

Androgens Are Differentially Associated with Ovarian Cancer Subtypes in the Ovarian Cancer Cohort Consortium



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Abstract

Invasive epithelial ovarian cancer (EOC) is the most lethal gynecologic malignancy. The etiology of EOC remains elusive; however, experimental and epidemiologic data suggest a role for hormone-related exposures in ovarian carcinogenesis and risk factor differences by histologic phenotypes and developmental pathways. Research on prediagnosis androgen concentrations and EOC risk has yielded inconclusive results, and analyses incorporating EOC subtypes are sparse. We conducted a pooled analysis of 7 nested case-control studies in the Ovarian Cancer Cohort Consortium to investigate the association between prediagnosis circulating androgens [testosterone, free testosterone, androstenedione, dehydroepiandrosterone sulfate (DHEAS)], sex hormone binding globulin (SHBG), and EOC risk by tumor characteristics (i.e., histology, grade, and stage). The final study population included 1,331 EOC cases and 3,017 matched con-

trols. Multivariable conditional logistic regression was used to assess risk associations in pooled individual data. Testosterone was positively associated with EOC risk (all subtypes combined, $OR_{log2} = 1.12$; 95% confidence interval 1.02–1.24); other endogenous androgens and SHBG were not associated with overall risk. Higher concentrations of testosterone and androstenedione associated with an increased risk in endometrioid and mucinous tumors [e.g., testosterone, endometrioid tumors, $OR_{log2} = 1.40$ (1.03–1.91)], but not serous or clear cell. An inverse association was observed between androstenedione and high grade serous tumors [$OR_{log2} = 0.76$ (0.60–0.96)]. Our analyses provide further evidence for a role of hormone-related pathways in EOC risk, with differences in associations between androgens and histologic subtypes of EOC. *Cancer Res*; 77(14); 3951–60. ©2017 AACR.

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Introduction

Reproductive history influences risk of ovarian cancer and it has been hypothesized that these associations are mediated by exposure to endogenous hormones, including androgens (1). Data from experimental studies link androgen-related signaling to ovarian cancer through increased cellular proliferation and reduced apoptotic rates (2–4). The relationship between androgens and epithelial ovarian cancer (EOC) risk has been examined in 7 nested case-control studies with the numbers of cases in these studies ranging from 31 to 1,052 (5–10); these studies predominantly investigated EOC as a composite outcome. Emerging data show heterogeneity in risk factors by histologic subtypes (e.g., serous, endometrioid, mucinous, clear cell) and by the hypothesized "dualistic pathway" of ovarian carcinogenesis (defined by differences in the genetic make-up and the morphological architecture of histologic phenotypes; refs. 11–18). The relationship between androgens and EOC risk by disease subtype has been minimally explored. Analyses to date suggest heterogeneity by subtype (9, 10); however, individual studies evaluating EOC by subtype were either limited by small case numbers in subtype analyses (9), or restricted to women pregnant at the time of serum sampling (10).

We pooled and harmonized available data from 6 nested case-control studies within the Ovarian Cancer Cohort Consortium (OC3), plus the Finnish Maternity Cohort (FMC), to investigate the relationship of pre-diagnosis concentrations of androgens [e.g., testosterone, free testosterone, androstenedione, dehydroepiandrosterone-sulfate (DHEAS)] and sex-hormone binding globulin (SHBG) with EOC risk, overall and by subtype. Subtype analyses included analyses by histology, grade and stage, and by the hypothesized dualistic model of EOC development, that is, type I versus type II (19). Our study represents the largest investigation to date including individual-level data from 1,331 EOC cases and 3,017 matched controls, with 61 (clear cell) to 667 (serous) cases represented in the major histologic subtypes.

Materials and Methods

Study population: ovarian cancer cohort consortium (OC3)

The OC3 has been described previously (12). For this investigation, eligible cohorts were required to have data on a defined set of *a priori* selected covariates (e.g., menopausal status at blood donation, oral contraceptive use at blood donation, parity) and prediagnosis measurements of testosterone, free testosterone, androstenedione, or DHEAS. In addition to the OC3 cohorts, the FMC, a cohort of women pregnant at blood collection, contributed data to this investigation (for contributing cohorts see Supplementary Table S1). Available biomarker and questionnaire data from each cohort were centrally collated and harmonized at the Data Coordinating Center at the Brigham and Women's Hospital.

Case characteristics

Eligible cases included women diagnosed with invasive EOC [International Classification of Disease Codes (ICD): ICD9 codes 183 and 158; ICD10 code C56] ascertained by self-report with medical record confirmation and/or linkage to cancer registries. Cases were individually matched to two or three controls (free of cancer and alive at the time of diagnosis of the index case) on age, date, menopausal status and day or phase of menstrual cycle at blood collection in premenopausal women, with the exception of

the FMC (matched on age and date at blood collection, parity at blood collection and at diagnosis/index date). Histomorphologic data were complete, and the majority of cases had data on stage (82%); grade was available for 36% of the cases. We used histology and grade to classify tumors into type I [(48%, $n = 291$); low-grade serous and endometrioid, all mucinous and clear cell] and type II [(52%, $n = 314$); high-grade serous, high-grade endometrioid; ref. 19]. Serous and endometrioid cases missing grade data were excluded from these analyses; mucinous and clear cell tumors were included regardless of grade data availability, as these tumors are classified as type I independent of grade. In a sensitivity analysis, all mucinous and clear cell cases missing grade were excluded from the type I subgroup (after exclusion, case $n = 77$). The proportion of type I tumors was higher than expected; however, we observed the expected distribution (type I: 28% vs. type II: 72%) after excluding women from the FMC (all missing grade; younger at diagnosis and more frequently diagnosed with mucinous tumors than cases from the other cohorts).

Laboratory methods

In all studies, case-control sets were measured in the same batch and technicians performing the assays were blinded to case-control status and quality control samples. Information on sample type, laboratory assays, and intra- and inter-batch coefficients of variations for each cohort is summarized in Supplementary Table S2. Free testosterone was calculated on the basis of measured concentrations of testosterone and SHBG, with albumin assumed to be a constant 40 g/L, according to the mass law of action (20).

Statistical analyses

Hormone measurements were standardized across studies based on the cohort-specific mean concentrations in controls (see Supplementary Methods; Supplementary Table S3). Conditional logistic regression was used to estimate ORs and 95% confidence intervals (CI). ORs were estimated using \log_2 -transformed biomarker concentrations and study-specific tertiles based on the distribution in controls. A continuous probit score, generating a rank for each participant in each cohort by hormone concentration, was used to test for trend across tertiles. We additionally evaluated associations in quintiles for EOC overall and the serous subtype. Multivariable models included: parity [never, ever, missing (2.8%)] and OC use [never, ever, missing (47%); excluding FMC 2.3% missing]. Additional adjustment for body mass index (BMI; kg/m^2) among women with data available ($n = 747$ cases), did not change the ORs (data not shown).

Statistical analyses were conducted using a two-stage approach: First, ORs were calculated within each cohort and pooled using DerSimonian and Laird random effects meta-analysis models to assess between-study heterogeneity (21). Second, ORs were calculated on the basis of pooled individual participant data (22). ORs estimated from meta-analysis and the data pooling method were similar, and we observed no significant between-study heterogeneity. Therefore, presented results are based on the pooled analysis. The assumption of linearity was tested using restricted cubic splines; no significant deviations from linearity were observed. Statistical heterogeneity of associations across subtypes was assessed via a likelihood ratio test comparing a model allowing the association for the risk factor of interest to vary by subtype versus one assuming the same association across subtype using polytomous conditional logistic regression (23).

We evaluated associations after stratification by menopausal status at blood collection (premenopausal vs. postmenopausal) and age at diagnosis (<55 vs. ≥55 years). Androgen concentrations are relatively stable in pregnancy (24); however, we excluded FMC members in sensitivity analyses given that all women were pregnant at the time they provided a blood sample. Finally, we conducted a sensitivity analysis after exclusion of women diagnosed within two years after blood donation. A more detailed description of statistical procedures is available in the Supplementary Methods.

SAS Statistical Software, version 9.3 (SAS Institute) was used for statistical analyses. *P* values <0.05 were considered as statistically significant; all statistical tests and *P* values were two-sided.

Results

In total 1,331 cases and 3,017 matched controls from 7 cohorts were included in this investigation (Table 1). Average age at blood collection ranged from 32 (FMC) to 61 years (CLUE II), and the majority of women were parous (89% cases, 94% controls; Table 1). Average age at diagnosis ranged from 45 (FMC) to 67 (CLUE II; Supplementary Table S4).

Androgens and overall EOC risk

A doubling of testosterone (i.e., 1-unit increase in log₂-transformed testosterone) was associated with a 12% increase in overall EOC risk (OR_{log2}, 1.12; 95% confidence interval (CI), 1.02–1.24], and a 25% increase in risk comparing top to bottom tertile (OR_{T3-T1} = 1.25; 95% CI, 1.06–1.48; *P*_{trend} = 0.03; Table 2). Free testosterone, androstenedione, DHEAS, and SHBG were not associated with overall risk of EOC. Results from analyses evaluating quintiles of androgen and SHBG concentrations were similar to those from models using tertiles (Supplementary Table S5); however, the OR comparing highest vs. lowest quintile of testosterone was not statistically significant (OR_{Q5-Q1}, 1.22; 95% CI, 0.99–1.52).

Histologic subtypes

The association between testosterone and EOC risk differed by histologic subtype (*P*_{het} = 0.06). Higher concentrations of circulating testosterone were associated with increased risk of endometrioid and mucinous tumors (e.g., endometrioid tumors: OR_{log2}, 1.40; 95% CI, 1.03–1.91), but not with serous or clear cell tumors (e.g., serous tumors: OR_{log2}, 0.96; 95% CI, 0.84–1.11). Free testosterone and androstenedione were associated with increased risk of mucinous tumors (e.g., androstenedione: OR_{log2}, 1.33; 95% CI, 1.03–1.72; Table 2), but not with any of the other histologic subtypes (e.g., androstenedione and endometrioid tumors: OR_{log2}, 1.04; 95% CI, 0.76–1.43). DHEAS and SHBG were not associated with any of the examined histologic subtypes.

Tumor grade and developmental pathways

We observed significant heterogeneity in the association between androstenedione and low grade EOC and high-grade serous disease; androstenedione was significantly inversely associated with high grade serous EOC (*P*_{het} = 0.02; all low-grade cases: OR_{log2}, 1.41; 95% CI, 0.86–2.31]; high-grade serous OR_{log2}, 0.76; 95% CI, 0.60–0.96; Table 3). The association between SHBG and EOC risk differed significantly by grade (*P*_{het} = 0.02); however, the individual effect estimates were not statistically significant.

The association between androgens and EOC risk differed by developmental pathway (type I vs. type II tumors, *P*_{het}, testosterone: 0.02; free testosterone: 0.01; androstenedione: <0.01; DHEAS: <0.01; Fig. 1). Overall, higher concentrations of androgens were associated with increased risk of type I tumors, and reduced risk of type II tumors (e.g., androstenedione: type I: OR_{log2}, 1.29; 95% CI, 1.05–1.60; cases *n* = 287; type II: OR_{log2}, 0.74; 95% CI, 0.59–0.92; cases *n* = 307; *P*_{het} < 0.01). Significant heterogeneity for androstenedione (*P* < 0.01) and DHEAS (*P* = 0.03) remained after exclusion of mucinous and clear cell

Table 1. Case and control characteristics in pooled analysis of prospective data on circulating androgens, SHBG and EOC risk: the Ovarian Cancer Cohort Consortium (OC3)

Cohort	Reference	No	Mean age at blood donation years (SD)	Nulliparous% ^a	Ever OC use, % ^a	Postmenopausal, % ^b	Mean BMI (SD)
Clue II ^c		Case	46	60.8 (13.0)	19%	20%	26.3 (5.8)
		Control	91	61.0 (12.9)	13%	13%	25.4 (4.6)
EPIC	Ose et al. 2015 (9)	Case	451	55.9 (8.5)	18%	37%	26.8 (4.9)
		Control	867	55.9 (8.6)	12%	45%	26.3 (4.7)
FMC	Schock et al. 2014 (10)	Case	576	32.5 (4.8)	0%	^d	^d
		Control	1,433	32.5 (4.7)	0%	^d	^d
NHS	Tworoger et al. 2008 (7)	Case	117	57.7 (6.5)	8%	41%	24.8 (4.8)
		Control	348	57.7 (6.5)	5%	47%	24.7 (4.1)
NHS II	Tworoger et al. 2008 (7)	Case	15	46.1 (4.4)	20%	93%	29.6 (9.8)
		Control	44	45.8 (4.3)	23%	86%	25.9 (5.8)
NYUWHS	Lukanova et al. 2003 (6)	Case	63	52.6 (8.6)	47%	29%	24.5 (3.8)
		Control	112	52.0 (8.5)	38%	36%	25.9 (4.3)
WHS	Tworoger et al. 2008 (7)	Case	63	55.7 (7.2)	25%	65%	24.5 (3.9)
		Control	122	55.5 (7.0)	15%	71%	25.1 (4.4)
Total		Case	1,331	45.8 (13.7)	11%	40%	26.2 (5.1)
		Control	3,017	44.8 (13.7)	6%	47%	25.8 (4.6)

Abbreviations: BMI, body mass index; CLUE, Washington County, MD Study "Give us a clue to cancer and heart disease"; EPIC, European Prospective Investigation into Cancer and Nutrition; FMC, Finnish Maternity Cohort; NHS, Nurses' Health Study; NYUWHS, New York University Women's Health Study; OC, oral contraceptive; OC3, Ovarian Cancer Cohort Consortium; SHBG, sex hormone binding globulin; WHS, Women's Health Study.

^aAmong women with data: parity 2.8% missing; OC use 47% missing (excluding FMC: 2.3% missing).

^bAt blood collection.

^cData from Clue II have not been published.

^dInformation on BMI and OC use was not collected in the FMC.

Table 2. ORs (95% CI) for EOC overall and by histologic subtypes in tertiles and for doubling of androgen concentrations: The Ovarian Cancer Cohort Consortium (OCC3)^a

	Invasive EOC			Serous			Endometrioid			Mucinous			Clear cell		
	Sets	OR (95%CI)	P _{trend}	Sets	OR (95%CI)	P _{trend}	Sets	OR (95%CI)	P _{trend}	Sets	OR (95%CI)	P _{trend}	Sets	OR (95%CI)	P _{trend}
Testosterone															
T1	398	ref		222	ref		35	ref		45	ref		15	ref	
T2	443	1.20 (1.02-1.41)		229	1.16 (0.92-1.46)		60	1.46 (0.88-2.42)		61	1.34 (0.86-2.08)		27	1.65 (0.73-3.73)	
T3 ^b	460	1.25 (1.06-1.48)	0.03	204	0.97 (0.76-1.24)	0.56	69	1.80 (1.08-3.01)	0.06	84	1.94 (1.25-3.02)	0.05	17	0.82 (0.34-2.00)	0.68
Doubling ^c	1,301	1.12 (1.02-1.24)	0.02	655	0.96 (0.84-1.11)	0.61	164	1.40 (1.03-1.91)	0.03	190	1.29 (1.01-1.66)	0.04	59	1.12 (0.69-1.80)	0.65
P _{het} ^d															0.06
Free testosterone															
T1	286	ref		155	ref		25	ref		35	ref		11	ref	
T2	287	1.05 (0.85-1.28)		151	1.04 (0.79-1.38)		32	0.94 (0.48-1.86)		48	1.46 (0.85-2.52)		10	0.82 (0.27-2.48)	
T3 ^b	292	1.06 (0.87-1.31)	0.04	129	0.83 (0.62-1.10)	0.63	36	1.03 (0.53-1.99)	0.45	50	1.50 (0.88-2.54)	0.03	20	2.02 (0.67-6.12)	0.26
Doubling ^c	865	1.10 (1.00-1.21)	0.05	435	0.97 (0.84-1.11)	0.61	93	1.11 (0.80-1.53)	0.53	133	1.33 (1.04-1.72)	0.03	41	1.32 (0.82-2.11)	0.12
P _{het} ^d															
Androstenedione															
T1	450	ref		235	ref		46	ref		56	ref		21	ref	
T2	387	0.86 (0.73-1.02)		204	0.88 (0.70-1.12)		45	0.67 (0.40-1.13)		51	0.90 (0.57-1.42)		15	0.93 (0.41-2.10)	
T3 ^b	470	1.07 (0.90-1.28)	0.13	217	0.99 (0.77-1.28)	0.79	73	0.99 (0.59-1.67)	0.82	84	1.57 (1.01-2.42)	0.03	24	0.81 (0.37-1.77)	0.62
Doubling ^c	1,307	1.08 (0.97-1.19)	0.16	656	0.97 (0.84-1.12)	0.69	164	1.04 (0.76-1.43)	0.82	191	1.33 (1.03-1.72)	0.03	60	1.07 (0.69-1.66)	0.77
P _{het} ^d															0.17
DHEAS															
T1	227	ref		128	ref		18	ref		8	ref		7	ref	
T2	245	1.08 (0.86-1.36)		127	1.06 (0.77-1.45)		23	0.81 (0.36-1.82)		16	1.04 (0.35-3.08)		12	3.36 (0.88-12.8)	
T3 ^b	219	0.95 (0.74-1.23)	0.87	111	0.83 (0.59-1.18)	0.45	28	1.02 (0.42-2.47)	0.85	20	1.53 (0.50-4.71)	0.29	14	3.50 (1.03-11.9)	0.10
Doubling ^c	691	0.99 (0.89-1.10)	0.82	366	0.93 (0.81-1.08)	0.36	69	1.05 (0.72-1.53)	0.78	44	1.34 (0.81-2.23)	0.26	33	1.52 (0.87-2.65)	0.14
P _{het} ^d															0.22
SHBG															
T1	311	ref		147	ref		30	ref		56	ref		19	ref	
T2	250	0.82 (0.67-1.00)		141	0.89 (0.67-1.18)		27	0.98 (0.49-1.93)		28	0.61 (0.36-1.05)		12	0.75 (0.30-1.91)	
T3 ^b	325	1.09 (0.89-1.33)	0.56	157	1.14 (0.86-1.52)	0.39	37	1.49 (0.78-2.85)	0.44	51	0.96 (0.58-1.57)	0.79	12	0.75 (0.29-1.97)	0.50
Doubling ^c	886	1.02 (0.91-1.14)	0.76	445	1.06 (0.91-1.25)	0.43	94	1.16 (0.80-1.67)	0.43	135	0.93 (0.68-1.28)	0.65	43	0.77 (0.46-1.30)	0.33
P _{het} ^d															0.43

NOTE: Bold numbers indicate statistically significant association.

Abbreviations: DHEAS, dehydroepiandrosterone sulfate; SHBG, sex hormone binding globulin.

^aResults were derived from conditional logistic regression models, additionally adjusted for OC use (never/ever/missing) and parity (never/ever/missing).

^bThe P value for trend across tertiles is based on a continuous probit score (generating a rank for each person in each cohort by hormone level).

^cLinear trends for doubling of hormone concentrations were estimated on log₂ scale.

^dPairwise heterogeneity tests were performed, using the likelihood ratio test comparing models assuming (i) the same association between exposure and outcomes compared with (ii) a model assuming different associations for each subtype.

Table 3. ORs (95% CI) for EOC for doubling of androgen concentrations and stratified by grade at diagnosis overall and for serous tumors: The Ovarian Cancer Cohort Consortium (OCC3)^a

	Sets	OR (95%CI)
Testosterone		
Low grade	55	1.28 (0.80–2.07)
High grade		
All	407	0.94 (0.79–1.12)
Serous	260	0.84 (0.67–1.04)
	P_{het}^b	0.25
	P_{het}^c	0.12
Free testosterone		
Low grade	38	1.34 (0.79–2.27)
High grade		
All	277	0.95 (0.80–1.13)
Serous	180	0.92 (0.74–1.13)
	P_{het}^b	0.24
	P_{het}^c	0.19
Androstenedione		
Low grade	55	1.41 (0.86–2.31)
High grade		
All	406	0.84 (0.69–1.01)
Serous	259	0.76 (0.60–0.96)
	P_{het}^b	0.05
	P_{het}^c	0.02
DHEAS		
Low grade	49	1.32 (0.89–1.97)
High grade		
All	374	0.93 (0.81–1.07)
Serous	234	0.91 (0.76–1.08)
	P_{het}^b	0.07
	P_{het}^c	0.06
SHBG		
Low grade	38	0.59 (0.33–1.03)
High grade		
All	286	1.12 (0.93–1.36)
Serous	185	1.17 (0.92–1.49)
	P_{het}^b	0.02
	P_{het}^c	0.02

NOTE: Bold numbers indicate statistically significant association.

Abbreviations: DHEAS, dehydroepiandrosterone sulfate; SHBG, sex hormone binding globulin.

^aResults were derived from conditional logistic regression models, additionally adjusted for OC use (never/ever/missing) and parity (never/ever/missing). Pairwise heterogeneity tests were performed, using the likelihood ratio test comparing models assuming (i) the same association between exposure and outcomes compared with (ii) a model assuming different associations for each subtype.^bComparing all high grade subtypes to low grade.^cComparing high grade serous to all low grade.

cases missing data on grade from the type I subgroup (before exclusion, $n = 291$ case-control sets; after exclusion, $n = 77$ case-control sets). However, although of the same general magnitude, the effect estimates were no longer statistically significant (Supplementary Fig. S1).

Sensitivity and subgroup analyses

We observed some evidence of heterogeneity for the androgens and SHBG and overall EOC by menopausal status at blood collection (androstenedione, $P_{\text{het}} = 0.05$; SHBG, $P_{\text{het}} = 0.02$) and age at diagnosis (<55 years vs. ≥ 55 years: androstenedione, $P_{\text{het}} = 0.02$; DHEAS, $P_{\text{het}} = 0.05$; SHBG, $P_{\text{het}} = 0.05$). Both androstenedione and SHBG were positively associated with risk only among women premenopausal at blood collection (androstenedione: premenopausal women, $OR_{\log 2}$, 1.18; 95% CI, 1.03–1.35; postmenopausal women $OR_{\log 2}$, 0.95; 95% CI, 0.82–1.12; SHBG:

premenopausal women, $OR_{\log 2}$, 1.18; 95% CI, 1.00–1.39; postmenopausal women $OR_{\log 2}$, 0.89; 95% CI, 0.76–1.04). No further significant heterogeneity was observed by menopausal status at blood collection. Androstenedione was associated with increased risk of EOC among women diagnosed before age 55 years, but not among women diagnosed at age 55 or older (<55 at diagnosis, $OR_{\log 2}$, 1.21; 95% CI, 1.05–1.40; ≥ 55 years at diagnosis, $OR_{\log 2}$, 0.95; 95% CI, 0.82–1.10). Although the association between DHEAS and SHBG and EOC differed by age at diagnosis, the ORs were not statistically significant in either age at diagnosis subgroup (e.g., SHBG, <55 at diagnosis, $OR_{\log 2}$, 1.16; 95% CI, 0.98–1.3; ≥ 55 years at diagnosis, $OR_{\log 2}$, 0.92; 95% CI, 0.79–1.07).

We observed no heterogeneity in analyses by stage at diagnosis. We observed an attenuation of the association between testosterone and EOC after excluding the FMC ($n = 576$ cases, 43% of sample; after exclusion: $OR_{\log 2}$, 1.06; 95% CI, 0.93–1.21). Overall, ORs were similar for the histologic subtypes after this exclusion, however, no longer statistically significant (e.g., testosterone and endometrioid tumors, before exclusion: $n = 164$, $OR_{\log 2}$, 1.40; 95% CI, 1.03–1.91; after exclusion: $n = 73$, $OR_{\log 2}$, 1.39; 95% CI, 0.81–2.36). The most substantial attenuation was for the association between androstenedione and mucinous tumors (before exclusion: $n = 191$ cases, $OR_{\log 2}$, 1.33; 95% CI, 1.03–1.72; after exclusion: $n = 49$ cases, $OR_{\log 2}$, 1.19; 95% CI, 0.74–1.92). Excluding women diagnosed within 2 years after blood donation did not meaningfully impact the results (data not shown).

Discussion

We investigated prediagnosis circulating concentrations of androgens and risk of EOC overall ($n = 1,331$ cases) and by subtype (case range, $n = 61$ clear cell to 667 serous), in a collaborative reanalysis of 7 nested case-control studies. The association between testosterone and risk of EOC differed by histologic subtype: endogenous androgens were predominantly associated with increased risk of endometrioid and mucinous tumors, whereas no significant associations were observed for serous or clear cell tumors, although some androgens were inversely associated with high-grade serous and endometrioid (type II) disease.

Ovarian cancer is comprised of four predominant histologic subtypes: serous, mucinous, endometrioid, and clear cell. These histologic subtypes differ substantially by molecular alterations at diagnosis and presumed tissue of origin. The majority of serous tumors are high-grade neoplasms; this subtype represents the majority of invasive EOCs. Separate etiologic pathways are hypothesized for low- and high-grade serous EOC. It is hypothesized that a proportion of low-grade serous carcinomas develop from distal epithelium of the fallopian tube that implants on the ovarian surface epithelium (~80%), whereas high-grade serous tumors may arise from serous tubal intraepithelial carcinomas (STIC) within the fimbriated end of the fallopian tube (25, 26). Mucinous carcinomas are hypothesized to develop from the gastrointestinal mucosa or from transitional-type epithelium located at the tubal-peritoneal junction; borderline mucinous ovarian tumors are established precursors for this subtype (19). Both endometrioid and clear cell tumors have been proposed to arise from endometrial tissue, and have been associated with endometriosis and retrograde menstruation (19, 27).

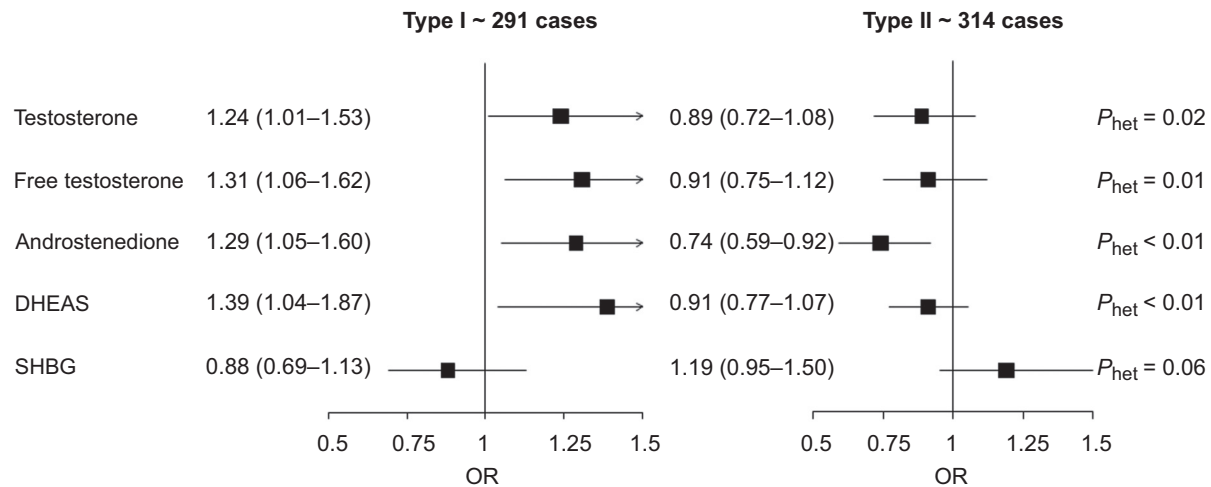


Figure 1.

ORs (95% CI) for EOC for doubling of androgen concentrations and EOC risk by the type I and type II classification: the Ovarian Cancer Cohort Consortium (OC3). Results were derived from conditional logistic regression models, additionally adjusted for OC use (never/ever/missing) and parity (never/ever/missing). Pairwise heterogeneity tests were performed, using the likelihood ratio test comparing models assuming (i) the same association between exposure and outcomes compared with (ii) a model assuming different associations for each subtype. DHEAS, dehydroepiandrosterone sulfate; SHBG, sex hormone binding globulin.

Beyond histologic subgroups, two hypothesized developmental pathways of tumorigenesis (type I and type II) have been defined using tumor molecular genetic characteristics (19, 25); in the absence of data on the tumor molecular profile, EOC is classified as type I or type II based on data on histology and grade. Type I tumors include low-grade serous, low-grade endometrioid, mucinous and malignant Brenner tumors (commonly present with *KRAS*, *BRAF*, *PTEN*, *PIK3CA*, *CTNNB1*, and *ERBB2* mutations)—subtypes that have been hypothesized to develop in a step-wise manner from borderline tumors or endometriosis within or on the surface of the ovary, and are typically diagnosed at earlier disease stage (27). Type II tumors include high-grade serous, high-grade endometrioid, malignant mixed and undifferentiated tumors (typically present with *TP53* mutations, but none of the mutations observed in type I disease; ref. 19). These latter tumors comprise the majority of EOCs, are aggressive, and typically present at an advanced stage.

Prior epidemiologic data suggest risk factor differences by EOC subtype defined by histology (e.g., refs. 12, 15–18) and the developmental pathway (11, 14). Consistent differences by histologic subtype of invasive EOC are observed for hormone-related risk factors, including duration of OC use (lower risk of all histologic subtypes but mucinous; refs. 12, 15), older age at menopause (higher risk of all but mucinous; ref. 12), smoking (higher risk of mucinous, lower risk of clear cell; refs. 12, 17), parity (more strongly protective in non-serous subtypes; ref. 12), postmenopausal hormone therapy (HT) use (higher risk of serous and endometrioid subtypes only; refs. 12, 18), and adiposity (among non-HT users; higher risk of serous and endometrioid subtypes only; ref. 16). Data by the type I/II classification are sparse, but consistently show stronger associations between parity and type I, relative to type II, disease (11, 14). Three prospective studies evaluated circulating estrogens (10, 28) and/or androgens (9, 10) and invasive EOC risk by subtype. Higher concentrations of both estrogens and androgens were associated with increased

risk of non-serous EOC subtypes (9, 10, 28), whereas higher concentrations of androstenedione had opposing effects on risk of type I (higher risk) and type II (lower risk) EOC (9).

In women, androgens are produced in the ovary, adrenal glands, and via peripheral conversion of androgen precursors (e.g., DHEA); in turn, androgens are the substrate for estrogen production by aromatase. DHEAS is a pre-androgen synthesized in the adrenal gland, and subsequently metabolized toward androstenedione and testosterone, or estradiol (29). Androstenedione, an intermediate between DHEA and DHEAS and testosterone, is produced in both the ovary (premenopausal women: 40%; postmenopausal women: 20%–30%) and the adrenal gland. In premenopausal women, approximately 25% of circulating testosterone originates in the ovary, 25% in the adrenal glands, and 50% is metabolized from precursors such as androstenedione in peripheral tissues (e.g., liver, adipose tissue; refs. 29, 30); the proportion of testosterone of ovarian origin is higher in postmenopausal women (~50%; ref. 29). These androgens are correlated with each other (e.g., $r = 0.54$ between DHEAS and androstenedione to $r = 0.69$ between DHEAS and testosterone; adjusted for menopausal status; ref. 6) and weakly correlated with estradiol [e.g., estradiol and testosterone: premenopausal women: $r = 0.08$; ref. 31; postmenopausal women, $r = 0.23$ – 0.38 ; refs. 32, 33; and body mass index ($r = 0.07$ – 0.13); refs. 31–33].

Androgens may (i) directly influence ovarian carcinogenesis through androgen receptor (AR) signaling, or (ii) impact risk through their role as estrogen precursors; associations with estrogens may be most evident in the context of progesterone insufficiency as observed in polycystic ovarian syndrome (PCOS). ARs and estrogen (ER) receptors are expressed in the normal ovary, including ovarian surface epithelial cells and cortical inclusion cysts, and the fallopian tube (34–36). *In vivo* data show that ovarian cancer preferentially develops in a hormonal milieu enriched with androgens (e.g., testosterone induces epithelial neoplasms in guinea pigs; ref. 37) or

estrogens (e.g., estrogen-induced tumor growth in high-grade serous ovarian cancers; refs. 38, 39). The hyperandrogenic PCOS is characterized by functional ovarian hyperandrogenism, with an excess of testosterone produced in the ovarian thecal cells (40); up to 45% of cases additionally present with adrenal hyperandrogenism (41). Estimates of PCOS prevalence range from 5% to 15% (30); the syndrome has highest prevalence among reproductive-age women. PCOS-related androgen excess is observed in both pre- and postmenopausal women (42). Progesterone deficiency is a hallmark of PCOS, resulting in a higher ratio of estrogens to progesterone. PCOS (43, 44) and relatively high levels of estrogens unopposed by progesterone are associated with increased endometrial cancer risk (i.e., estrogen-alone HT; ref. 45; relatively high endogenous estrogens in postmenopausal women; refs. 33, 46). These associations with endometrial cancer may be most relevant to the endometrioid or clear cell EOC, given endometrial tissue is a proposed tissue of origin for these subtypes. PCOS itself has not consistently been associated with ovarian cancer (43, 44, 47), although data by subtype are limited. Data to date suggest both estrogen-alone and estrogen plus progesterone HT are associated with increased risk of endometrioid EOC (18).

In the current study, we evaluated three members of the androgen synthesis pathway, DHEAS, androstenedione, testosterone, and EOC risk by histology (i.e., accounting for hypothesized differences in cell of origin) and developmental pathway (i.e., "less" relative to "more" aggressive disease). We observed a significant positive association between testosterone and risk of endometrioid ovarian cancer. There is limited *in vitro* evidence to support a role of androgens in the etiology of endometrioid EOC (34, 48). However, given the possible common tissue of origin, it is plausible that androgens impact risk similarly in both endometrial cancer and endometrioid EOC. With respect to endometrial cancer, recent *in vivo* data have demonstrated that androgens induce epithelial proliferation in the mouse uterus (49), and epidemiologic data provide some support for an association between androgens and endometrial cancer risk (50). Together, these data on endometrial cancer provide indirect evidence supporting an association between androgens and endometrioid EOC. Androgens are an intermediate on the estrogen-synthesis pathway, and estrogen exposure unopposed by progesterone may be the underlying biological mechanism linking androgens to endometrioid EOC, particularly if in the context of progesterone deficiency, as in PCOS and in postmenopausal women. Prior research has linked higher early pregnancy estradiol concentrations to a 2.5-fold increase in risk of endometrioid EOC (10), and postmenopausal HT use (12, 18) and adiposity (16) are associated with increased risk of this subtype. We adjusted for BMI in a sensitivity analysis, given (i) the association between PCOS and obesity and (ii) adipose tissue is a key site of metabolism of androgens to estrogens in postmenopausal women. Adjustment for BMI did not impact the results. Data on history of PCOS were not available.

Higher concentrations of all investigated androgens, except DHEAS, were significantly associated with increased risk of mucinous tumors. Emerging data suggest the ovarian stroma proximal to mucinous EOC has higher concentrations of sex-steroid producing enzymes than distant stroma, providing support for a role for sex steroids in the development of mucinous disease (35). Androgens (directly, or after conversion to estrogens) may contribute to growth promotion in the early stages of

mucinous disease; however, to our knowledge, the androgen responsiveness of mucinous tumors is not well characterized, and data on ER expression are limited (51, 52). The precise biological mechanisms underlying the observed associations between androgens and mucinous tumors remain an open question.

In line with two prior prospective studies (9, 10), both included in this analysis, we observed no association with pre-diagnosis androgen concentrations and increased risk of serous carcinomas. Recent data on estrogens and ovarian cancer are in line with our results on androgens, with no association observed between estrogens and risk of invasive serous tumors in the FMC (first-trimester estrogens; ref. 10) or among postmenopausal women in the Women's Health Initiative (28). We observed no associations with clear cell disease. However, sample size for this subtype was limited.

We observed significant heterogeneity in the strength of associations between androgens and risk of type I vs. type II tumors; higher androgen concentrations were associated with higher risk of type I, but lower risk of type II (predominantly high grade serous), tumors. These results are in agreement with the single prior study on endogenous androgens and EOC risk using the dualistic model classification (9); these data from the European Prospective Investigation into Cancer and Nutrition (EPIC) were included in the current analysis. There is indirect evidence for differences in hormone dependency in type I and type II tumors, based on the variation of ER expression between low-grade (ER expression: 58%) and high-grade serous carcinomas (ER expression: 27%; ref. 53). However, the mechanisms linking androgen concentrations to lower risk of type II tumors in our study are unclear. Although chance and residual confounding may explain the results, future work should explicitly examine the impact of androgens on type II tumors.

Given the large sample size, our study was powered to investigate risk associations for less common tumors (e.g., mucinous tumors) and by developmental pathway (type I/type II). A general weakness of pooled analyses is the difference in data availability of covariates and differences in laboratory methods. In this investigation, data from each cohort were centrally compiled and harmonized and we addressed differences in absolute biomarker concentrations (i) using study-specific tertiles and (ii) standardizing hormone measurements using study-specific mean concentrations. Results were robust regardless of whether we calculated ORs from the pooling of individual data or from meta-analysis. For some of the investigated hormones, the number of sets that could be used was reduced for subgroup analyses, which resulted in reduced power. In our primary analyses using the developmental pathway classification, we included all mucinous and clear cell tumors in the "type I" classification, as their classification is independent of grade. If there were systematic differences in the observed associations with type I disease in cases with and without grade data, this may result in a biased interpretation of the differences between type I and type II EOC. However, the associations observed in our primary analysis and in a sensitivity analysis restricted to women with complete data on grade were of similar magnitude. Many statistical tests are reported; therefore, some significant observations may be due to chance. However, all statistical analyses were hypothesis driven. In line with the majority of other epidemiological studies, a single measurement of biomarkers was used to assess risk associations. This single measurement may not reflect long-term average concentrations and the storage time and conditions may impact the true value of the

biochemical indicators. However, the stability of androgen measurements over time has been shown previously for a period over at least 2–3 years: (i) premenopausal women [ICC ranged from 0.58 (androstenedione) up to 0.81 (DHEAS; ref. 54) and (ii) postmenopausal women [ICC ranged from 0.66 (androstenedione) up to 0.92 (SHBG; ref. 55).

The testosterone synthesis pathway (e.g., DHEAS, androstenedione, testosterone) may play an important role in the onset and progression of a subset of epithelial invasive ovarian carcinomas. Androgens may either have a direct impact on ovarian carcinogenesis, or act through increased synthesis of other steroid hormones (e.g., estrogens); this is an area for future epidemiologic research. Although androgens were associated with increased risk of non-serous tumors, we observed an inverse association between androstenedione and high grade serous tumors. In addition to providing novel findings on hormone-related pathways in ovarian carcinogenesis, this study supports emerging data on the heterogeneity of epithelial invasive ovarian cancer and underscores the importance of examining etiologic differences for subtypes.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Disclaimer

The authors assume full responsibility for analyses and interpretation of these data.

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