Androgens are differentially associated with ovarian cancer subtypes in the Ovarian Cancer Cohort Consortium

Jennifer Ose[1,2], Elizabeth M. Poole[3], Helena Schock[1], Matti Lehtinen[4], Alan A. Arslan[5], Anne Zeleniuch-Jacquotte[6], Kala Visvanathan[7], Kathy Helzlsouer[7], Julie E. Buring[8,9], I-Min Lee[8,9], Anne Tjønneland[10], Laure Dossus[11], Antonia Trichopoulou[12,13], Giovanna Masala[14], N. Charlotte Onland-Moret[15], Elisabete Weiderpass[16,17,18,19], Eric J. Duell[20], Annika Idahl[21], Ruth C. Travis[22], Sabina Rinaldi[23], Melissa A. Merritt[24], Britton Trabert[25], Nicolas Wentzensen[25], Shelley S. Tworoger[3,9], Rudolf Kaaks[1], Renée T. Fortner[1]

[1]Division of Cancer Epidemiology, German Cancer Research Center, Heidelberg, Baden-Württemberg, Germany
[2]Department of Population Sciences, Huntsman Cancer Institute, University of Utah, Salt Lake City, Utah, USA
[3]Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA
[4]Karolinska Institutet, Department of Laboratory Medicine, Huddinge, Sweden
[5]Departments of Obstetrics and Gynecology, Population Health, and Environmental Medicine, New York University School of Medicine; Perlmutter Cancer Center, New York University, New York, New York
[6]Departments of Population Health, and Environmental Medicine, New York University School of Medicine; Perlmutter Cancer Center, New York University, New York, New York
[7]Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland
[8]Division of Preventive Medicine, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA
[9]Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA, USA
[10]Unit of Diet, Genes and Environment, Danish Cancer Society Research Center, Copenhagen, Denmark
[11]INSERM, Centre for research in Epidemiology and Population Health (CESP), U1018, Nutrition, Hormones and Women's Health team, Villejuif, France
[12]Hellenic Health Foundation, Athens, Greece
[13]WHO Collaborating Center for Nutrition and Health, Unit of Nutritional Epidemiology and Nutrition in Public Health, Dept. of Hygiene, Epidemiology and Medical Statistics, School of Medicine, National and Kapodistrian University of Athens, Greece
[14]Molecular and Nutritional Epidemiology Unit, Cancer Research and Prevention Institute – ISPO, Florence – Italy
[15] Department of Epidemiology, Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, The Netherlands
[16]Department of Community Medicine, Faculty of Health Sciences, University of Tromsø, The Arctic University of Norway, Tromsø, Norway.
[17]Department of Research, Cancer Registry of Norway, Oslo, Norway
[18]Department of Medical Epidemiology and Biostatistics, Karolinska Institute, Stockholm, Sweden
[19]Genetic Epidemiology Group, Folkhälsan Research Center, Helsinki, Finland
[20]Unit of Nutrition and Cancer, Cancer Epidemiology Research Program, Catalan Institute of Oncology (ICO-IDIBELL), Barcelona, Spain
[21]Department of Clinical Sciences, Obstetrics and Gynecology, Umea University, Umea, Sweden
[22]Cancer Epidemiology Unit, Nuffield Department of Population Health, University of Oxford, Oxford, UK
[23]Section of Nutrition and Metabolism, International Agency for Research on Cancer (IARC), Lyon, France
[24]Department of Epidemiology and Biostatistics, School of Public Health, Imperial College London, London, UK
[25]Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Department of Health and Human Services, Bethesda, MD, USA

Running title: Circulating androgens and ovarian cancer risk by subtype

Key words: ovarian cancer, androgens, histologic subtypes, developmental pathways

Grant Support
The OC3 is supported by Department of Defense Ovarian Cancer Research Program Grant No. W81XWH-12-1-0561. RT Fortner was supported by a Marie Curie International Incoming Fellowship of the European Commission's Seventh Framework Programme (MC-IIF-623984).

The coordination of EPIC is financially supported by the European Commission (DG-SANCO) and the International Agency for Research on Cancer. The national cohorts are supported by Danish Cancer Society (Denmark); Ligue Contre le Cancer, Institut Gustave Roussy, Mutuelle Générale de l'Education Nationale, Institut National de la Santé et de la Recherche Médicale (INSERM) (France); German Cancer Aid, German Cancer Research Center (DKFZ), Federal Ministry of Education and Research (BMBF), Deutsche Krebshilfe, Deutsches Krebsforschungszentrum and Federal Ministry of Education and Research (Germany); the Hellenic Health Foundation (Greece); Associazione Italiana per la Ricerca sul Cancro-AIRC-Italy and National Research Council (Italy); Dutch Ministry of Public Health, Welfare and Sports (VWS), Netherlands

Cancer Registry (NKR), LK Research Funds, Dutch Prevention Funds, Dutch ZON (Zorg Onderzoek Nederland), World Cancer Research Fund (WCRF), Statistics Netherlands (The Netherlands); ERC-2009-AdG 232997 and Nordforsk, Nordic Centre of Excellence programme on Food, Nutrition and Health (Norway); Health Research Fund (FIS), PI13/00061 to Granada; PI13/01162 to EPIC-Murcia), Regional Governments of Andalucía, Asturias, Basque Country, Murcia and Navarra, ISCIII RETIC (RD06/0020) (Spain); Swedish Cancer Society, Swedish Research Council and County Councils of Skåne and Västerbotten (Sweden); Cancer Research UK (14136 to EPIC-Norfolk; C570/A16491 and C8221/A19170 to EPIC-Oxford), Medical Research Council (1000143 to EPIC-Norfolk, MR/M012190/1 to EPIC-Oxford) (United Kingdom). For information on how to submit an application for gaining access to EPIC data and/or bio-specimens, please follow the instructions at http://epic.iarc.fr/access/index.php

Support was provided by the following National Institutes of Health/NCI grants: R01 CA120061 (Finnish Maternity Cohort); UM1 CA186107, P01 CA87969, UM1 CA176726, and R01 CA67262 (Nurses' Health Study, Nurses' Health Study II); HL043851, HL080467, and HL099355 (Women's Health Study).

CLUEII was supported by National Cancer Institute grant numbers: CA-97857, CA-86308; Grant sponsor: M. Jean Goutal (donation): Grant sponsors: The Woodrow Wilson Foundation/Johnson and Johnson, The Lloyds TSB Charitable Foundation for the Channel Islands.

Corresponding author: Renée T. Fortner,
Division of Cancer Epidemiology,
German Cancer Research Center (DKFZ)
Im Neuenheimer Feld 280
69120 Heidelberg, Germany.
Phone: + 49 (0) 6221 42 2241
Fax: + 49 (0) 6221 42 2203
E-mail: r.fortner@dkfz.de

Conflicts of Interest: The authors report no conflicts of interest.

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 pre-diagnosis 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 pre-diagnosis 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 controls. Multivariable conditional logistic regression was used to assess risk associations in pooled individual data. Testosterone was positively associated with EOC risk (all subtypes combined, Odds Ratio $(OR)_{log2}$=1.12 [95% Confidence Interval (CI) 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.

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 signalling 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) (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, i.e., type I vs. 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.

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 pre-diagnosis 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). Histomorphological data was 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) (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 Supplemental Table S2. Free testosterone was calculated based on measured concentrations of

testosterone and SHBG, with albumin assumed to be a constant 40g/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 supplemental methods; Supplemental Table S3). Conditional logistic regression was used to estimate odds ratios (OR) and 95% confidence intervals (CI). ORs were estimated using log2-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 based on 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 supplemental methods.

SAS Statistical Software, version 9.3 (SAS Institute, Cary NC, USA) 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) (Supplemental Table S4).

Androgens and overall EOC risk

A doubling of testosterone (i.e., 1-unit increase in log_2-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 [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 (Supplemental Table S5); however, the OR comparing highest vs. lowest quintile of testosterone was not statistically significant (OR_{Q5-Q1}=1.22 [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 [1.03-1.91]), but not with serous or clear cell tumors (e.g., serous tumors: OR_{log2}=0.96 [0.84-1.11]). Free testosterone and androstenedione were associated with increased risk of mucinous tumors (e.g., androstenedione: OR_{log2}=1.33 [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 [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 [0.86-2.31]; high grade serous OR_{log2}=0.76 [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) (Figure 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 [1.05-1.60]; cases n=287; type II: OR_{log2}=0.74 [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 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, while of the same general magnitude, the effect estimates were no longer statistically significant (Supplemental Figure 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_{het}=0.05; SHBG, p_{het}=0.02) and age at diagnosis (<55 years vs ≥55 years: androstenedione, p_{het}=0.02; DHEAS, p_{het}=0.05; SHBG, p_{het}=0.05). Both androstenedione and SHBG were positively associated with risk only among women premenopausal at blood collection (androstenedione: premenopausal women, OR_{log2}=1.18 [1.03-1.35], postmenopausal women OR_{log2}=0.95 [0.82-1.12]; SHBG: premenopausal women, OR_{log2}=1.18 [1.00-1.39], postmenopausal women OR_{log2}=0.89 [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_{log2}=1.21 [1.05-1.40], ≥55 years at diagnosis, OR_{log2}=0.95 [0.82-1.10]). While 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_{log2}=1.16 [0.98-1.38], ≥55 years at diagnosis, OR_{log2}=0.92 [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_{log2}=1.06 [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_{log2}=1.40 [1.03 - 1.91]; after exclusion: n=73, OR_{log2}=1.39 [0.81 - 2.36]. The most substantial attenuation was for the association between androstenedione and mucinous tumors (before exclusion: n=191 cases, OR_{log2}=1.33 [1.03 - 1.72]; after exclusion: n=49 cases, OR_{log2}=1.19 [0.74 - 1.92]). Excluding women diagnosed within two years after blood donation did not meaningfully impact the results (data not shown).

Discussion

We investigated pre-diagnosis 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 re-analysis 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, while 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%), while 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).

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) (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. (12, 15-18)) and 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; (12, 15)), older age at menopause (higher risk of all but mucinous; (12)), smoking (higher risk of mucinous, lower risk of clear cell; (12, 17)), parity (more strongly protective in non-serous subtypes; (12)), postmenopausal hormone therapy (HT) use (higher risk of serous and endometrioid subtypes only; (12, 18)), and adiposity (among non-HT users; higher risk of serous and endometrioid subtypes only); (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) (29, 30); the proportion of testosterone of ovarian origin is higher in postmenopausal women (~50%) (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 (6)) and weakly correlated with estradiol (e.g., estradiol and testosterone: premenopausal women: r=0.08 (31); postmenopausal women, r=0.23-0.38; (32, 33)) and body mass index (r=0.07-0.13; (31-33)).

Androgens may (1) directly influence ovarian carcinogenesis through androgen receptor (AR) signaling, or (2) 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 (37)) or estrogens (e.g., estrogen-induced tumor growth in high-grade serous ovarian cancers) (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 (45), relatively high endogenous estrogens in postmenopausal women (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), though 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, this data on endometrial cancer provides 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 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 (1) the association between PCOS and obesity and

(2) 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) (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%) (53). However, the mechanisms linking androgen concentrations to lower risk of type II tumors in our study are unclear. While 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: (1) premenopausal women [ICC ranged from 0.58 (androstenedione) up to 0.81 (DHEAS), (54) and (2) postmenopausal women [ICC ranged from 0.66 (androstenedione) up to 0.92 (SHBG) (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. While 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.

Acknowledgements
We thank the participants and staff of the Nurses' Health Study and Nurses' Health Study II for their valuable contributions as well as the following state cancer registries for their help: Alabama, Arizona, Arkansas, California, Colorado, Connecticut, Delaware, Florida, Georgia, Idaho, Illinois, Indiana, Iowa, Kentucky, Louisiana, Maine, Maryland, Massachusetts, Michigan, Nebraska, New Hampshire, New Jersey, New York, North Carolina, North Dakota, Ohio, Oklahoma, Oregon, Pennsylvania, Rhode Island, South Carolina, Tennessee, Texas, Virginia, Washington, and Wyoming.

We thank the participants and staff of CLUE II for their contributions as well as the Maryland Cancer Registry, Center for Cancer Surveillance and Control, Department of Health and Mental Hygiene, 201 W. Preston Street, Room 400, Baltimore, MD 21201, http://phpa.dhmh.maryland.gov/cancer, 410-767-4055. We acknowledge the State of Maryland, the Maryland Cigarette Restitution Fund, and the National Program of Cancer Registries of the Centers for Disease Control and Prevention for the funds that support the collection and availability of the cancer registry data.

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

References

1. Lukanova A, Kaaks R. Endogenous hormones and ovarian cancer: epidemiology and current hypotheses. Cancer Epidemiol Biomarkers Prev. 2005;14:98-107.
2. Risch HA. Hormonal etiology of epithelial ovarian cancer, with a hypothesis concerning the role of androgens and progesterone. J Natl Cancer Inst. 1998;90:1774-86.
3. Modugno F, Laskey RA, Smith AL, Andersen CL, Haluska P, Oesterreich S. Hormone response in ovarian cancer: time to reconsider as a clinical target? Endocrine-related cancer. 2012.
4. Edmondson RJ, Monaghan JM, Davies BR. The human ovarian surface epithelium is an androgen responsive tissue. Br J Cancer. 2002;86:879-85.
5. Helzlsouer KJ, Alberg AJ, Gordon GB, Longcope C, Bush TL, Hoffman SC, et al. Serum gonadotropins and steroid hormones and the development of ovarian cancer. JAMA. 1995;274:1926-30.
6. Lukanova A, Lundin E, Akhmedkhanov A, Micheli A, Rinaldi S, Zeleniuch-Jacquotte A, et al. Circulating levels of sex steroid hormones and risk of ovarian cancer. International journal of cancer. 2003;104:636-42.
7. Tworoger SS, Lee IM, Buring JE, Hankinson SE. Plasma androgen concentrations and risk of incident ovarian cancer. Am J Epidemiol. 2008;167:211-8.
8. Rinaldi S, Dossus L, Lukanova A, Peeters PH, Allen NE, Key T, et al. Endogenous androgens and risk of epithelial ovarian cancer: results from the European Prospective Investigation into Cancer and Nutrition (EPIC). Cancer Epidemiol Biomarkers Prev. 2007;16:23-9.
9. Ose J, Fortner RT, Rinaldi S, Schock H, Overvad K, Tjonneland A, et al. Endogenous androgens and risk of epithelial invasive ovarian cancer by tumor characteristics in the European Prospective Investigation into Cancer and Nutrition. International journal of cancer. 2015;136:399-410.
10. Schock H, Surcel HM, Zeleniuch-Jacquotte A, Grankvist K, Lakso HA, Fortner RT, et al. Early pregnancy sex steroids and maternal risk of epithelial ovarian cancer. Endocrine-related cancer. 2014;21:831-44.
11. Fortner RT, Ose J, Merritt MA, Schock H, Tjonneland A, Hansen L, et al. Reproductive and hormone-related risk factors for epithelial ovarian cancer by histologic pathways, invasiveness and histologic subtypes: Results from the EPIC cohort. International journal of cancer. 2015;137:1196-208.
12. Wentzensen N, Poole EM, Trabert B, White E, Arslan AA, Patel AV, et al. Ovarian Cancer Risk Factors by Histologic Subtype: An Analysis From the Ovarian Cancer Cohort Consortium. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2016;34:2888-98.
13. Prat J. Ovarian carcinomas: five distinct diseases with different origins, genetic alterations, and clinicopathological features. Virchows Arch. 2012;460:237-49.
14. Merritt MA, De Pari M, Vitonis AF, Titus LJ, Cramer DW, Terry KL. Reproductive characteristics in relation to ovarian cancer risk by histologic pathways. Human Reproduction. 2013;28:1406-17.
15. Collaborative Group on Epidemiological Studies of Ovarian C, Beral V, Doll R, Hermon C, Peto R, Reeves G. Ovarian cancer and oral contraceptives: collaborative reanalysis of data from 45 epidemiological studies including 23,257 women with ovarian cancer and 87,303 controls. Lancet. 2008;371:303-14.
16. Collaborative Group on Epidemiological Studies of Ovarian C. Ovarian cancer and body size: individual participant meta-analysis including 25,157 women with ovarian cancer from 47 epidemiological studies. PLoS medicine. 2012;9:e1001200.
17. Collaborative Group on Epidemiological Studies of Ovarian C, Beral V, Gaitskell K, Hermon C, Moser K, Reeves G, et al. Ovarian cancer and smoking: individual participant meta-analysis including 28,114 women with ovarian cancer from 51 epidemiological studies. The Lancet Oncology. 2012;13:946-56.

18. Collaborative Group On Epidemiological Studies Of Ovarian Cancer. Menopausal hormone use and ovarian cancer risk: individual participant meta-analysis of 52 epidemiological studies. Lancet. 2015.
19. Kurman RJ, Shih Ie M. Molecular pathogenesis and extraovarian origin of epithelial ovarian cancer--shifting the paradigm. Hum Pathol. 2011;42:918-31.
20. Sodergard R, Backstrom T, Shanbhag V, Carstensen H. Calculation of free and bound fractions of testosterone and estradiol-17 beta to human plasma proteins at body temperature. J Steroid Biochem. 1982;16:801-10.
21. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials. 1986;7:177-88.
22. Smith-Warner SA, Spiegelman D, Ritz J, Albanes D, Beeson WL, Bernstein L, et al. Methods for pooling results of epidemiologic studies: the Pooling Project of Prospective Studies of Diet and Cancer. Am J Epidemiol. 2006;163:1053-64.
23. Wang M, Spiegelman D, Kuchiba A, Lochhead P, Kim S, Chan AT, et al. Statistical methods for studying disease subtype heterogeneity. Statistics in medicine. 2016;35:782-800.
24. Schock H, Zeleniuch-Jacquotte A, Lundin E, Grankvist K, Lakso HA, Idahl A, et al. Hormone concentrations throughout uncomplicated pregnancies: a longitudinal study. BMC Pregnancy Childbirth. 2016;16:146.
25. Nik NN, Vang R, Shih Ie M, Kurman RJ. Origin and pathogenesis of pelvic (ovarian, tubal, and primary peritoneal) serous carcinoma. Annu Rev Pathol. 2014;9:27-45.
26. Zeppernick F, Meinhold-Heerlein I, Shih Ie M. Precursors of ovarian cancer in the fallopian tube: serous tubal intraepithelial carcinoma--an update. J Obstet Gynaecol Res. 2015;41:6-11.
27. Seidman JD, Cho KR, Ronnett BM, Kurman RJ. Surface Epithelial Tumors of the Ovary. In: Kurman RJ, Hedrick Ellenson L, Ronnett BM, editors. Blaustein's Pathology of the Female Genital Tract, 6th ed. USA: Springer; 2011.
28. Trabert B, Brinton LA, Anderson GL, Pfeiffer RM, Falk RT, Strickler HD, et al. Circulating Estrogens and Postmenopausal Ovarian Cancer Risk in the Women's Health Initiative Observational Study. Cancer Epidemiol Biomarkers Prev. 2016;25:648-56.
29. Yen SS, Jaffe RB, Barbieri RL. Reproductive Endocrinology. 4th ed. Philadelphia: W.B. Saunders Company; 1999.
30. Rosenfield RL, Ehrmann DA. The Pathogenesis of Polycystic Ovary Syndrome (PCOS): The Hypothesis of PCOS as Functional Ovarian Hyperandrogenism Revisited. Endocrine reviews. 2016;37:467-520.
31. Kaaks R, Berrino F, Key T, Rinaldi S, Dossus L, Biessy C, et al. Serum Sex Steroids in Premenopausal Women and Breast Cancer Risk Within the European Prospective Investigation into Cancer and Nutrition (EPIC). Journal of the National Cancer Institute. 2005;97:755-65.
32. James RE, Lukanova A, Dossus L, Becker S, Rinaldi S, Tjønneland A, et al. Postmenopausal serum sex steroids and risk of hormone receptor-positive and -negative breast cancer: a nested case-control study. Cancer Prevention Research. 2011;4:1626-35.
33. Lukanova A, Lundin E, Micheli A, Arslan A, Ferrari P, Rinaldi S, et al. Circulating levels of sex steroid hormones and risk of endometrial cancer in postmenopausal women. International journal of cancer. 2004;108:425-32.
34. Gibson DA, Simitsidellis I, Collins F, Saunders PT. Evidence of androgen action in endometrial and ovarian cancers. Endocr Relat Cancer. 2014;21:T203-18.
35. Blanco LZ, Jr., Kuhn E, Morrison JC, Bahadirli-Talbott A, Smith-Sehdev A, Kurman RJ. Steroid hormone synthesis by the ovarian stroma surrounding epithelial ovarian tumors: a potential mechanism in ovarian tumorigenesis. Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc. 2017.
36. Kyriakidis I, Papaioannidou P. Estrogen receptor beta and ovarian cancer: a key to pathogenesis and response to therapy. Archives of gynecology and obstetrics. 2016;293:1161-8.
37. Silva EG, Tornos C, Fritsche HA, Jr., el-Naggar A, Gray K, Ordonez NG, et al. The induction of benign epithelial neoplasms of the ovaries of guinea pigs by testosterone stimulation: a

potential animal model. Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc. 1997;10:879-83.
38. Silva EG, Tornos C, Deavers M, Kaisman K, Gray K, Gershenson D. Induction of epithelial neoplasms in the ovaries of guinea pigs by estrogenic stimulation. Gynecologic oncology. 1998;71:240-6.
39. Ciucci A, Zannoni GF, Buttarelli M, Lisi L, Travaglia D, Martinelli E, et al. Multiple direct and indirect mechanisms drive estrogen-induced tumor growth in high grade serous ovarian cancers. Oncotarget. 2016;7:8155-71.
40. Ehrmann DA. Polycystic ovary syndrome. The New England journal of medicine. 2005;352:1223-36.
41. Luque-Ramirez M, Escobar-Morreale HF. Adrenal Hyperandrogenism and Polycystic Ovary Syndrome. Current pharmaceutical design. 2016;22:5588-602.
42. Markopoulos MC, Kassi E, Alexandraki KI, Mastorakos G, Kaltsas G. Hyperandrogenism after menopause. European journal of endocrinology. 2015;172:R79-91.
43. Gottschau M, Kjaer SK, Jensen A, Munk C, Mellemkjaer L. Risk of cancer among women with polycystic ovary syndrome: a Danish cohort study. Gynecologic oncology. 2015;136:99-103.
44. Barry JA, Azizia MM, Hardiman PJ. Risk of endometrial, ovarian and breast cancer in women with polycystic ovary syndrome: a systematic review and meta-analysis. Human reproduction update. 2014;20:748-58.
45. Beral V, Bull D, Reeves G, Million Women Study C. Endometrial cancer and hormone-replacement therapy in the Million Women Study. Lancet. 2005;365:1543-51.
46. Allen NE, Key TJ, Dossus L, Rinaldi S, Cust A, Lukanova A, et al. Endogenous sex hormones and endometrial cancer risk in women in the European Prospective Investigation into Cancer and Nutrition (EPIC). Endocrine-related cancer. 2008;15:485-97.
47. Harris HR, Titus LJ, Cramer DW, Terry KL. Long and irregular menstrual cycles, polycystic ovary syndrome, and ovarian cancer risk in a population-based case-control study. International journal of cancer. 2017;140:285-91.
48. Maliqueo MA, Quezada S, Clementi M, Bacallao K, Anido M, Johnson C, et al. Potential action of androstenedione on the proliferation and apoptosis of stromal endometrial cells. Reprod Biol Endocrinol. 2004;2:81.
49. Simitsidellis I, Gibson DA, Cousins FL, Esnal-Zufiaurre A, Saunders PT. A Role for Androgens in Epithelial Proliferation and Formation of Glands in the Mouse Uterus. Endocrinology. 2016;157:2116-28.
50. Clendenen TV, Hertzmark K, Koenig KL, Lundin E, Rinaldi S, Johnson T, et al. Premenopausal Circulating Androgens and Risk of Endometrial Cancer: results of a Prospective Study. Horm Cancer. 2016;7:178-87.
51. Tkalia IG, Vorobyova LI, Svintsitsky VS, Nespryadko SV, Goncharuk IV, Lukyanova NY, et al. Clinical significance of hormonal receptor status of malignant ovarian tumors. Exp Oncol. 2014;36:125-33.
52. Sieh W, Kobel M, Longacre TA, Bowtell DD, deFazio A, Goodman MT, et al. Hormone-receptor expression and ovarian cancer survival: an Ovarian Tumor Tissue Analysis consortium study. The Lancet Oncology. 2013;14:853-62.
53. Escobar J, Klimowicz AC, Dean M, Chu P, Nation JG, Nelson GS, et al. Quantification of ER/PR expression in ovarian low-grade serous carcinoma. Gynecologic oncology. 2013;128:371-6.
54. Missmer SA, Spiegelman D, Bertone-Johnson ER, Barbieri RL, Pollak MN, Hankinson SE. Reproducibility of plasma steroid hormones, prolactin, and insulin-like growth factor levels among premenopausal women over a 2- to 3-year period. Cancer Epidemiol Biomarkers Prev. 2006;15:972-8.
55. Hankinson SE, Manson JE, Spiegelman D, Willett WC, Longcope C, Speizer FE. Reproducibility of plasma hormone levels in postmenopausal women over a 2-3-year period. Cancer Epidemiol Biomarkers Prev. 1995;4:649-54.

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 %[1]	Ever OC use, %[1]	Postmenopausal, %[2]	Mean BMI (SD)
Clue II	≠	Case	46	60.8 (13.0)	19%	20%	85%	26.3 (5.8)
	≠	Control	91	61.0 (12.9)	13%	13%	86%	25.4 (4.6)
EPIC	Ose et al. 2014	Case	451	55.9 (8.5)	18%	37%	77%	26.8 (4.9)
		Control	867	55.9 (8.6)	12%	45%	77%	26.3 (4.7)
FMC	Schock et al. 2014	Case	576	32.5 (4.8)	0%	≠≠	0%	≠≠
		Control	1,433	32.5 (4.7)	0%	≠≠	0%	≠≠
NHS	Tworoger et al. 2007	Case	117	57.7 (6.5)	8%	41%	79%	24.8 (4.8)
		Control	348	57.7 (6.5)	5%	47%	79%	24.7 (4.1)
NHS II	Tworoger et al. 2007	Case	15	46.1 (4.4)	20%	93%	20%	29.6 (9.8)
		Control	44	45.8 (4.3)	23%	86%	18%	25.9 (5.8)
NYUWHS	Lukanova et al. 2002	Case	63	52.6 (8.6)	47%	29%	56%	24.5 (3.8)
		Control	112	52.0 (8.5)	38%	36%	54%	25.9 (4.3)
WHS	Tworoger et al. 2007	Case	63	55.7 (7.2)	25%	65%	75%	24.5 (3.9)
		Control	122	55.5 (7.0)	15%	71%	70%	25.1 (4.4)
Total		Case	1,331	45.8 (13.7)	11%	40%	42%	26.2 (5.1)
		Control	3,017	44.8 (13.7)	6%	47%	39%	25.8 (4.6)

[1]Among women with data: parity 2.8% missing; OC use 47% missing (excluding FMC: 2.3% missing)

[2]At blood collection

BMI = body mass index; OC = oral contraceptive; OC3 = Ovarian Cancer Cohort Consortium; SHBG = sex hormone binding globulin; SD = standard deviation; 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; WHS = Women's Health Study.

≠ Data from Clue II have not been published.

≠≠ Information on BMI and OC use was not collected in the FMC

Table 2: Odds ratios (95% CI) for EOC overall and by histologic subtypes in tertiles and for doubling of androgen concentrations: the Ovarian Cancer Cohort Consortium (OC3)[1]

	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[2]	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[3]	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}[4]															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[2]	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[3]	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)	0.26
p_{het}[4]															0.12
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 (0.4 - 1.13)		51	0.90 (0.57 - 1.42)		15	0.93 (0.41 - 2.10)	
T3[2]	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[3]	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}[4]															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[2]	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[3]	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}[4]															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[2]	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[3]	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}[4]															0.43

[1]Results were derived from conditional logistic regression models, additionally adjusted for OC use (never/ever/missing) and parity (never/ever/missing); [2]The 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); [3]Linear trends for doubling of hormone concentrations were estimated on $\log_2$ scale; [4] Pair-wise heterogeneity tests were performed, using the likelihood ratio test comparing models assuming (1) the same association between exposure and outcomes compared to (2) a model assuming different associations for each subtype.

DHEAS=dehydroepiandrosterone sulfate; SHBG=sex hormone binding globulin

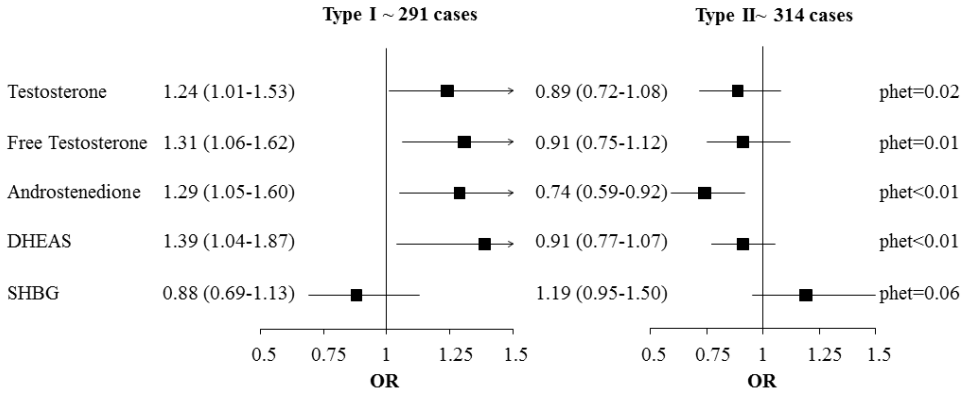
Table 3: Odds ratios (95% CI) for EOC for doubling of androgen concentrations and stratified by grade at diagnosis overall and for serous tumors: the Ovarian Cancer Cohort Consortium (OC3) [1]

	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}[2]	0.25
	p_{het}[3]	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}[2]	0.24
	p_{het}[3]	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}[2]	0.05
	p_{het}[3]	**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}[2]	0.07
	p_{het}[3]	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}[2]	**0.02**
	p_{het}[3]	**0.02**

[1]Results were derived from conditional logistic regression models, additionally adjusted for OC use (never/ever/missing) and parity (never/ever/missing). Pair-wise heterogeneity tests were performed, using the likelihood ratio test comparing models assuming (1) the same association between exposure and outcomes compared to (2) a model assuming different associations for each subtype. [2]Comparing all high grade subtypes to low grade. [3]Comparing high grade serous to all low grade. DHEAS=dehydroepiandrosterone sulfate; SHBG=sex hormone binding globulin

Figure 1.

Title: Odds ratios (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). Pair-wise heterogeneity tests were performed, using the likelihood ratio test comparing models assuming (1) the same association between exposure and outcomes compared to (2) a model assuming different associations for each subtype. DHEAS=dehydroepiandrosterone sulfate; SHBG=sex hormone binding globulin

Online-Only Supplemental Material

Study population and methods

The study population was based on the following cohorts: the 'Washington Country, MD Study 'Give us a clue to cancer and heart disease' (CLUE) II, European Prospective Investigation into Cancer and Nutrition (EPIC), the Finnish Maternity Cohort (FMC), the Nurses' Health Study (NHS), NHS II, the Harvard Women's Health Study (WHS) and the New York University Women's Health Study (NYUWHS) (Table S1). In all cohorts, cases were individually matched to two (CLUE II, EPIC, NYUWHS, WHS) or up to three controls (FMC, NHS, NHS II) on age, date (or follow-up time in EPIC), menopausal status at blood collection and day or phase of menstrual cycle in premenopausal women (with exception of the FMC, which was restricted to currently pregnant women).

Selection of case patients and control participants

EOC cases in the participating cohorts are ascertained by (1) self-report with subsequent medical record confirmation (2) and/or linkage to cancer registries that in each study generally is estimated to be >95% complete. Analyses were limited to women diagnosed with invasive ovarian carcinomas and with data on histologic subtype.

Data on tumor characteristics

The OC3 database contained complete information on histomorphology: 50% of tumors were of serous histology (n=667), 15% mucinous (n=193), 12% endometrioid (n=166), 5% clear cell (n=61) and 18% other (malignant epithelial neoplasms, carcinoma, malignant mixed Müllerian or malignant Brenner tumors; n=244). Information on tumor stage was 82% complete and cases with local disease were classified as low stage (23%), whereas cases with regional or metastatic disease were classified as high stage (77%). Data on grade were provided by CLUE II, EPIC, NHS, NHS II, WHS and NYUWHS, and were available for 36% of cases. Well differentiated tumors were classified as low grade (12%); moderately and

poorly/undifferentiated tumors as high grade (88%). In the absence of data on molecular genetics and immunohistochemistry, information on histology and grade can be used to classify tumors as put forward by Kurman and colleagues [1].

Assessment of reproductive factors and lifestyle characteristics

Data on reproductive and lifestyle characteristics at blood collection were collected from participating cohorts. Available data from each cohort were sent to the coordinating center at the Brigham and Women's Hospital for centralized harmonization. Information was requested for (1) general data (e.g., ID, matched Case-Set ID, matching variables, sample types and laboratory batches), (2) lifestyle related data (e.g., BMI, smoking status) and (3) reproductive and hormone-related data (e.g., parity, menopausal status at blood collection, phase of menstrual cycle in premenopausal women, OC or postmenopausal hormone therapy (HT) use).

To account for potential interference of exogenous hormones with circulating concentrations [2], women using OC or HT at the time of blood collection were either: (1) excluded a priori (e.g., EPIC, NHS II premenopausal) or (2) cases and controls were matched on HT use at blood donation (e.g., CLUE II, NHS, NHS II postmenopausal or WHS). It was presumed that FMC participants (pregnant at blood collection) were not using exogenous hormones at the time of blood donation.

Laboratory methods

All participating studies used a nested case-control design, with assays arranged so that case-control sets were measured in the same batch and technicians performing the assays were blinded to case-control status and quality control samples. Hormone concentrations were measured in serum in EPIC (except Sweden), FMC and the NYUWHS; heparin plasma specimens were used in CLUEII and the NHS and NHS II; and EDTA plasma was used for Harvard WHS and Swedish participants from the EPIC cohort. The assays used, along with

intra- and interbatch coefficients, are presented in Table S2. Free testosterone was calculated for CLUE II, EPIC, FMC and NYUWHS, based on measured concentrations of testosterone and SHBG, with albumin assumed to be a constant 40g/L, according to the mass law of action [3].

Statistical analyses

Outliers were identified and removed using the ESD approach [4]. As biomarker data deviated from the normal distribution, we applied the log2 transformation to limit heteroscedasticity. To account for differences in study-specific mean concentrations and a slightly different case-control ratio between studies (1:2 vs. 1:3), data were standardized based on the cohort-specific mean concentrations in controls (Table S3).

Statistical analyses were conducted using a two-stage approach (I) using random effect meta-analyses and (II) an aggregated data approach based on individual participant data. First, the log2 relative risks were calculated from conditional logistic regression models within each cohort and pooled using DerSimonian and Laird random effects models (random effects pooling, [5]). Heterogeneity between cohort-specific effect estimates was tested by DerSimonian and Lairds Q statistic [5] and conducted for all analyses (invasive EOC, by histologic subtype, stage, grade, type I / type II model, menopausal status at blood donation, age at diagnosis and exclusion of women diagnosed within 2 years after blood donation). NHS, NHS II and WHS data were combined for meta-analysis, as these studies were evaluated together in a previous publication [6], and specimens were analyzed in the same laboratory. Based on the relatively small case numbers in some cohorts (e.g., CLUE II: cases n=46) meta-analyses were performed in the crude model (accounting for matching factors), and limited to cohorts contributing more than 5 cases for any given subgroup analyses.

Second, in the aggregated approach, individual participant data from all cohorts were pooled and a combined effect estimate was calculated from a conditional logistic regression model [7]. The original matched sets were retained for all statistical analyses.

Data analysis was conducted using the Unix SAS system to access data remotely on the external servers at the study coordinating center at Brigham and Women's Hospital (SAS Statistical Software, version 9.3 (SAS Institute, Cary NC, USA)). P-values<0.05 were considered as statistically significant; all statistical tests and corresponding p-values were two-sided. Forest plots were prepared using the R software (package 'rmeta', function 'forestplot') version 2.15.2 (R Core Team 2014).

References

1. Kurman RJ, Shih Ie M. Molecular pathogenesis and extraovarian origin of epithelial ovarian cancer--shifting the paradigm. Hum Pathol 2011;42(7):918-31.
2. Zimmerman Y, Foidart JM, Pintiaux A*, et al.* Restoring testosterone levels by adding dehydroepiandrosterone to a drospirenone containing combined oral contraceptive: II. Clinical effects. Contraception 2015;91(2):134-42.
3. Sodergard R, Backstrom T, Shanbhag V*, et al.* Calculation of free and bound fractions of testosterone and estradiol-17 beta to human plasma proteins at body temperature. J Steroid Biochem 1982;16(6):801-10.
4. Rosner B. PERCENTAGE POINTS FOR A GENERALIZED ESD MANY-OUTLIER PROCEDURE. Technometrics 1983;25(2):165-172.
5. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials 1986;7(3):177-88.
6. Tworoger SS, Lee IM, Buring JE*, et al.* Plasma androgen concentrations and risk of incident ovarian cancer. Am J Epidemiol 2008;167(2):211-8.
7. Smith-Warner SA, Spiegelman D, Ritz J*, et al.* Methods for pooling results of epidemiologic studies: the Pooling Project of Prospective Studies of Diet and Cancer. Am J Epidemiol 2006;163(11):1053-64.
8. Lunn M, McNeil D. Applying Cox regression to competing risks. Biometrics 1995;51(2):524-32.
9. Prat J. Ovarian carcinomas: five distinct diseases with different origins, genetic alterations, and clinicopathological features. Virchows Arch 2012;460(3):237-49.

Table S1: Basic information on the participating cohort studies for the pooled analysis on androgens and EOC: the Ovarian Cancer Cohort Consortium (OC3)

Cohort (cases)	Population	Recruitment period	Fasting status	Storage	Matching criteria				
					Controls per case	Age at blood donation	Date of blood sample	Day of cycle	Menopausal status	Other criteria
Clue II (46)	Residents of Washington Country, USA	1989	Non-fasting	-70°C	1:2	± 1 years	± 14 days	± 1 day	Menopausal status at blood collection	Current OC / HT use
EPIC (451)	Volunteers in Denmark, France, Germany, Greece, Italy, Netherlands, Spain, Sweden and UK	1992-2000	Matched	-196°C[1]	1:2	± 6 months	No (incidence density sampling)	5 phases	Menopausal status at blood collection	Recruitment center, Time of the day of blood collection,
FMC (576)	Population based maternity cohort	1986-2007	Not available	-25°C	1:3	± 6 months	± 3 months	Not applicable	Not available	Parity (1,2,>2), parity at diagnosis (1,2,>2)
NHS and II[2] (138)	Registered nurses in the USA	1996-99	Matched	-130°C	1:3	± 2 years	± 2 months	± 1 day for luteal blood sample[3]	Menopausal status at baseline and diagnosis	Time of day, use of postmenopausal hormones at blood collection
WHS (63)	US female health professionals; RCT[4]	1992-95	Matched	-170°C	1:2	± 1 year	± 3 months	5 phases and day	Menopausal status at baseline / diagnosis	Postmenopausal hormones at baseline /diagnosis, time since randomization (± 6 months),
NYUWHS (63)	Women attending breast cancer screening center, NY USA	1985-91	Non-fasting	-80°C	1:2	± 6 months	± 3 months	day of menstrual cycle	Menopausal status at blood donation	Number of blood donations

CLUE II = 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. NYU WHS = New York University Women's Health Study. [1]Most samples were stored in liquid nitrogen at -196°C, apart from Denmark and Sweden were samples were stored locally at -150°C and -70°C. [2]NHS phase 1 (1999-2003 follow-up cycles) and phase 2 (2005-09 follow-up cycles). [3]Patients were asked to provide follicular sample at 3-5 days and luteal sample at 7-9 days before anticipated start of the next cycle. [4] RCT = Randomized Controlled Trial.

Table S2: Laboratory assays and Intra- and Inter-batch CVs for the participating cohorts: the Ovarian Cancer Cohort Consortium (OC3)

Biomarker	Sample	Assay	Intra-Batch CV (%)	Inter-Batch CV (%)
Testosterone				
CLUE II	heparin plasma	direct RIA	12.9	22.2
EPIC phase 1	serum	direct RIA[1]	6.6	11.0
EPIC phase 2		direct RIA[1]	12.7	7.6
FMC	serum	HPLC tandem mass spec.	9.7	8.1
NHS	heparin plasma	liquid chromatography/mass spec.	13.3*	-
NHS II				-
WHS	EDTA plasma	Liquid chromatography/mass spec.		-
NYUWHS	serum	Direct RIA[1]	9.6	14
DHEAS				
CLUE II	heparin plasma	direct RIA	< 3	<10
EPIC phase 1	serum	direct RIA[1]	3.4	11.6
EPIC phase 2		direct RIA[1]	8.2	6.2
FMC	-	-	-	-
NHS	heparin plasma	chemiluminescent immunoassay	3.8*	-
NHS II	heparin plasma	chemiluminescent immunoassay		-
WHS	EDTA plasma	chemiluminescent immunoassay		-
NYUWHS	serum	direct RIA[1]	4.6	11.5
Androstenedione				
CLUE II	heparin plasma	double-antibody RIA	8.6	10.0
EPIC phase 1	serum	direct RIA[3]	3.0	8.4
EPIC phase 2		direct RIA[3]	20.5	10.4
FMC	serum	HPLC tandem mass spectrometry	8.3	7.7
NHS	heparin plasma	Liquid chromatography/mass spec.	9.4*	-
NHS II		Liquid chromatography/mass spec.		-
WHS	EDTA plasma	Liquid chromatography/mass spec.		-
NYUWHS	serum	double-antibody RIA[3]	7.0	13.8
SHBG				
CLUE II	heparin plasma	direct "sandwich" immunoradiometric	1.4	22.2
EPIC phase 1	serum	direct "sandwich" immunoradiometric[5]	4.2	10.7
EPIC phase 2	serum	direct "sandwich" immunoradiometric[5]	5.9	3.2
FMC	serum	chemiluminescence	8.7	3.7
NHS	-	-	-	-
NHS II	-	-	-	-
WHS	-	-	-	-
NYUWHS	serum	direct 'sandwich'immunoradiometric[5]	6.2	11.5

[1]Radio-Immuno-Assay (RIA) Immunotech, Marseille, France; [2]Beckman Coulter, Brea, California; [3]Diagnostic System Laboratories (DSL), Webster, Texas, USA; [4]Beckman and Coulter, Brea, California, USA; [5]CIS-Bio, Gif-sur-Yvette, France; [6]Enzyme-linked immunosorbent assay (ELISA); DSL, Webster, Texas, USA; [7]Immunodiagnostics Systems, Germany. *average intra-batch coefficient from NHS / NHS II and WHS

Table S3. Geometric means of hormone concentrations (95% CI) by cohort and case-control status after log2 transformation and standardization: the Ovarian Cancer Cohort Consortium (OC3)

Study			Testosterone (ng/ml)	Free Testosterone (nmol/l)	Androstenedione (ng/ml)	DHEAS (ug/dl)	SHBG (nmol/l)
Clue II	**Cases**	46	1.10 (0.96-1.27)	1.11 (0.92-1.32)	1.10 (0.95-1.28)	1.01 (0.83-1.23)	1.04 (0.90-1.22)
	Controls	91	1.00 (0.90-1.11)	1.00 (0.88-1.13)	1.00 (0.90-1.11)	1.00 (0.87-1.15)	1.00 (0.90-1.11)
EPIC	**Cases**	451	1.00 (0.96-1.05)	1.04 (0.98-1.10)	0.98 (0.94-1.03)	1.03 (0.97-1.10)	0.97 (0.92-1.02)
	Controls	867	1.00 (0.97-1.03)	1.00 (0.96-1.04)	1.00 (0.97-1.04)	1.00 (0.96-1.05)	1.00 (0.97-1.03)
FMC	**Cases**	576	1.07 (1.03-1.11)	1.07 (1.00-1.14)	1.07 (1.03-1.12)	≠≠	1.04 (0.98-1.10)
	Controls	1,433	1.00 (0.97-1.03)	1.00 (0.96-1.04)	1.00 (0.97-1.03)	≠≠	1.00 (0.90-1.11)
NHS	**Cases**	117	1.05 (0.96-1.15)	≠≠	0.93 (0.84-1.02)	0.87 (0.77-0.99)	≠≠
	Controls	348	1.00 (0.95-1.05)	≠≠	1.00 (0.94-1.06)	1.00 (0.93-1.07)	≠≠
NHS II	**Cases**	15	1.17 (0.91-1.50)	≠≠	1.03 (0.79-1.36)	0.94 (0.67-1.34)	≠≠
	Controls	45	1.00 (0.86-1.16)	≠≠	1.00 (0.85-1.18)	1.00 (0.82-1.22)	≠≠
NYU WHS	**Cases**	63	0.98 (0.87-1.11)	0.92 (0.79-1.08)	0.95 (0.84-1.09)	≠≠	1.08 (0.95-1.23)
	Controls	112	1.00 (0.91-1.10)	1.00 (0.89-1.12)	1.00 (0.91-1.10)	≠≠	1.00 (0.91-1.10)
WHS	**Cases**	63	0.90 (0.80-1.02)	≠≠	1.01 (0.88-1.15)	1.00 (0.85-1.19)	≠≠
	Controls	122	1.00 (0.92-1.09)	≠≠	1.00 (0.91-1.09)	1.00 (0.89-1.13)	≠≠

Table S4. Tumor characteristics in pooled analysis of prospective data on circulating androgens, SHBG and EOC risk: the Ovarian Cancer Cohort Consortium (OC3)

	Clue II	**EPIC**	**FMC**	**NHS**	**NHS II**	**NYUWHS**	**WHS**	**Total**
References	≠	Ose et al. 2014	Schock et al. 2014	Tworoger et al. 2008	Tworoger et al. 2008	Lukanova et al. 2002	Tworoger et al. 2008	
No	46	451	576	117	15	63	63	1,331
Age at dx, yrs [1]	67.4 (13.0)	62.5 (8.9)	44.7 (8.1)	65.0 (7.3)	48.8 (3.8)	59.8 (8.8)	60.1 (8.0)	54.8 (12.4)
Lag time, yrs [1]	6.6 (3.0)	6.6 (3.6)	12.3 (6.8)	7.3 (4.0)	2.7 (1.9)	7.2 (3.5)	4.3 (2.6)	9.0 (6.0)
Histology								
Serous	19 (41%)	238 (53%)	263 (46%)	62 (53%)	5 (33%)	38 (60%)	42 (67%)	667 (50%)
Endometrioid	5 (11%)	45 (10%)	92 (16%)	11 (9%)	4 (27%)	4 (6%)	5 (8%)	166 (12%)
Mucinous	2 (4%)	30 (7%)	143 (25%)	9 (8%)	1 (7%)	6 (10%)	2 (3%)	193 (15%)
Clear cell	2 (4%)	25 (6%)	23 (4%)	4 (3%)	2 (13%)	5 (8%)	-	61 (5%)
Others	18 (39%)	113 (25%)	55 (10%)	31 (27%)	3 (20%)	10 (16%)	14 (22%)	244 (18%)
Grade [2]								
Low grade	1 (4%)	31 (12%)	-	11 (12%)	3 (25%)	7 (14%)	3 (7%)	56 (12%)
High grade	24 (96%)	220 (88%)	-	79 (88%)	9 (75%)	43 (86%)	42 (93%)	417 (88%)
Stage [2]								
Low stage	3 (9%)	57 (14%)	150 (31%)	27 (23%)	7 (47%)	13 (24%)	-	257 (23%)
High stage	29 (91%)	341 (86%)	332 (69%)	88 (77%)	8 (53%)	41 (76%)	-	839 (77%)
Type [2]								
Type I	5 (24%)	76 (32%)	166	20 (24%)	6 (55%)	14 (30%)	4 (11%)	291 (48%)
Type II	16 (76%)	163 (68%)	-	65 (76%)	5 (45%)	33 (70%)	32 (89%)	314 (52%)

[1] presented as mean (SD)

[2] Among cases with data. grade missing for 64%, stage missing for 18%, Type I/II missing for 55%

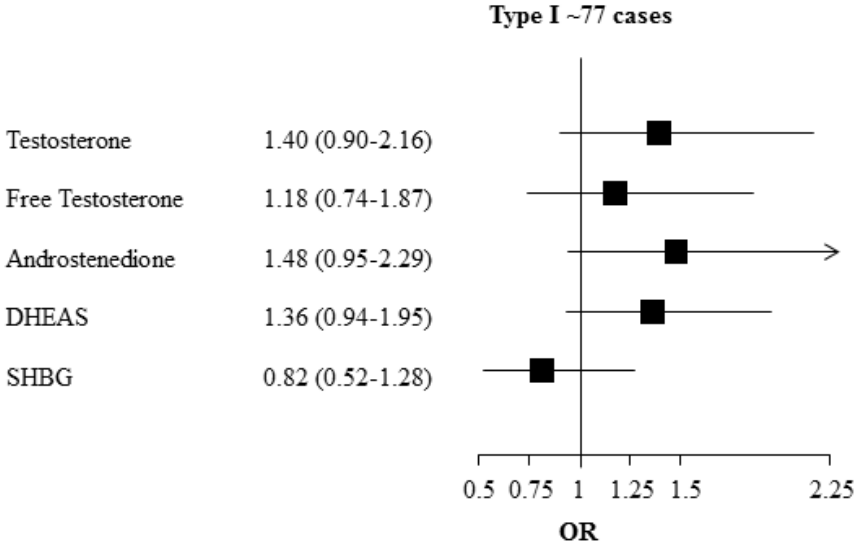
≠ Data from Clue II have not been published.

Table S5. Odds ratios (95% CI) for invasive EOC overall and the serous subtype in quintiles of androgen and SHBG concentrations: OC3[1]

	Invasive EOC			Serous EOC		
	Sets	**OR (95% CI)**	**p_{trend}[2]**	**Sets**	**OR (95% CI)**	**p_{trend}[2]**
Testosterone						
Q1	254	ref		145	ref	
Q2	250	1.00 (0.81-1.23)		128	0.88 (0.66-1.18)	
Q3	251	1.10 (0.89-1.36)		140	1.13 (0.84-1.50)	
Q4	267	1.12 (0.90-1.38)		121	0.88 (0.65-1.19)	
Q5	279	1.22 (0.99-1.52)	0.03	121	0.88 (0.65-1.20)	0.56
Free Testosterone						
Q1	159	ref		84	ref	
Q2	179	1.08 (0.83-1.41)		96	1.04 (0.72-1.49)	
Q3	177	1.15 (0.87-1.50)		90	1.10 (0.76-1.60)	
Q4	149	0.97 (0.73-1.28)		85	0.95 (0.65-1.38)	
Q5	201	1.29 (0.99-1.68)	0.04	80	0.88 (0.60-1.29)	0.63
Androstenedione						
Q1	260	ref		138	ref	
Q2	265	1.07 (0.87-1.33)		141	1.02 (0.76-1.37)	
Q3	220	0.88 (0.70-1.10)		114	0.77 (0.57-1.06)	
Q4	276	1.10 (0.89-1.37)		145	1.14 (0.74-1.54)	
Q5	286	1.20 (0.95-1.51)	0.13	118	0.90 (0.64-1.25)	0.79
DHEAS						
Q1	135	ref		74	ref	
Q2	133	0.97 (0.73-1.31)		74	1.01 (0.68-1.50)	
Q3	158	1.18 (0.88-1.58)		78	1.22 (0.81-1.82)	
Q4	116	0.88 (0.64-1.20)		66	0.93 (0.61-1.41)	
Q5	149	1.13 (0.82-1.55)	0.87	74	0.99 (0.63-1.54)	0.45
SHBG						
Q1	186	ref		85	ref	
Q2	183	0.99 (0.77-1.28)		96	1.18 (0.83-1.69)	
Q3	137	0.77 (0.59-1.00)		76	0.89 (0.62-1.28)	
Q4	180	0.99 (0.76-1.27)		92	1.05 (0.73-1.50)	
Q5	200	1.14 (0.88-1.48)	0.56	96	1.31 (0.90-1.89)	0.39

[1]Results were derived from conditional logistic regression models, additionally adjusted for OC use (never/ever/missing) and parity (never/ever/missing); [2]The p value for trend across quintiles is based on a continuous probit score (generating a rank for each person in each cohort by hormone level). DHEAS=dehydroepiandrosterone sulfate; SHBG=sex hormone binding globulin

Figure S1. Odds ratios (95% CI) for doubling of androgen concentrations and Type I EOC restricted to cases with data on tumor grade (p_{het} comparing type I and type II: testosterone, 0.09; free testosterone, 0.38; androstenedione, <0.01; DHEAS, 0.03; SHBG, 0.14; type II ORs shown in Figure 1): 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). Pair-wise heterogeneity tests were performed, using the likelihood ratio test comparing models assuming (1) the same association between exposure and outcomes compared to (2) a model assuming different associations for each subtype. DHEAS=dehydroepiandrosterone sulfate; SHBG=sex hormone binding globulin