quiescent until the surge of estrogens and progesterone at puberty when the basal cells of the originally thin columnar epithelium transform into squamous cells in a physiologic process termed squamous metaplasia. This transformation area, called the squamous–columnar junction, is the preferential location of ICC. Eversion of the squamous–columnar lesion occurs during pregnancy and oral contraceptive use, facilitating direct exposure to and growth of HPV. Epidemiologic studies have shown that multiparity (3), recent oral contraceptive (OC) use (4), and, for not completely clear reasons, smoking (5) increase the risk of progression from cervical HPV infection to ICC. In addition, ICC incidence rates in unscreened populations stop increasing at around age 45 years (6), when sex steroid hormone levels start declining and the squamous–columnar junction withdrawing into the endocervix (7). A similar slowing in the increase of incidence rates in the perimenopausal period has also been observed for cancer of the breast (in unscreened populations), endometrium, and ovary whose development is clearly influenced by sex steroids (8). Although new cervical HPV infections continue to occur after middle age, the risk of CIN3 is decreased compared with younger women (9). These epidemiologic findings implicate sex steroid hormone levels in the development of ICC and CIN3. In vivo and in vitro laboratory studies also suggest that sex steroid hormones, especially estrogens, are required for the onset of atypical metaplasia in the squamous–columnar junction and ICC (10, 11).

To date, the association between serum or plasma levels of sex steroid hormones and the risk of ICC or CIN2 or 3 has been only evaluated in 3 case–control studies yielding inconsistent results (12, 13, 14). The aim of the present study was to assess the relationship between prediagnostic levels of sex steroid hormones and the risk of ICC and CIN3 in women enrolled in a prospective study, the European Prospective Investigation into Cancer and Nutrition (EPIC).

Materials and Methods

Study population and blood sample collection

The EPIC cohort included about 370,000 women, mainly aged 35 to 69 years, recruited between 1992 and 1998 in 23 centers in 10 European countries (Denmark, France, Germany, Greece, Italy, Norway, Spain, Sweden, the Netherlands, and the United Kingdom). Details on the cohort population and data collection procedures have been described elsewhere (15). In brief, extensive standardized questionnaire data were collected on habitual diet and lifestyle variables, including questions about reproductive and menstrual histories, current and past use of OCs and menopausal replacement therapy. Height, weight, and waist and hip circumferences were measured for most women while they were visiting the enrollment centers. However, anthropometric variables were self-reported in Norway and parts of the French and United Kingdom cohorts.

Blood samples were collected from about 65% of women in EPIC. In France, Germany, Greece, Italy, Norway, Spain, the Netherlands and the United Kingdom, blood was collected according to a standardized protocol: 30 mL of blood was withdrawn from each participant (16, 17). After centrifugation, samples were aliquotted into 0.5 mL straws of serum, plasma, red blood cells and buffy coat, and stored in liquid nitrogen containers ($-196°C$). A mirror half of these aliquots was stored locally in each of the centers whereas the other half was sent to the central bio-bank at the International Agency for Research on Cancer (IARC). In Denmark and Sweden, samples were stored only locally. In Denmark, samples were taken and aliquotted into 1 mL tubes, which were then stored in liquid nitrogen vapors ($-150°C$). In Sweden, samples were taken and stored into 1 mL aliquots in freezers at $-80°C$. The stability of steroid hormones and SHBG measurements on samples stored over long time periods at $-80°C$ or lower temperature has been documented (18).

Follow-up for cancer incidence and vital status

Follow-up for vital status was collected through record linkage with regional/national cancer registries in all countries with the exception of France, Germany, and Greece, where these data were collected through an active follow-up. Incident ICC cases were identified through record linkage with regional cancer registries in Denmark, Norway, Spain, Sweden, the Netherlands, the United Kingdom, and in most of the Italian centers (complete to December 2005). In France, Germany, Greece, and Naples (Italy), follow-up (complete to December 2006) was based on a combination of methods, including health insurance records, cancer and pathology registries, and active follow-up through study subjects and their next-of-kin. For each EPIC study center, closure dates of the study period were defined as the latest dates of complete follow-up for both cancer incidence and vital status. Contrary to ICC cases, CIN3 cases were not systematically collected and consistently reported by all cancer registries and centers in EPIC and are, therefore, substantially fewer than expected (9).

Determination of menopausal status and phase of the menstrual cycle at blood donation

Women were considered as postmenopausal if they had reported at enrollment: (i) no menses over the past 12 months; (ii) bilateral ovariectomy; or (iii) age older than 55 years. Women were considered as premenopausal if they had reported: (i) regular menses over the past 12 months;

or (ii) age less than 42 years. Women between 42 and 55 years who reported hysterectomy without ovariectomy or for whom information on menopausal status was not available were classified as unknown menopausal status and excluded from the present study (16, 17).

In premenopausal women, the phase of the menstrual cycle at blood donation was determined as previously reported (16, 17). In brief, 2 different methods were used: "forward dating" counted forward from the woman's reported start date of her last menses (information collected from the baseline questionnaires), and "backward dating" counted backward from the start date of her next menses after blood donation (information obtained from prepaid postcard that the woman sent back after her blood collection). When both types of information were available (Italy, Spain, and Oxford, United Kingdom), backward dating was used as the length of the second half of the cycle is generally more constant than that of the first half. The phase of a woman's menstrual cycle was defined as: follicular (days 0–11 of the cycle), periovulatory (days 12–16), and luteal (from day 17 to the subsequent menstrual period).

Nested case–control design and participation

Cases were defined as women who had donated blood and developed ICC or CIN3 after enrollment into the EPIC study and before the end of the follow-up. Ineligibility criteria for the present study included: (i) use of OCs or menopausal replacement therapy at the time of blood donation (247 cases); (ii) history of cancer other than nonmelanoma skin cancer (11 cases); (iii) unknown menopausal status, or, among premenopausal women, unknown menstrual phase (71 cases). Twenty cervical neoplasias of uncertain malignant potential were also excluded.

Ninety-nine ICC cases and 121 CIN3 cases were included in the present analyses (Table 1). The distribution of ICC/CIN3 by country was as follows: 9/0 in Denmark; 0/2 in France; 13/4 in Greece; 13/22 in Germany; 11/10 in Italy; 11/3 in the Netherlands; 5/7 in Norway; 18/17 in Spain; 10/24 in Sweden; and 9/32 in the United Kingdom.

For each case of ICC or CIN3, 2 matched control women were chosen at random among appropriate risk sets consisting of all cancer-free cohort women who had reported no history of hysterectomy or bilateral ovariectomy at enrollment. An incidence density sampling procedure was used, that is, controls could be sampled more than once and may include women who became cases at a later time. Matching criteria were: center of enrollment; age (±6 months); time of the day and fasting status (<3 hours; 3–6 hours, >6 hours) at blood collection; date of entry; duration of follow-up time; menopausal status (pre-, postmenopausal) and, among premenopausal women, phase of the menstrual cycle (follicular, periovulatory, or luteal). All participants gave their consent for a broad range of use of their blood samples and the study was approved by the Institutional Review Board of IARC and all participating centers.

Table 1. Comparison of cases of invasive cervical cancer and CIN3 and their matched controls by selected characteristics and menopausal status

| | Invasive cervical cancer | | | | CIN3 | | | |
| | Premenopausal women | | Postmenopausal women | | Premenopausal women | | Postmenopausal women | |
	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
Number	46	90	53	100	71	140	50	100
Age at blood collection[a]	43.1 (35.3–51.0)	43.1 (35.8–51.5)	58.0 (49.9–65.4)	57.9 (49.7–65.3)	39.1 (28.3–48.2)	39.1 (28.7–49.0)	57.0 (48.1–69.9)	57.0 (48.2–69.9)
Age at diagnosis[a]	46.7 (38.7–57.4)	—	62.2 (51.9–73.1)	—	42.4 (32.5–51.5)	—	60.2 (51.7–70.4)	—
Years between blood collection and diagnosis[a]	3.6 (0.31–8.0)	—	4.2 (0.42–10.0)	—	3.4 (0.36–8.0)	—	3.2 (0.23–6.6)	—
Completed secondary school, %	47	46	28	26	51	46	33	31
BMI[a]	25.5 (20.7–32.4)	25.8 (19.3–33.3)	26.8 (19.9–36.5)	27.5 (19.7–38.5)	23.0 (19.6–28.7)	24.6 (19.5–31.3)	24.8 (18.3–31.7)	25.5 (19.2–32.1)
Ever smokers, %	61	48	62	38	67	41	64	51

[a]Mean and 5th to 95th percentiles.

Laboratory assays

Sera were used for laboratory assays except in Norway (citrated plasma) and Sweden (EDTA and heparin plasma). Sex hormones can be accurately measured in serum as well as in plasma samples (19).

Total testosterone (T), dehydroepiandrosterone sulfate (DHEAS), and progesterone (premenopausal women only) levels were measured using a radioimmunoassay from Beckmann Coulter. Total estradiol (E_2) levels were measured by a radioimmunoassay from DiaSorin. Concentrations of sex hormone binding globulin (SHBG) were measured by a solid phase "sandwich" immunoradiometric assay (Cis-Bio International). Validity of the measurements of T and E_2 was previously reported (20). Free estradiol (fE_2) and free T (fT), that is, the fraction of these hormones that was not bound to either albumin or SHBG, were calculated from the total concentrations of T and E_2 by using mass action equations, assuming a constant concentration of albumin of 43 g/L. Validity of these equations in pre- and postmenopausal women was previously reported (21). Because of insufficient sample volume, DHEAS, E_2, and progesterone could not be measured in samples from Denmark.

All assays were carried out at IARC, without knowing case–control status. Samples belonging to matched case–control sets were always tested in the same batch. On the basis of results obtained for quality-control samples (containing low, medium, and high hormone concentrations, all measured in each of the analytical batches), the intrabatch coefficients of variation were estimated. Intrabatch coefficients of variation were 8.5% for T at a concentration of 2.7 nmol/L; 8.8% for DHEAS at 4.0 μmol/L; 2.3% for E_2 at 220 pmol/L; 4.4% for progesterone at 30.4 nmol/L; and 7.2% for SHBG at 40.0 nmol/L.

Statistical analyses

All statistical analyses were carried out separately for ICC and CIN3 and by menopausal status. Partial Spearman's correlation coefficients between hormones and body mass index (BMI, kg/m^2) were computed among control women only and adjusted for age and batch. The association between hormones, BMI and current smoking (yes or no) were estimated using point-biserial correlation coefficients, with the same adjustments.

Tertile categories were based on the distribution of hormone and SHBG levels among pre- or postmenopausal control women. In premenopausal women, E_2, and progesterone levels showed important variations across the menstrual cycle. To control for this variation, locally weighted polynomial regression models were fitted to the log hormone levels in control women, with cycle day as the predictor variable. Tertile categories were based on the residuals from these models (i.e., the dimensionless relative deviations of sex steroid hormone levels from the predicted average on a given cycle day among controls; ref. 22). Because of the extremely low levels of progesterone in the follicular phase and the start of the periovulatory phase, the analysis of progesterone among premenopausal women was restricted to women who gave blood in the luteal phase.

Odds ratio (OR) estimates and corresponding 95% confidence intervals (CI) were computed using Bayesian conditional logistic regression, with the default prior of Gelman and colleagues (23). This gives stable, regularized OR estimates in small samples. All logistic regression models were conditioned on the matching variables, that is, center, age, time of day at blood collection, fasting status, duration of follow-up, menopausal status, and in premenopausal women, phase of menstrual cycle. Potential confounders (i.e., BMI as a continuous variable; and smoking, never/former/current) were adjusted for. Additional adjustments for number of full-term pregnancies, age at menarche and first full-term pregnancy (in continuous), and past use of OCs were made but were not retained in the final model as they did not substantially alter the observed associations.

Trend tests were conducted by assigning consecutive scores (from 1 to 3) to the tertile categories. Tests for heterogeneity in the trend between hormone and SHBG levels and ICC or CIN3 risk by time interval between blood donation and disease diagnosis were done by including an interaction term in the regression model. All statistical tests were 2-sided.

Results

Basic demographic and lifestyle characteristics of ICC and CIN3 cases and corresponding controls are shown in Table 1, separately by menopausal status. Mean age at blood collection varied between 39.1 years in premenopausal CIN3 cases to 58.0 for postmenopausal ICC cases. The mean interval between blood collection and tumor diagnosis ranged from 3.2 years for postmenopausal CIN3 cases to 4.2 years for postmenopausal ICC cases. ICC cases were significantly more often current or former smokers (Table 1) and past OC users (data not shown) than their matched control women. CIN3 cases were significantly leaner and more often current or former smokers than their matched control women.

Table 2 shows Spearman's correlation coefficients between levels of sex steroid hormones and SHBG and BMI among control women, separately by menopausal status and adjusted for age and batch. In both pre- and postmenopausal women, the strongest positive correlations were found between T and DHEAS (0.61 and 0.67, respectively) and the strongest negative correlations between SHBG and BMI (−0.38 and −0.47). In premenopausal women, SHBG was positively correlated with E_2 (0.28) and negatively correlated with DHEAS (−0.36). In postmenopausal women, E_2 was positively correlated with T (0.42), DHEAS (0.21), and BMI (0.28) but inversely correlated with SHBG (−0.22). Smoking was positively correlated with SHBG (0.23) and negatively correlated with BMI (−0.24). Progesterone level in premenopausal women in the luteal phase was positively associated with

Table 2. Partial Spearman's correlation coefficients between levels of sex steroid hormones and SHBG and BMI by menopausal status[a] among control women, adjusted for age and batch

	Testosterone	DHEAS	SHBG	Estradiol[b]	BMI	Smoking[c]	Progesterone[b]
Testosterone		*0.61*	*−0.16*	*0.03*	*0.18*	*−0.04*	*0.15*
DHEAS	**0.67**		*−0.36*	*−0.10*	*0.16*	*0.05*	*0.10*
SHBG	**0.03**	**−0.11**		*0.28*	*−0.38*	*0.00*	*0.27*
Estradiol	**0.42**	**0.21**	**−0.22**		*−0.14*	*−0.04*	*0.29*
BMI	**0.12**	**0.02**	**−0.47**	**0.28**		*0.06*	*0.03*
Smoking	**0.01**	**0.10**	**0.23**	**−0.07**	**−0.24**		*−0.01*

[a]Premenopausal women in italics and postmenopausal women bold faced.
[b]Calculated on residuals in premenopausal women and restricted to luteal phase for progesterone.
[c]Point-biserial correlation for current smoking (yes/no).

SHBG (0.27) and E_2 (0.29). All other correlations were 0.20 or lower (Table 2).

Table 3 shows ORs for ICC by level of total and free sex steroid hormones and SHBG, separately by menopausal status. Among premenopausal women, fT level was significantly associated with ICC risk (OR for the highest vs. lowest tertile = 5.16, 95% CI, 1.50–20.1). A statistically significant inverse trend was observed for SHBG level,

Table 3. ORs and corresponding 95% CIs for invasive cervical cancer by tertile of circulating sex steroid hormone and SHGB levels and menopausal status

		Premenopausal women			Postmenopausal women		
		Case:control[a]	OR (95% CI)[b]	P_{trend}	Case:control[a]	OR (95% CI)[b]	P_{trend}
Testosterone, nmol/L	<1.11	19:39	ref.		11:32	ref.	
	1.11–1.67	13:29	0.99 (0.40–2.37)		16:33	1.59 (0.60–4.44)	
	>1.67	14:20	1.57 (0.56–4.48)	0.43	25:33	3.14 (1.21–9.37)	0.02
Free testosterone, pmol/L	<11.16	11:40	ref.		15:31	ref.	
	11.16–19.99	19:29	2.66 (1.08–7.19)		17:36	1.52 (0.58–4.04)	
	>19.99	15:17	5.16 (1.50–20.1)	0.005	20:31	2.08 (0.81–5.64)	0.12
DHEAS, µmol/L	<3.27	14:34	ref.		14:34	ref.	
	3.27–4.93	11:25	1.12 (0.41–3.10)		15:27	1.72 (0.64–4.73)	
	>4.93	15:19	2.37 (0.84–7.36)	0.10	12:21	1.72 (0.57–5.25)	0.29
Estradiol, pmol/L	<0.78[c]	11:30	ref.		11:26	ref.	
	0.78–1.23	17:23	3.23 (1.06–11.1)		11:31	1.23 (0.41–3.81)	
	>1.23	17:37	1.50 (0.60–4.09)	0.48	20:27	2.91 (0.90–10.7)	0.08
Free estradiol, pmol/L	<0.78[c]	11:30	ref.		13:27	ref.	
	0.78–1.24	15:23	2.27 (0.78–7.09)		16:27	1.26 (0.49–3.30)	
	>1.24	18:36	1.71 (0.67–4.54)	0.29	13:30	1.26 (0.40–4.11)	0.64
Progesterone, nmol/L	<0.76[c]	6:13	ref.		—	—	
	0.76–1.30	5:9	3.50 (0.57–34.1)		—	—	
	>1.30	4:12	0.46 (0.04–3.58)	0.51	—	—	
SHBG, nmol/L	<59.26	21:23	ref.		12:33	ref.	
	59.26–88.62	12:34	0.30 (0.09–0.86)		19:33	1.29 (0.53–3.20)	
	>88.62	12:31	0.35 (0.11–1.04)	0.04	22:34	1.22 (0.46–3.36)	0.68

[a]Some figures do not add up to the total because of missing variables.
[b]From conditional logistic regression analysis, conditioned on center, age, date, and time of day at blood collection, fasting status and, in premenopausal women, on phase of menstrual cycle (follicular, ovulatory or luteal); additional adjustments for BMI and smoking status.
[c]Residuals from locally weighted regression models were used in premenopausal women; restricted to women in the luteal phase for progesterone. Residuals are not expressed in pmol/L as they are dimensionless.

Table 4. ORs and corresponding 95% CIs for CIN3 by tertile of circulating sex steroid hormone and SHGB level and menopausal status

		Premenopausal women				Postmenopausal women		
		Case:control[a]	OR (95% CI)[b]	P_{trend}		Case:control[a]	OR (95% CI)[b]	P_{trend}
Testosterone, nmol/L	<1.11	17:37	ref.		<0.80	13:33	ref.	
	1.11–1.67	19:46	0.87 (0.36–2.09)		0.80–1.21	18:31	1.51 (0.59–4.09)	
	>1.67	35:55	1.41 (0.55–3.67)	0.44	>1.21	17:31	1.82 (0.64–5.43)	0.98
Free testosterone, pmol/L	<11.16	18:33	ref.		<9.35	18:34	ref.	
	11.16–19.99	25:43	0.91 (0.40–2.02)		9.35–15.73	15:28	1.12 (0.42–2.99)	
	>19.99	25:55	1.02 (0.44–2.48)	0.96	>15.73	15:33	1.16 (0.41–3.48)	0.87
DHEAS, µmol/L	<3.27	16:34	ref.		<2.11	18:27	ref.	
	3.27–4.93	19:42	0.88 (0.38–2.07)		2.11–3.28	10:34	0.39 (0.13–1.11)	
	>4.93	28:48	1.44 (0.60–3.65)	0.38	>3.28	22:39	0.71 (0.25–1.96)	0.77
Estradiol, pmol/L	<0.78[c]	19:47	ref.		<30.79	19:34	ref.	
	0.78–1.23	25:53	1.00 (0.43–2.41)		30.79–39.32	8:28	0.54 (0.18–1.49)	
	>1.23	26:40	1.60 (0.64–4.18)	0.31	>39.32	20:32	1.40 (0.55–3.63)	0.56
Free estradiol, pmol/L	<0.78[c]	21:46	ref.		<0.66	21:33	ref.	
	0.78–1.24	27:52	1.10 (0.52–2.36)		0.66–0.96	9:32	0.51 (0.17–1.37)	
	>1.24	20:39	0.94 (0.40–2.25)	0.93	>0.96	17:29	1.12 (0.41–3.03)	0.68
Progesterone, nmol/L	<0.76[c]	4:11	ref.		—	—	—	
	0.76–1.30	9:15	1.30 (0.32–6.01)		—	—	—	
	>1.30	7:12	0.99 (0.13–7.78)	0.93	—	—	—	
SHBG, nmol/L	<59.26	19:51	ref.		<41.84	11:34	ref.	
	59.26–88.62	25:39	1.43 (0.59–3.50)		41.84–69.80	18:34	1.16 (0.45–3.10)	
	>88.62	24:42	1.05 (0.42–2.64)	0.97	>69.80	21:32	1.38 (0.52–3.89)	0.98

[a]Some figures do not add up to the total because of missing variables.
[b]From conditional logistic regression analysis, conditioned on center, age, date, and time of day at blood collection, fasting status and, in premenopausal women, on phase of menstrual cycle (follicular, ovulatory, or luteal); additional adjustments for BMI and smoking status.
[c]Residuals from locally weighted regression models were used in premenopausal women; restricted to women in the luteal phase for progesterone. Residuals are not expressed in pmol/L as they are dimensionless.

with both medium (OR = 0.30, 95% CI, 0.09–0.86) and high (OR = 0.35, 95% CI, 0.11–1.04) levels associated with lower ICC risk. Positive associations were also observed for E_2, fE_2, and DHEAS levels but there was no statistically significant trend. No significant relationship was found between ICC risk and progesterone level in premenopausal women who gave blood in the luteal phase. Among postmenopausal women, positive associations with ICC were found with T (OR = 3.14; 95% CI, 1.21–9.37 for highest vs. lowest tertile; Table 3). Positive associations were also observed for fT and E_2 but there was no statistically significant trend. SHBG concentration was unrelated to ICC risk in postmenopausal women.

No substantial heterogeneity in the association between hormone or SHBG levels and ICC risk was observed by

time interval between blood donation and ICC diagnosis (<3 vs. ≥3 years; results not shown).

Table 4 shows the relationship between levels of sex steroid hormones and SHBG and CIN3 risk, separately by menopausal status. No statistically significant associations were observed in either pre- or postmenopausal women.

Discussion

In the present prospective study, the first to date on the relationship between prediagnostic circulating levels of sex steroid hormones and ICC risk, we found a significant positive association with T or fT level in both pre- and postmenopausal women. On account of the different

behavior of SHBG level (inversely associated with ICC risk in pre- but not in postmenopausal women), fT level seemed to be a somewhat stronger predictor of ICC risk than total T in premenopausal women but not in post-menopausal women. Positive associations between ICC risk and DHEAS, E_2, and fE_2 level were also observed but there was no significant trend. We assessed progesterone levels only in premenopausal women who had their blood collected in the luteal phase, and found progesterone unrelated to ICC risk although the corresponding OR had a very broad CI. No associations were found between sex steroid hormone or SHBG levels and CIN3 risk in either pre- or postmenopausal women although, again, the CI of the corresponding ORs do not allow us to rule out associations in either direction.

The relationship between circulating sex steroid hormones and the risk of cancerous or precancerous cervical lesions has been previously investigated in 3 case–control studies (12, 13, 14). Zheng included 51 postmenopausal women with ICC and 52 cancer-free postmenopausal women (12). ICC cases had statistically significant higher serum levels of estrone and estriol than control women whereas no differences were reported for E_2. Wang and colleagues compared 141 ICC cases with 2 control groups (137 women with uterine myoma and 129 healthy women; ref. 13). Serum E_2 levels were significantly higher among ICC cases than in either control group whereas progesterone level did not differ. Shields and colleagues compared cross-sectionally plasma E_2, estrone, SHBG, DHEAS, and progesterone levels in 110 women with CIN2, 3, or ICC to 440 women with normal cytology or CIN1 selected within the Guanacaste cohort study, Costa Rica (14). Half of the control women were chosen among HPV-positive women. No significant associations were found between the risk of CIN2 or worse lesions and levels of sex steroid hormones or SHBG.

The partial disagreement between our findings and those of Shields and colleagues (14) may be explained by the fact that cases in their study mainly included CIN2 and CIN3 (85 of 110), whereas the association we found with T and fT and, possibly, E_2 levels were restricted to ICC. Lack of association for CIN2 and CIN3 may suggest that sex steroid hormones act during the late stages of cervical carcinogenesis. However, the interpretation of our present CIN3-related findings is hampered by the incomplete follow-up for CIN3 in the EPIC study and our inability to adjust for screening history. A diagnosis of CIN3 requires a woman to have been screened whereas the screening history of control women matched with CIN3 cases was unknown. No organized screening programs existed and no information on individual screening history was collected in a fraction of the EPIC centers.

The conclusion on whether androgens, estrogens or both may be involved in cervical carcinogenesis is difficult because the 2 hormones are biochemically closely related. Long-term T level can be inferred from one-time measurement much more reliably than E_2 level (16, 17, 24). Contrary to T (25), E_2 level in premenopausal women

varies enormously throughout the menstrual cycle, making a single measurement insufficient to classify long-term exposure accurately (7). In postmenopausal women, the comparison of the effect of T and E_2 is complicated by the fact that T is the main source of E_2 after ovarian estrogen production has stopped and T levels are much higher than E_2 levels (24).

A negative association between ICC risk and SHBG level in premenopausal but not in postmenopausal women had never been reported before. Conversely, negative associations with SHBG levels have been consistently reported with postmenopausal breast cancer risk (24). Low SHBG level implies higher bioavailability of estrogens and may indicate higher levels of insulin or insulin-like growth factor-1 (26). A direct inhibitory role of SHBG on breast cancer cell proliferation has also been proposed (27).

In transgenic mice that express HPV16 E6 and E7 oncogenes, estrogen is required for the onset of ICC (10, 11). Estrogens have a profound influence on the cervical squamous–columnar junction from which ICC arises in mice and in humans (28), and the estrogen receptor α has been shown to be necessary for cervical cancer to arise in mice (11). Drugs that interfere with the function of estrogen receptor α (e.g., raloxifene) have been shown to prevent the onset or induce the regression of ICC in transgenic mice (11) but corresponding data in humans are scant (29).

Epidemiologic data on the association of full-term pregnancies and recent OC use with ICC risk, and the cessation of the increase in ICC incidence rates after menopause also suggest an involvement of high estrogen levels but do not allow to rule out an influence of progesterone or androgens (30). The 2 best correlates of high unopposed estrogen levels in postmenopausal women (i.e., overweight/obesity (31) and the use of estrogen-only menopausal replacement therapy (32, 33) have been little studied in respect to ICC, and their evaluation is severely confounded by intense screening in menopausal replacement therapy users (34).

The present study has strengths and weaknesses. It derives from a large cohort study but it includes relatively few ICC and CIN3 cases due, respectively, to the low incidence of ICC among the European women who voluntarily joined the EPIC study, and severely incomplete follow-up for CIN3. In addition, a substantial number of women had to be excluded, notably because their menopausal status or menstrual cycle phase were ill-defined or they were taking OCs or menopausal replacement therapy at the time of blood collection. Reliance on a single serum measurement of hormone has certainly led to an underestimate of any true association with the disease, more notably for E_2. Although EPIC participants may not be representative of the corresponding general female populations, the association of certain characteristics (i.e., smoking status and past OC use) with ICC in the present report from the EPIC study is consistent with previous literature findings on the

disease (4, 5). Lack of adjustment for HPV infection is not of great concern as all ICC cases can be assumed to harbor a long-duration infection (1). Conversely, HPV positivity at one point in time among control women is difficult to interpret as it may be either a recent or long-duration infection (4). All hormonal analyses were adjusted for BMI and smoking status as these factors can modify levels of sex steroid hormones and SHBG. Indeed, hormonal modifications may be one of the mechanisms through which BMI and smoking affect ICC risk. Finally, null findings for CIN3 should be interpreted very cautiously.

Notwithstanding these limitations, our present findings are a strong reminder that, despite the unique role of HPV infection in cervical cancer (1), the cervix epithelium is a hormone-dependent epithelium (35). Although the hormonal balance responsible for risk increase varies by cancer site, sex steroid hormones are likely to be implicated in ICC etiology as it has been more convincingly shown for cancer of the breast (24, 36), endometrium, and ovary (8).

Biobanks from existing prospective studies should be used to confirm our present limited data on the influence of sex hormone levels on cancerous and precancerous cervical lesions and new mechanistic studies on the effect of estrogens, progesterone, and androgens on HPV-infected cervical cells should be started (30). ICC can be effectively prevented through screening and HPV vaccination programs, and treated with surgery and radiotherapy (37). Elucidating the dependence of cervical lesions on sex steroid hormones (29, 30) may allow, however, the discovery of hormone modulators that could further improve ICC control by, for example, decreasing recurrences of cancerous and precancerous lesions.

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