Endogenous Androgens and Risk of Epithelial Ovarian Cancer: Results from the European Prospective Investigation into Cancer and Nutrition (EPIC)

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Abstract

Few epidemiologic studies have examined the hypothesis that circulating androgens are involved in the development of ovarian cancer. We investigated the association between prediagnostic serum levels of androgens and sex hormone–binding globulin (SHBG) and ovarian cancer risk in a case-control study nested within the European Prospective Investigation into Cancer and Nutrition cohort. One hundred and ninety-two ovarian cancer cases and 346 matched controls not using exogenous hormones at baseline blood donation were eligible for the study. Serum levels of testosterone, androstenedione, dehydroepiandrosterone sulfate, and SHBG were measured by direct immunoassays. Free testosterone (fT) was calculated according to mass action laws. Multivariate conditional logistic regression was used to estimate odds ratios adjusted for possible confounders. Overall, there was no association between serum concentrations of androgens or SHBG and ovarian cancer risk. In postmenopausal women, fT concentrations were inversely related to risk [highest versus lowest tertile odds ratio 0.45 (0.24-0.86); P trend = 0.01]. Among women diagnosed before the age of 55 years, there was a negative association with SHBG and a positive association with fT and ovarian cancer risk, although these associations were not statistically significant. The present study suggests that circulating androgens and SHBG levels are not strongly associated with ovarian cancer risk, although levels of fT may be associated with an increased risk among women diagnosed at relatively young age. The heterogeneity of results on the associations of fT with ovarian cancer risk in postmenopausal women deserves further investigation. (Cancer Epidemiol Biomarkers Prev 2007;16(1):23–9)
Introduction

Ovarian cancer is the sixth most common cancer and the fifth most common cause of cancer death in women in developed countries (1). Established epidemiologic risk factors for ovarian cancer are infertility, low parity, and family history, although use of oral contraceptives, hysterectomy, breast-feeding, or tubal ligation decrease the risk (2, 3). Several hypotheses on the etiology of ovarian cancer have been proposed, including incessant ovulation (4), inflammation (5), and excessive stimulation by gonadotropins (6). The gonadotropin hypothesis suggests that an excessive production of gonadotropins (such as luteinizing hormone) can stimulate proliferation and malignant transformations of ovarian epithelium either directly (7) or indirectly through increased ovarian production of androgens (2). Suggestions for an involvement of androgens in ovarian cancer come from in vitro studies and animal experiments (8-11), in which androgens were found to stimulate ovarian epithelial cell proliferations. This hypothesis is also corroborated by the association of polycystic ovary syndrome (a syndrome associated with increased ovarian androgen secretion) with an increased risk of ovarian cancer (3) and the protective effect of oral contraceptives, which suppress androgen synthesis.

Only two small prospective epidemiologic studies have been published thus far on the association between circulating androgens and ovarian cancer risk. The first was a prospective cohort study of 31 cases (13 premenopausal and 18 postmenopausal) that showed an increasing risk of ovarian cancer with increasing levels of androstenedione (Δ4) and dehydroepiandrosterone (12). No significant association between ovarian cancer and prediagnostic androgens was observed in the second study, which included 44 premenopausal and 88 postmenopausal cases (13). However, in both studies, the numbers of cases have been quite small, and relative risk estimates were imprecise.

We conducted a case-control study nested within the European Prospective Investigation into Cancer and Nutrition (EPIC; ref. 14) to examine further the relationship between prediagnostic levels of testosterone (T), dehydroepiandrosterone sulfate (DHEAS), Δ4, and sex hormone–binding globulin (SHBG) and ovarian cancer risk. With 192 cases and 346 controls, this is the largest prospective study published to date on this topic.

Materials and Methods

Study Population. The EPIC cohort consists of about 360,000 women and 150,000 men, mostly ages 35 to 69, recruited between 1992 and 1998 in 23 research centers in 10 Western European countries (15). Extensive standardized questionnaire data on dietary and nondietary variables and anthropometric measurements were collected from all subjects, whereas blood samples were collected from 250,000 women and 140,000 men. Questionnaires included detailed questions about habitual diet and physical activity, history of previous illness and surgical operations and, for women, menstrual and reproductive history, current and past use of oral contraceptives, and postmenopausal hormone replacement therapy. Anthropometric indexes (as height, weight, and waist and hip circumferences) were also measured according to standardized protocols, except for the Oxford cohort, where height, weight, and body circumferences were mostly self-reported.

The present study includes ovarian cancer cases (occurred after blood donation) and matched control subjects from 19 recruitment centers in eight of the participating countries: Denmark, France, Germany, Greece, Italy, the Netherlands, Spain, and the United Kingdom. Norway was not included in the present study because blood samples have been collected only recently on a subsample of cohort participants and only very few cases of epithelial ovarian cancer have been accumulated thus far; Sweden was not included because a parallel study on ovarian cancer and endogenous sex steroid concentrations has already been undertaken within the Swedish cohort (13).

Collection and Storage of Blood Samples. In France, the Netherlands, the United Kingdom, Germany, Spain, Italy, and Greece, blood samples were collected according to a standardized protocol (14). From each subject, ~30 mL of nonfasting blood were drawn, and serum, plasma, red cells, and buffy coat were aliquoted in plastic straws of 0.5 mL each, which were heat sealed and stored under liquid nitrogen (−196°C). In Denmark, blood fractions were aliquoted into 1 mL tubes and stored in the vapor phase in liquid nitrogen containers (−150°C).

Follow-up for Cancer Incidence and Vital Status. In Denmark, the Netherlands, the United Kingdom, Spain, and in most of the Italian centers, incident cancer cases were identified through record linkage with regional cancer registries. In Germany, France, Greece, and Naples, follow-up 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. Data on vital status in most EPIC study centers were collected from mortality registries at the regional or national level in combination with data collected by active follow-up (Greece). 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 (dates varied between centers, between June 1999 and December 2003).

Determination of Menopausal Status at Blood Donation and Definition of Phase of Menstrual Cycle (Premenopausal Women). Women were considered as premenopausal when they reported they were menstruating regularly over the past 12 months. If this information was missing, women were considered to be premenopausal if they were <42 years of age at recruitment (among EPIC women who had complete data, 99.5% of those below age 42 years were premenopausal). Women were considered postmenopausal when they reported not having had any menses over the past 12 months or when they reported bilateral ovariectomy or when they were >55 years of age. Women who were between 42 and 55 years of age and who had missing or incomplete questionnaire data or who reported previous hysterectomy (without ovariectomy) were classified as unknown and excluded from the study because of the possible presence of women with polycystic ovaries.

A detailed description of the determination of the phase of menstrual cycle in premenopausal women has been reported previously (16). In brief, two different dating methods were used: ‘forward’ dating counted forward from the woman’s reported date of the start of her last menses and/or ‘backward’ dating counted backward from the date of the start of her next menstruation after blood donation, which the woman reported on a prepaid postcard that she sent back to the recruitment center after her visit to donate a blood sample. When both dating methods were available, the backward dating method was used to determine the menstrual cycle phase. In France, the Netherlands, Greece, and Germany, data were available only for forward dating of the phase of menstrual cycle at blood donation, whereas for the vast majority of cohort participants in Italy, Spain, and Oxford data were also available for backward dating. In Denmark and in Cambridge, no information on phase of menstrual cycle was collected.

Selection of Case and Control Subjects. Case subjects were selected among women who developed epithelial ovarian cancer after their recruitment into the EPIC study and before the end of the study period (defined for each study center by
the latest end date of follow-up). Cases were coded according to the 10th Revision of the International Statistical Classification of Diseases, Injuries, and Cause of Death. Women who used any hormone replacement therapy at the time of blood donation or any exogenous hormones for contraception or medical purposes and who had a previous diagnosis of cancer (except non-melanoma skin cancer) were excluded from the study. Women whose ovarian cancers were not primary cancers or who had a diagnosis of non-epithelial tumors or who reported (unilateral or bilateral) ovariectomy or hysterectomy were also excluded.

A total of 192 incident cases of epithelial ovarian cancer was identified (56 among women who were premenopausal at blood donation and 136 among women who were postmenopausal at blood donation). Among these, 92 (48%) were classified as serous, 14 (7%) as mucinous, 25 (13%) as endometrioid, 7 (4%) as clear cell, and 54 (28%) as other (11 as missing, 40 as unspecified, and 3 as undifferentiated). The 192 incident cases included 38 cases in Denmark, 43 in Italy, 31 in Spain, 28 in the United Kingdom, 26 in the Netherlands, 12 in Greece, 7 in France, and 7 in Germany.

For each case subject with ovarian cancer, two control subjects were chosen at random among appropriate risk sets consisting of all cohort members alive and free of cancer (except non-melanoma skin cancer) at the time of diagnosis of the index case. An incidence density sampling protocol for control selection was used such that controls could include subjects who became a case later in time, although each control subject could also be sampled more than once. Matching characteristics for cases and controls were the study center where the subjects were enrolled in the cohort, menopausal status (premenopausal, postmenopausal), age (26 months) at enrollment, time of the day at blood collection (±1 h), fasting status (<3 h, 3-6 h, >6 h), and, for premenopausal women, phase of menstrual cycle (‘early follicular’ (days 0-7 of the cycle), ‘late follicular’ (days 8-11), ‘periiovulatory’ (days 12-16), ‘midluteal’ (days 20-24), and ‘other luteal’ (days 17-19 or days 25-40; ref. 16]). Cases with missing information on phase of menstrual cycle were matched with controls with missing information. For the present analysis, 346 control subjects were matched to 192 ovarian cancer cases. All participants had given their consent for future analyses of their blood samples, and the Internal Review Board of IARC had approved the hormone analyses.

**Laboratory Assays.** All hormone assays were done at the IARC (Nutrition and Hormones Group). Serum samples from cases and matched controls were always analyzed within the same analytic batch. \( \Delta_4 \) was measured by direct double-antibody RIAs from Diagnostic Systems Laboratories (Webster, TX), whereas T and DHEAS were measured by direct antibody RIAs from Immunotech (Marseille, France). SHBG was measured by direct double-antibody RIAs from Diagnostic Systems Laboratories (Webster, TX), whereas T and DHEAS were measured by direct antibody RIAs from Diagnostic Systems Laboratories (Webster, TX), whereas T and DHEAS were measured by direct antibody RIAs from Immunotech (Marseille, France). SHBG was measured by direct antibody RIAs from Immunotech (Marseille, France).

Serum concentrations of free testosterone [fT; i.e., the fractions of hormones not linked to binding proteins in blood] were calculated from the concentrations of total T and SHBG using equations based on mass action law, assuming a constant serum albumin concentration of 43 g/L. These equations have been previously validated by theoretical simulations and by comparison with fT measurements obtained by equilibrium dialysis in postmenopausal women (18). Theoretical sensitivity analyses done in our laboratory, as well as in previous publications (19, 20), showed that the same equations would give valid results also when applied to premenopausal women.

**Statistical Analyses.** Measurements of sex steroids and SHBG were transformed using the natural logarithm to normalize their distributions. Correlations among hormones and SHBG adjusting for age, case-control status, and batch were calculated as Pearson’s partial correlation coefficients, on the full data set of cases and controls, by menopausal status.

A pairwise \( t \) test was used to test for mean case-control differences in age at blood donation, age at diagnosis, height, weight, waist-to-hip ratio, body mass index (BMI; calculated as kilograms divided by the square of the height expressed in meters), age at first full-term pregnancy, number of full-term pregnancies, cumulative duration of oral contraceptive use, age at menarche, and hormone levels. A \( x^2 \) test was used to test for case-control differences in ever having had a full-term pregnancy, percentage of past hormone users, previous oral contraceptive use, smoking status, ever had fertility problems, and ever breast-feeding. Relative risks [odds ratios (OR)] for epithelial ovarian cancer in relation to serum hormone levels were calculated by conditional logistic regression models using the PHREG procedure of the Statistical Analysis System software package version 9 (SAS Institute, Cary, NC). To allow for adequate numbers of subjects in each category, hormone levels were categorized into two groups (above and below median levels) for premenopausal women and into thirds for postmenopausal women. The cut-off points were based on the hormone variable distributions in the controls. Likelihood ratio tests were used to assess linear trends in ORs over the tertiles, scoring the tertile categories quantitatively as 1, 2, and 3 or using continuous values for exposure variables. All statistical tests and corresponding \( P \) values were two sided, and \( P \) values of <0.05 were considered statistically significant. Only for analysis of statistical heterogeneity, between study center/countries, or between subgroups of age at diagnosis and BMI, ORs were estimated for continuous measurements of sex steroids and SHBG transformed on the log\(_2\) scale. In this scale, a unit increase corresponds to a doubling of hormone concentrations. When stratifying data by BMI, statistical analyses were done by using an unconditional logistic regression analyses adjusted for relevant matching criteria (age, center, time at blood donation, and fasting status). Formal tests of heterogeneity between the ORs in different EPIC subgroups were based on \( x^2 \) statistics calculated as the deviations of logistic \( \beta \)-coefficients observed in each of the subgroups relative to the overall \( \beta \)-coefficient.

Multivariate conditional logistic regression was used to estimate ORs adjusted for possible confounders other than those controlled for by the matching criteria, including ever having had a full-term pregnancy (nulliparous, parous, missing), number of full-term pregnancies (0, 1, 2, 3+, or missing), BMI (continuous), past use of oral contraceptive (never, previous, missing), oral contraceptive use duration (never users, 0-1 year, 2-5 years, 6-10 years, >10 years, missing), and smoking (never smoker, ex-smoker, current smoker, missing). Only BMI and the variable for ever having had a full-term pregnancy were included in the final model.

All statistical analyses were done using the Statistical Analysis System software package version 9.

**Results**

Baseline characteristics of the study population are shown in Table 1. Mean age at recruitment was 46.1 and 45.8 years for cases and controls, respectively, for women who were
premenopausal at blood donation and 60.9 for cases and controls for women who were postmenopausal at blood donation. Mean age at diagnosis was 48.9 years for premenopausal and 63.8 years for postmenopausal women. On average, cases were diagnosed after 3.4 years from blood donation and 29% were premenopausal at blood donation.

Among the premenopausal women, the median BMI was 24.7, whereas among the postmenopausal women the median BMI was 26.8. In premenopausal women, case subjects were heavier than controls (P = 0.02) and were more often nulliparous (P = 0.01; Table 1). In postmenopausal women, cases were taller than control subjects (P = 0.03) but no other statistically significant differences could be observed. The variables of ever having had a full-term pregnancy and number of full-term pregnancies were inversely associated with ovarian cancer risk in premenopausal women only [OR, 0.26 (0.09-0.75); P = 0.01 for ever versus never and OR, 0.10 (0.01-0.96); P = 0.05 for women having more than five children versus women with no children, respectively], whereas duration of use of oral contraceptives was inversely associated with ovarian cancer risk in postmenopausal women only [OR, 0.17 (0.04-0.74); P = 0.02]. Smoking was not associated with risk in the overall population [OR, 0.88 (0.54-1.44) for current smokers versus never smokers] or in premenopausal or postmenopausal women separately [OR, 0.92 (0.54-1.57) and OR, 0.90 (0.50-1.63), respectively, for current smokers versus never smokers].

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Case subjects (n = 56)</th>
<th>Control subjects (n = 109)</th>
<th>pdiff*</th>
</tr>
</thead>
<tbody>
<tr>
<td>T (nmol/L)</td>
<td>1.47 (1.30-1.66)</td>
<td>1.40 (1.28-1.53)</td>
<td>0.47</td>
</tr>
<tr>
<td>fT (pmol/L)</td>
<td>20.1 (17.1-23.7)</td>
<td>18.2 (16.2-20.4)</td>
<td>0.15</td>
</tr>
<tr>
<td>Δ4 (nmol/L)</td>
<td>5.17 (4.66-5.75)</td>
<td>4.99 (4.63-5.39)</td>
<td>0.55</td>
</tr>
<tr>
<td>DHEAS (μmol/L)</td>
<td>3.26 (2.84-3.73)</td>
<td>2.91 (2.64-3.21)</td>
<td>0.23</td>
</tr>
<tr>
<td>SHBG (nmol/L)</td>
<td>46.6 (41.0-53.1)</td>
<td>49.9 (45.4-54.8)</td>
<td>0.41</td>
</tr>
</tbody>
</table>

Table 2. Geometric means (and 95% confidence interval) of steroid hormones and SHBG for ovarian cancer case and control subjects by menopausal status at time of blood donation

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Case subjects (n = 136)</th>
<th>Control subjects (n = 237)</th>
<th>pdiff*</th>
</tr>
</thead>
<tbody>
<tr>
<td>T (nmol/L)</td>
<td>1.16 (1.06-1.26)</td>
<td>1.20 (1.13-1.29)</td>
<td>0.41</td>
</tr>
<tr>
<td>fT (pmol/L)</td>
<td>16.2 (14.5-18.1)</td>
<td>17.4 (15.9-18.9)</td>
<td>0.27</td>
</tr>
<tr>
<td>Δ4 (nmol/L)</td>
<td>3.13 (2.90-3.37)</td>
<td>3.34 (3.16-3.54)</td>
<td>0.19</td>
</tr>
<tr>
<td>DHEAS (μmol/L)</td>
<td>1.78 (1.58-2.00)</td>
<td>1.84 (1.68-2.01)</td>
<td>0.82</td>
</tr>
<tr>
<td>SHBG (nmol/L)</td>
<td>42.2 (38.6-46.3)</td>
<td>42.6 (39.7-45.6)</td>
<td>0.95</td>
</tr>
</tbody>
</table>

*Paired t test.

No differences in concentrations of hormones and SHBG were observed between cases and controls in either premenopausal or postmenopausal women (Table 2) nor within younger (<55 years at diagnosis) or older women (>55 years at diagnosis; results not shown). Duration of oral contraceptive use and the variable of ever having had a full-term pregnancy were not significantly associated with serum hormone levels. In premenopausal women, androgens and SHBG concentrations did not vary over the menstrual cycle.

Serum levels of T, fT, Δ4, and DHEAS were positively correlated with each other in both premenopausal and postmenopausal women (Pearson’s r between 0.43 and 0.87), whereas SHBG was negatively correlated with T (Pearson’s r = −0.24 and −0.15, respectively, in premenopausal and postmenopausal women), fT (Pearson’s r = −0.72 and −0.62, respectively, in premenopausal and postmenopausal women), DHEAS (Pearson’s r = −0.15 and −0.17, respectively, in premenopausal and postmenopausal women), and Δ4 (Pearson’s r = −0.12) in postmenopausal women only, there was no correlation between SHBG and Δ4 in premenopausal women. BMI was weakly positively correlated with T and fT in premenopausal and postmenopausal women (Pearson’s r = 0.21 and 0.41, respectively, for premenopausal women and r = 0.13 and 0.33, respectively, for postmenopausal women) and inversely correlated with SHBG (r = −0.45 in premenopausal women and r = −0.41 for postmenopausal

NOTE: Mean (5th-95th percentiles) or percentages.
Abbreviations: OC, oral contraceptive; HRT, hormone replacement therapy.
*Paired t test.
†χ² test.
women), whereas no association was found with Δ4 and DHEAS. T, fT, Δ4 and DHEAS were inversely correlated with age in premenopausal women (Pearson’s r = −0.33, −0.24, −0.29, and −0.19, respectively). In postmenopausal women, DHEAS was inversely correlated with age (r = −0.18); there was no correlation for all other hormones and SHBG.

Conditional logistic regression analyses on the full set of ovarian cancer cases and controls (all age groups combined) showed no statistically significant association between serum levels of androgens and SHBG and ovarian cancer risk (results not shown).

In women who were premenopausal at blood donation, there were no statistically significant associations between serum levels of androgens and SHBG and ovarian cancer risk either before or after adjustment for BMI and ever having had a full-term pregnancy (Table 3). However, in women who had a diagnosis of ovarian cancer when they were <55 years (n = 57), serum fT concentration was positively associated with ovarian cancer risk and fT, although of borderline significance only [OR, 2.49 (0.97-6.43); P = 0.06, highest versus lowest tertile, for a model adjusted for BMI and ever having had a full-term pregnancy]; SHBG concentrations showed an inverse association with risk [OR, 0.35 (0.12-0.94), highest versus lowest tertile] of borderline significance (P = 0.06), and no relationship was observed for Δ4, T, and DHEAS.

In postmenopausal women, fT concentrations were negatively associated with a decrease in ovarian cancer risk [OR, 0.45 (0.24-0.86); P = 0.01; Table 4], whereas no statistically significant association was observed for all the other hormones and SHBG. Virtually the same results were obtained when restricting analyses to ovarian cancer cases who were postmenopausal at blood donation and >55 years at diagnosis (results not shown).

In the total study population, BMI was significantly associated with a linear increase in epithelial ovarian cancer risk [OR, 1.86 (1.16-2.99); P = 0.01, highest versus lowest tertile]. This increase was more marked in premenopausal women [OR, 2.36 (1.02-5.43); P = 0.05, highest versus lowest tertile] than in postmenopausal women, where this association did not reach statistical significance [OR, 1.40 (0.80-2.45); P = 0.24, highest versus lowest tertile].

We observed no overall heterogeneity in OR estimate by time since blood donation (<2 years versus ≥2 years) for any of the three androgens or SHBG in the overall population or for premenopausal women (results not shown). However, in postmenopausal women, a statistically significant heterogeneity in OR estimate by time since blood donation was observed for SHBG: women who gave blood <2 years before cancer diagnosis had an OR of 1.70 (0.94-3.06) for a doubling of SHBG concentrations, whereas women who gave blood at least 2 years before cancer diagnosis had an OR of 0.81 (0.57-1.17; P = 0.04) for a doubling in SHBG levels. Virtually the same results were obtained when stratifying the analyses by age at diagnosis for the subgroup of women who had a diagnosis when >55 years.

When statistical analyses were stratified by BMI, a strong heterogeneity in the association of SHBG with ovarian cancer risk was observed in postmenopausal women between women with a BMI below the median of the population (BMI, <26.8) and women with a BMI above the median: SHBG was strongly inversely associated to ovarian cancer risk in leaner women [OR, 0.31 (0.14-0.68), on a continuous log scale] and strongly directly associated to ovarian cancer risk in heavier women [OR, 2.48 (1.31-4.71), on a continuous log scale; P = 0.0001]. Heterogeneity of association was also observed for fT (a measure that is strongly inversely associated to SHBG concentrations) but not for other hormones. The same heterogeneity of results was observed on the overall population.

In all statistical analyses, results remained virtually the same when using country-specific cut-off points or cohort-wide cut-off points. No heterogeneity in OR estimate has been observed among the different countries (P = 0.48 for fT, P = 0.71 for fT, P = 0.76 for Δ4, P = 0.47 for DHEAS, and P = 0.66 for SHBG). Exclusion of women who developed ovarian cancer <1 year after recruitment of the study did not change the level of association of hormones with ovarian cancer risk.

### Table 3. ORs of ovarian cancer (95% confidence intervals) by levels of sex steroids and SHBG in premenopausal women and their matched controls (in two exposure levels)

<table>
<thead>
<tr>
<th>Hormone concentration</th>
<th>Low</th>
<th>High</th>
<th>P&lt;sub&gt;association&lt;/sub&gt;</th>
<th>P&lt;sub&gt;trend&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>&lt;1.42 nmol/L</td>
<td>≥1.42 nmol/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases/controls</td>
<td>23/48</td>
<td>29/49</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude</td>
<td>1.00</td>
<td>1.27 (0.60-2.69)</td>
<td>0.53</td>
<td>0.46</td>
</tr>
<tr>
<td>Adjusted&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.00</td>
<td>1.17 (0.53-2.38)</td>
<td>0.71</td>
<td>0.48</td>
</tr>
<tr>
<td>Adjusted&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.00</td>
<td>1.27 (0.56-2.89)</td>
<td>0.57</td>
<td>0.51</td>
</tr>
<tr>
<td>Δ4</td>
<td>&lt;4.90 nmol/L</td>
<td>≥4.90 nmol/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases/controls</td>
<td>26/52</td>
<td>28/52</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude</td>
<td>1.00</td>
<td>1.11 (0.55-2.24)</td>
<td>0.77</td>
<td>0.55</td>
</tr>
<tr>
<td>Adjusted&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.00</td>
<td>1.16 (0.56-2.41)</td>
<td>0.69</td>
<td>0.45</td>
</tr>
<tr>
<td>Adjusted&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.00</td>
<td>1.06 (0.49-2.00)</td>
<td>0.87</td>
<td>0.63</td>
</tr>
<tr>
<td>DHEAS</td>
<td>&lt;3.09 μmol/L</td>
<td>≥3.09 μmol/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases/controls</td>
<td>25/54</td>
<td>31/55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude</td>
<td>1.00</td>
<td>1.26 (0.63-2.51)</td>
<td>0.52</td>
<td>0.20</td>
</tr>
<tr>
<td>Adjusted&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.00</td>
<td>1.22 (0.59-2.50)</td>
<td>0.59</td>
<td>0.16</td>
</tr>
<tr>
<td>Adjusted&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.00</td>
<td>1.20 (0.58-2.46)</td>
<td>0.63</td>
<td>0.29</td>
</tr>
<tr>
<td>SHBG</td>
<td>&lt;50.6 nmol/L</td>
<td>≥50.6 nmol/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases/controls</td>
<td>31/51</td>
<td>23/51</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude</td>
<td>1.00</td>
<td>0.71 (0.36-1.41)</td>
<td>0.33</td>
<td>0.29</td>
</tr>
<tr>
<td>Adjusted&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.00</td>
<td>0.95 (0.45-2.01)</td>
<td>0.89</td>
<td>0.84</td>
</tr>
<tr>
<td>Adjusted&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.00</td>
<td>0.97 (0.45-2.08)</td>
<td>0.93</td>
<td>0.77</td>
</tr>
</tbody>
</table>

<sup>a</sup>Linear trend on continuous (log transformed) variable.
<sup>b</sup>Further adjustment for BMI.

### Discussion

In this prospective study, the largest to date conducted on the relationship between epithelial ovarian cancer risk and circulating levels of androgen and SHBG, there were no statistically significant associations between androgens and SHBG concentrations and ovarian cancer risk. However, we observed a relatively strong inverse association between fT concentrations and risk of ovarian cancer in postmenopausal women, whereas we did not observe any relationship between androgens and SHBG concentrations and cancer risk in premenopausal women.

These findings differ with the findings by Helzlsouer et al. (12), in which Δ4 and DHEAS seemed to be directly associated with ovarian cancer risk in both premenopausal and postmenopausal women. This study, however, included only 31 cancer cases, so findings should be interpreted carefully. The overall lack of association of endogenous DHEAS concentrations with ovarian cancer risk in our study confirms a previous observation that the contribution of adrenally produced hormones in ovarian cancer risk is most likely of little importance (13).

When analyses were restricted to premenopausal women at blood donation, we did not observe any association between...
Table 4. ORs of ovarian cancer (95% confidence intervals) by tertiles of sex steroids and SHBG in postmenopausal women and their matched controls

<table>
<thead>
<tr>
<th>T</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>Crude†</th>
<th>Adjusted†</th>
<th>Crude‡</th>
<th>Adjusted‡</th>
<th>Crude§</th>
<th>Adjusted§</th>
<th>Crude∥</th>
<th>Adjusted∥</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases/controls</td>
<td>41/67</td>
<td>51/68</td>
<td>44/70</td>
<td>54/61</td>
<td>32/64</td>
<td>54/61</td>
<td>32/64</td>
<td>46/73</td>
<td>35/76</td>
<td>46/73</td>
<td>35/76</td>
</tr>
<tr>
<td>Tertiles</td>
<td>&lt;1.01 nmol/L</td>
<td>1.01-1.45 nmol/L</td>
<td>≥1.46 nmol/L</td>
<td>1.00</td>
<td>0.80 0.47-1.37</td>
<td>0.81 0.47-1.37</td>
<td>0.59 0.34-0.92</td>
<td>0.54 0.29-0.98</td>
<td>0.45 0.23-0.85</td>
<td>0.65 0.37-1.14</td>
<td>0.67 0.35-1.20</td>
</tr>
<tr>
<td>fT</td>
<td>&lt;14.3 pmol/L</td>
<td>14.3-23.6 pmol/L</td>
<td>≥23.7 pmol/L</td>
<td>1.00</td>
<td>0.82 0.48-1.40</td>
<td>0.82 0.48-1.40</td>
<td>0.80 0.45-1.41</td>
<td>0.60 0.35-1.04</td>
<td>0.60 0.35-1.04</td>
<td>0.60 0.35-1.04</td>
<td>0.60 0.35-1.04</td>
</tr>
<tr>
<td>Δ4</td>
<td>&lt;2.74 nmol/L</td>
<td>2.74-3.85 nmol/L</td>
<td>≥3.86 nmol/L</td>
<td>1.00</td>
<td>0.81 0.47-1.37</td>
<td>0.81 0.47-1.37</td>
<td>0.81 0.47-1.37</td>
<td>0.60 0.35-1.04</td>
<td>0.60 0.35-1.04</td>
<td>0.60 0.35-1.04</td>
<td>0.60 0.35-1.04</td>
</tr>
<tr>
<td>Cases/controls</td>
<td>51/77</td>
<td>40/76</td>
<td>43/79</td>
<td>46/73</td>
<td>35/76</td>
<td>46/73</td>
<td>35/76</td>
<td>46/73</td>
<td>35/76</td>
<td>46/73</td>
<td>35/76</td>
</tr>
<tr>
<td>SHBG</td>
<td>&lt;36.3 nmol/L</td>
<td>36.3-52.0 nmol/L</td>
<td>≥52.1 nmol/L</td>
<td>1.00</td>
<td>0.80 0.47-1.37</td>
<td>0.81 0.47-1.37</td>
<td>0.81 0.47-1.37</td>
<td>0.80 0.45-1.41</td>
<td>0.80 0.45-1.41</td>
<td>0.80 0.45-1.41</td>
<td>0.80 0.45-1.41</td>
</tr>
<tr>
<td>Cases/controls</td>
<td>50/70</td>
<td>35/72</td>
<td>54/73</td>
<td>50/70</td>
<td>35/72</td>
<td>54/73</td>
<td>50/70</td>
<td>35/72</td>
<td>54/73</td>
<td>50/70</td>
<td>35/72</td>
</tr>
<tr>
<td>DHEAS</td>
<td>&lt;1.52 μmol/L</td>
<td>1.52-2.58 μmol/L</td>
<td>≥2.59 μmol/L</td>
<td>1.00</td>
<td>0.80 0.47-1.37</td>
<td>0.81 0.47-1.37</td>
<td>0.81 0.47-1.37</td>
<td>0.80 0.45-1.41</td>
<td>0.80 0.45-1.41</td>
<td>0.80 0.45-1.41</td>
<td>0.80 0.45-1.41</td>
</tr>
<tr>
<td>Cases/controls</td>
<td>54/61</td>
<td>32/64</td>
<td>54/61</td>
<td>54/61</td>
<td>32/64</td>
<td>54/61</td>
<td>32/64</td>
<td>54/61</td>
<td>32/64</td>
<td>54/61</td>
<td>32/64</td>
</tr>
</tbody>
</table>

*Linear trends in ORs by scoring the tertiles according to the quantitative score 1, 2, and 3 for tertile categories.
†Linear trend on continuous (log transformed) variable.
‡Analysis matched on EPIC recruitment center, age at blood donation, time of the day at blood donation, and fasting status.
§Further adjustment for BMI.
∥Further adjustment for ever having had a full-term pregnancy.

Endogenous androgens and SHBG and epithelial ovarian cancer risk. This finding contrasts with the results of the two prospective studies published previously (12, 13), in which, in premenopausal women, Δ4 concentrations were associated with an increase in risk of ovarian cancer. However, the number of cases in this subgroup of women still remains relatively low, also in our study, and confidence intervals are wide.

When analyses were stratified by age at diagnosis rather than by menopausal status at blood donation, we observed a direct association of Δ4 and an inverse association of SHBG, with ovarian cancer risk in women who were <55 years at diagnosis, although both associations were of borderline significance. This may suggest that circulating free androgen levels are important in ovarian cancer development/progression in women who develop this cancer at a relatively young age. Future work is needed to confirm this finding.

In postmenopausal women (and in women who were postmenopausal at blood donation and >55 years at diagnosis), Δ4 was inversely associated with ovarian cancer risk. Although this association did not appreciably change after the adjustment for BMI, there was some evidence of heterogeneity in the association between ovarian cancer risk and SHBG and Δ4 concentrations when analyses were stratified by BMI in leaner women (BMI, <26.8). SHBG was inversely related to cancer risk and, therefore, Δ4 was mildly directly related to risk, whereas in overweight women SHBG was directly associated to cancer risk and Δ4 was inversely associated to risk. The number of cases in each BMI category, however, remains limited so we should interpret these findings carefully. The association of Δ4 and ovarian cancer risk and its possible modification by BMI needs to be examined in greater detail in further studies with larger subjects.

Several studies have examined the relationship between BMI and ovarian cancer risk, with inconsistent results (21-25). Increased BMI is generally associated with a lowering of SHBG and with an increase in Δ4 concentrations in women, which may lead to an increased ovarian cancer risk, according to the hypothesis of Risch (3). In our study, BMI was significantly associated with an increase in ovarian cancer risk only in premenopausal women and not in postmenopausal women. This could be related to the fact that premenopausal women were much leaner than postmenopausal women (median BMI of 24.7 in premenopausal women versus 26.8 in postmenopausal women) and that, in leaner women, SHBG was inversely related to ovarian cancer risk, whereas Δ4 was directly associated with risk. In premenopausal women, the association between BMI and cancer risk was attenuated after adjustment for Δ4 and SHBG, suggesting that the relationship of BMI with cancer risk could be partially mediated through SHBG and Δ4 levels. This would support the hypothesis of an implication of Δ4 in ovarian cancer development at least in this population. Unfortunately, the lack of statistical association between T and Δ4 and ovarian cancer in the current study does not corroborate this hypothesis further, but, as mentioned previously, this could be partially explained by the lack of statistical power due to small sample size.

To check whether results were modified by time to cancer diagnosis, we stratified our statistical analyses by time since blood donation. No heterogeneity of data was observed for androgens, whereas some heterogeneity could be observed for SHBG: postmenopausal women who gave blood <2 years before cancer diagnosis had an increase in risk for a doubling of SHBG concentrations, whereas women who gave blood at least 2 years before cancer diagnosis had a decrease in risk for a doubling in SHBG levels. We do not have a clear explanation
for these findings. However, one could speculate that women who had the diagnosis of cancer <2 years after blood donation could have experienced some weight loss due to the presence of the undiagnosed tumor, leading to an increase in SHBG levels and therefore to a direct association between SHBG concentration and cancer risk.

Hormone concentrations at tissue levels could be substantially different from the concentrations found in blood. The importance of intracrine compared with endocrine exposure has already been discussed for breast cancer (26), and it might be of particular importance for ovarian cancer because the epithelium that covers the ovaries is not vascular, and it is likely that cells are more exposed to a paracrine rather than to an endocrine hormone environment (2). This might partially account for the heterogeneity of results on ovarian cancer risk and circulating hormone concentrations.

The current study is the largest study to date on epithelial ovarian cancer risk and endogenous androgens and SHBG, although its sample size remains limited, above all for subgroup analyses. Its prospective design, furthermore, very much reduces the possibility that circulating hormone levels could have been influenced by the presence or diagnosis of the disease. Our ability to exclude cases diagnosed within the first 2 years of follow-up further minimizes this bias. In addition, standardized protocols were followed for recruitment and blood collection, questionnaire data, and hormone measurements across subpopulations with heterogeneous lifestyles and cancer risks. A relative limitation of the study might be that only a single blood sample was obtained for each study participant. However, it has been observed that the within-subject reproducibility of serum androgen and SHBG concentrations over a relatively long period (11-60 months) is quite high (intra-assay correlations between 0.57 for Δ4 to 0.89 for SHBG and DHEAS; ref. 13). Other limitations are the lack of information about family history of ovarian cancer and BRCA-1 and BRCA-2 mutation carriers (factors that are strongly related to ovarian cancer risk) and the lack of information of menopausal status of the case subjects at cancer diagnosis: it could have happened that women, who were recruited as being premenopausal women at blood donation, could have become postmenopausal women at the time of the cancer diagnosis. However, given the short lag time between blood donation and diagnosis for premenopausal women on average 2.8 years (95th–95th percentile range: 0.2-7.6 years)], these women would have gone into menopause only for a very short period.

In conclusion, there is no strong evidence that circulating levels of androgens and SHBG are associated with an increased risk of ovarian cancer, although levels of Δ4 may be associated with an increased risk of ovarian cancer among women who were diagnosed at a relatively young age. The heterogeneity of results on the associations of SHBG and Δ4 with ovarian cancer risk in postmenopausal women by BMI and by time since blood donation deserves further investigations. Only studies with larger sample sizes will help to finally address these issues.

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References

Endogenous Androgens and Risk of Epithelial Ovarian Cancer: Results from the European Prospective Investigation into Cancer and Nutrition (EPIC)

Sabina Rinaldi, Laure Dossus, Annekatrin Lukanova, et al.


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