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Circulating androgens and postmenopausal ovarian cancer risk in the Women's Health Initiative Observational Study

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Abstract

Our knowledge of epidemiologic risk factors for ovarian cancer supports a role for androgens in the pathogenesis of this disease; however, few studies have examined associations between circulating androgens and ovarian cancer risk. Using highly sensitive LC-MS/MS assays, we evaluated associations between pre-diagnostic serum levels of 12 androgens, including novel androgen metabolites that reflect androgen activity in tissues, and ovarian cancer risk among postmenopausal women in a nested case-control study in the Women's Health Initiative (WHI) Observational Study (OS). We frequency-matched 169 ovarian cancer cases to 410 controls from women enrolled in WHI-OS who were not using menopausal hormones at enrollment/blood draw. We estimated associations overall and by subtype (n=102 serous/67 non-serous) using multivariable adjusted logistic regression. Androgen/androgen metabolite levels were not associated with overall ovarian cancer risk. In analyses by subtype, women with increased levels of androsterone-glucuronide (ADT-G) and total 5-α reduced glucuronide metabolites (markers of tissue-level androgenic activity) were at increased risk of developing non-serous ovarian cancer: ADT-G tertile (T)3 versus T1 odds ratio [OR] (95% confidence interval [CI]) 4.36 (1.68–11.32), p-heterogeneity 0.002; total glucuronide metabolites 3.63 (1.47–8.95), 0.002. Risk of developing serous tumors was unrelated to these markers.

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Availability of data: The data that support the findings of this study are available from https://www.whi.org/researchers/SitePages/Home.aspx. Restrictions apply to the availability of these data, which were used under license for this study.

ADT-G and total glucuronide metabolites, better markers of tissue-level androgenic activity in women than testosterone, were associated with an increased risk of developing non-serous ovarian cancer. Our work demonstrates that sex steroid metabolism is important in the etiology of non-serous ovarian cancers and supports a heterogeneous hormonal etiology across histologic subtypes of ovarian cancer.

Keywords

Endogenous androgens; androgen metabolites; androgenic activity; ovarian cancer risk; nested case-control study; heterogeneity

INTRODUCTION

An understanding of the etiology of ovarian cancer remains elusive. However, experimental and epidemiologic data suggest a role for hormone-related exposures and risk factor differences by histologic subtype[1]. A role for androgens stimulating epithelial cell proliferation has been suggested in the pathogenesis of ovarian cancer. Epidemiologic studies demonstrate that factors associated with high androgen levels, such as testosterone treatments, are related to increased ovarian cancer risk[2]; while oral contraceptives, which suppress testosterone,[3] are associated with reduced risk[4].

In postmenopausal women, elevated levels of prediagnostic circulating androgens or androgen precursors (androstenedione, testosterone, dehydroepiandrosterone (DHEA), and its metabolite dehydroepiandrosterone sulfate (DHEAS)), have been associated with ovarian cancer risk inconsistently across studies[5–11]. In a recent pooled analysis of these studies, testosterone was associated with increased risk of ovarian cancer overall and androstenedione was associated with increased ovarian cancer risk in premenopausal women, but not in postmenopausal women[11]. Furthermore, testosterone and androstenedione results suggested heterogeneity by subtype, with increased risks for endometrioid and mucinous tumors, and null associations for clear cell and serous tumors[11]. At present, it is unclear whether the androgens measured in these studies (e.g., testosterone, androstenedione, DHEA, DHEAS) adequately reflect underlying androgenic activity and local tissue production of bioactive androgens[12], and for postmenopausal women, whether they simply reflect a reservoir of precursor substrates for estrogens.

In postmenopausal women, metabolism of androgens and their precursors occurs in tissues primarily via 5α-reductase[13]; the derived 5α-reduced metabolites (Figure 1), including dihydrotestosterone (DHT), are potent local bioactive agents. However, serum DHT is difficult to measure at the low levels found in postmenopausal women, and recent studies suggest that it may not adequately reflect the activity of more distant 5α-reduced androgen metabolites. Studies of prostate cancer, as well as polycystic ovary syndrome (PCOS) and hirsutism in women, have measured androsterone glucuronide (ADT-G), a distal metabolite of DHT, as a marker of peripheral androgen activity[14, 15]. These studies suggested that circulating levels of ADT-G plus androstanediol glucuronide (found as two isomers: 5α-androstane-3α,17β diol-3-glucuronide (3α-diol-3G) and 5α-androstane-3α,17β diol-17-glucuronide (3α-diol-17G)) taken together may be a better marker of androgenic activity in

tissue than their precursors (testosterone and androstenedione) alone[12, 13]. Further, they estimated that ADT-G accounts for 93% of the total 5α-reduced androgen glucuronide derivatives and proposed that this metabolite alone could replace measures of serum testosterone as a circulating measure of androgenicity in postmenopausal women[12]. Another potentially important androgen conjugate is dihydrotestosterone sulfate (DHTS), which may form an inactive reservoir for the highly potent DHT. As such, the measurement of 5α-reduced glucuronide metabolites may be relevant in the study of ovarian carcinogenesis.

To date, no epidemiologic studies have evaluated measures of the 5α-reduced androgen metabolites in relation to ovarian cancer risk. Therefore, we conducted a nested case-control study within the Women's Health Initiative (WHI)-Observational Study (OS), to evaluate the associations between the 5α-reduced androgen metabolites, other major androgens, and their adrenal precursors,[16] and ovarian cancer risk--with the goal of evaluating the glucuronide derivates as better markers of tissue-level androgenic activity than their precursors. Existing data on estrogens[17] enabled further evaluation of whether the associations with androgens are independent of their influence on parent estrogen production. Given increasing evidence of etiologic heterogeneity of epithelial ovarian cancers—especially with respect to the associations with hormonal risk factors (e.g. body mass index (BMI), menopausal hormone therapy use[1])—we also evaluated associations by tumor subtype.

MATERIALS AND METHODS

Study population

Details of the WHI-OS[18, 19] and the nested case-control study of ovarian cancer used in these analyses[17] have been described previously. Briefly, the WHI-OS is a prospective cohort that enrolled 93,676 postmenopausal women ages 50 to 79 years at 40 centers throughout the United States between 1993 and 1998[18, 19]. Women were excluded from the OS if they were participating in a WHI clinical trial; if they had medical conditions with a predicted survival of less than 3 years; or if they had retention issues. The nested case-control study included incident ovarian cancer cases (diagnoses of incident primary epithelial ovarian, fallopian tube, or peritoneal cancer) that were diagnosed between study initiation and May 2012; we refer to this case group collectively as "ovarian cancers" throughout the remainder of the manuscript. All cancer diagnoses were centrally adjudicated at the WHI Clinical Coordinating Center according to SEER guidelines. Both cases and controls met the following criteria to be eligible: no history of cancer at baseline other than non-melanoma skin cancer; no current use of exogenous hormones; no history of bilateral oophorectomy; and at least 1.1 mL of available pre-diagnostic serum.

Among the cases, the mean time from serum collection to diagnosis was 6.9 years (standard deviation = 3.8 years; range = 352 days – 14.8 years). Controls were eligible WHI-OS cohort members selected from strata defined by age at blood draw (5-year categories), year at blood draw (1993–1996, 1997–1998), race/ethnicity (white, black, Hispanic, other/unknown), hysterectomy status at baseline or during follow-up (yes/no), and time since last menopausal hormone use (≤1 year, >1 year/never). Controls were drawn from the set of eligible cohort members in each stratum containing ovarian cancer cases that were alive at

the time of diagnosis of their matched case, and were selected with a ratio of at least 2 controls per case per stratum.

We excluded women with unconjugated estrone concentrations greater than or equal to 184 pmol/L (~50 pg/mL; n=10), which is typically indicative of exogenous hormone use, as well as 2 control women who did not have sufficient serum to measure circulating androgens after estrogen metabolites were measured in a prior analysis[17]. The present study included 169 ovarian cancer cases and 410 matched controls. Among ovarian cancer cases, 102 had serous tumors and the remaining 67 had non-serous cancers (13 endometrioid, 11 clear cell, 9 mucinous, and 34 other-epithelial subtypes). Approval for conducting WHI was obtained from human subjects review at the Fred Hutchinson Cancer Research Center (WHI Clinical Coordinating Center) and all 40 clinical centers. The current project was reviewed and exempted by the Office of Human Subjects Research at the U.S. National Cancer Institute. Written informed consent was obtained from study participants.

Laboratory assays

Stable isotope dilution high performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used to quantify 12 androgens and androgen metabolites (Figure 1) including the principal androgens secreted by the adrenals (DHEA and DHEAS) and the ovaries (androstenedione and testosterone), as well as their 5α-reduced androgen metabolites: 5α-androstane-3,17-dione (5α-androstanedione), DHT, androsterone (ADT), DHTS, 3α-diol-3G, 3α-diol-17G, and ADT-G; and the 5β-reduced metabolite etiocholanolone-glucuronide. Details of the method have been published previously[16]. We included etiocholanolone-glucuronide in our assay since it is recognized, along with ADT-G, as one of the major inactive metabolites of testosterone; it also serves as a marker of 5β-reduced androgen levels. The other 5β-reduced androgen metabolites were not included either because of low abundance in serum, lack of internal standards, or because they do not bind to or bind only weakly to the androgen receptor (e.g., 5β-DHT). LC-MS/MS analysis was performed using updated instrumentation, a Thermo TSQ™ Quantiva triple quadrupole mass spectrometer (Thermo Fisher, San Jose, CA) coupled with a NexeraXR LC system (Shimadzu Scientific Instruments, Columbia, MD). Both the chromatographer and mass spectrometer were controlled by Xcalibur™ software (Thermo Fisher, San Jose, CA). Nine stable isotope labeled unconjugated and conjugated androgens were used to account for losses during sample preparation and analysis, which included: dehydroepiandrosterone-2,2,3,4,4-d_5 (d_5-DHEA), androstenedione-2,3,4-$^{13}C_3$ ($^{13}C_3$-A), testosterone-2,3,4-$^{13}C_3$ ($^{13}C_3$-T), dihydrotestosterone-16,16,17-d_3 (d_3-DHT), androsterone-2,2,4,4-d_4 (d_4-ADT), dehydroepiandrosterone sulfate-2,2,3,4,4-d_5 (d_5-DHEAS), dihydrotestosterone sulfate-16,16,17-d_3 (d_3-DHTS), and androsterone glucuronide-2,2,4,4-d_4 (d_4-ADT-G) obtained from Cerilliant Corporation (Round Rock, TX); 5α-androstane-3α,17β-diol-17-glucuronide-16,16,17-d_3 (d_3-3α-diol-17G) was purchased from 13C Molecular, Inc. (Fayetteville, NC). Estrogens were previously quantified using an independent LC-MS/MS assay[17].

The limits of quantitation for the unconjugated androgens and conjugated androgens were as follows: 0.01 ng/mL for A and T; 0.05 ng/mL for DHEA, DHT, and ADT; 0.1 ng/mL for

5α-A; 0.05 ng/mL for DHEAS and DHTS; 0.1 ng/mL for 3α-diol-3G, 3α-diol-17G, ADT-G, and etiocholanolone-glucuronide. No samples in the current study had undetectable levels for any of the hormones measured. Laboratory coefficients of variation (CVs) of blinded duplicate samples within and across batches were <11.0% for all hormones measured. Intraclass correlation coefficients (ICCs) ranged from 0.77–0.997 with a mean value of 0.94 (median 0.99).

Statistical analysis

Androgens and androgen metabolites were analyzed individually and as ratios. We calculated a measure of the total (5α-reduced) glucuronide metabolites as the sum of ADT-G, 3α-diol-3G, and 3α-diol-17G, as described by Labrie and colleagues[20]. All androgen measures (individual androgens, ratios, measure of total glucuronide metabolites) were categorized into tertiles based on the distribution in controls. First, we estimated overall associations (all ovarian cancer cases) and then those stratified by subtype (serous/non-serous). Conditional logistic regression models were used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for ovarian cancer risk conditioning on matching factors: age at blood draw, calendar year of blood draw, race/ethnicity, hysterectomy status, and time since last menopausal hormone use, and further adjusted for potential confounding factors chosen *a priori,* based on knowledge of the literature: gravidity (ever, never), BMI (<25, 25–29.9, ≥30 kg/m^2), cigarette smoking status (never, former, current), and duration of oral contraception use (never, <5, 5-<10, ≥10 years). Tests for trend were based on the Wald statistic using the median concentrations from each tertile of a given androgen as a continuous variable.

For analyses stratified by case characteristics (subtype and time between blood draw and diagnosis) we used multinomial logistic regression models, with the controls as the reference group and adjusted for matching factors and the *a priori* selected potential confounding factors. In these models, time since menopausal hormone therapy use was modeled as: never, ≤1 year, >1 year. Chi-square p-values for heterogeneity across subgroup associations were estimated from models that treated the largest subgroup as the reference and excluded non-cases. We also evaluated associations stratified by age at blood draw (<65 years old, ≥65 years old). We conducted the following sensitivity analyses: 1) excluding potential outliers (concentrations greater than five standard deviations above the median; median number of excluded subject per hormone measure n=6 (min-max: 2–19)), 2) excluding women who reported a history of diabetes at baseline (n=28), and 3) excluding women who reported prior use of menopausal hormones (n=209). All p-values were based on two-sided tests and a p-value<0.05 was considered statistically significant.

RESULTS

Participants were on average 64 years of age at blood draw and predominantly white (90%). Women who developed serous cancers were slightly older (average age at blood draw, 64.7 years) than women who developed non-serous cancers (average 63.2 years) (Table 1). Median androgen and androgen metabolite levels were not substantially different among women who developed cancer or did not (Table 2). There were some differences in median

concentrations across histologic subtypes. We observed higher circulating levels of ADT-G and total glucuronide metabolites among women who developed non-serous cancers than in women who developed serous cancers or among control women, levels of DHEAS, DHEA, and androstenedione were also higher in case women than control women.

The individual androgens and androgen metabolites as well as the ratios of androgens/metabolites were not associated with overall ovarian cancer risk (Table 3). We noted statistically significant heterogeneity in the associations for ADT-G as well as the circulating measure of total glucuronide metabolites across histologic subtypes of ovarian cancer (Table 3). Women with increased levels of ADT-G or total glucuronide metabolites were at increased risk of developing non-serous ovarian cancers [ADT-G OR (95% CI) for the highest versus lowest tertile (T3 vs. T1): 4.36 (1.68–11.32), p-het 0.002; total glucuronide metabolites: 3.63 (1.47–8.95), p-het 0.002], while risk of developing serous ovarian cancers was unrelated to levels of these two markers (Table 3). Given almost complete correlation between levels of ADT-G and total glucuronide metabolites (see Supplementary Table S1), associations with these two markers of androgenic activity are likely dependent. Although not statistically significant, there were notable elevations in OR estimates for non-serous tumors for the pro- androgens (DHEA, DHEAS, androstenedione, and testosterone), and 5-α reduced androgens (DHTS and 3α-diol-3G), while ORs for serous tumors at or less than 1.0 for these markers.

After mutual adjustment of both ADT-G and estradiol, the increased risk of developing non-serous cancer with higher levels of ADT-G remained [OR (95% CI) for T3 vs. T1 ADT-G: 4.02 (1.53–10.55)] as did the association between increased levels of unconjugated estradiol and non-serous tumors [OR (95% CI) T3 vs T1 unconjugated estradiol without adjustment for ADT-G: 2.57 (1.18–5.60) and with adjustment for ADT-G: 2.25 (1.01–4.99)] (results not tabled).

Results did not differ by time between blood draw and diagnosis (Supplementary Table S2) or by age at blood draw (Supplementary Table S3). Median androgen and androgen metabolite levels were generally consistent across non-serous tumor subtypes (Supplementary Table S4). In sensitivity analyses excluding outliers for individual androgen measurements, excluding individuals with a history of diabetes at baseline, or excluding former hormone users, effect estimates were largely unchanged (results not shown).

DISCUSSION

There is strong *in vitro* and *in vivo* evidence[21–23], as well as some epidemiologic data[11, 17], demonstrating that sex steroids play a role in ovarian carcinogenesis. Androgenic activity via androgen receptor signaling is responsible for healthy functioning of many organs in women. Data from experimental studies link androgen-related signalling to ovarian cancer through increased cellular proliferation and reduced apoptotic rates[21–23]. However, previous epidemiologic studies have not consistently shown associations between circulating androgens and overall ovarian cancer risk. In the current study, we identified significant heterogeneity in associations by ovarian cancer subtype for ADT-G and total glucuronide metabolites—markers of androgenic activity in tissues as opposed to increased estrogenic

activity, because androgens cannot be aromatized into estrogens after they are metabolized via 5α-reductase. We did not find associations with serous cancers, the most common and fatal subtype, which explains the lack of association with overall ovarian cancer risk. To our knowledge, we are the first to provide evidence suggesting that ADT-G and total glucuronide metabolites--circulating markers of tissue-level androgenic activity in postmenopausal women--are associated with increased risk of developing non-serous ovarian cancer. ORs were elevated for non-serous ovarian cancers for a number of other androgens measured, including other 5-α reduced metabolites: DHTS and 3α-diol-3G, which further support the plausibility of a role for androgens in the etiology of non-serous ovarian cancer. Associations with serous cancers for these markers were null. Finally, the increased risk of non-serous ovarian cancer associated with androgens was independent of circulating estrogen levels[17]. Taken together, the current work and our prior research evaluating estrogen metabolites in the same study population[17] suggests a role for both androgenic and estrogenic metabolites in the development of non-serous ovarian tumors.

In a recent pooled analysis of cohort studies, higher levels of testosterone were related to increased risk of ovarian cancer[11]. Also, high-levels of testosterone and androstenedione were associated with increased risks of specific non-serous subtypes, namely endometrioid and mucinous tumors. The OR for the highest versus lowest tertiles of testosterone levels and ovarian cancer risk in our study (1.32) was similar to that in the pooled analysis (1.25)[11]. Further, the pattern of elevated risk for non-serous subtypes with higher levels of androstenedione and testosterone in our study was consistent (albeit imprecise) with that in the pooled analysis[11].

We detected substantial increased risk of non-serous ovarian cancers with increasing levels of androgen biomarkers previously unmeasured in ovarian cancer studies: ADT-G and total glucuronide metabolites, both of which are proposed to better reflect tissue level androgen activity than either testosterone or androstenedione[12]. The findings from the prior studies (summarized in Ose *et al.*[11]) are not all directly comparable to our results that were restricted to postmenopausal women, given their inclusion of both pre- and perimenopausal women as well as postmenopausal women. Further some of the prior studies measured androgens using direct RIA or chemiluminescent immunoassays[5, 6, 8, 9], which have recognized limitations in terms of sensitivity and specificity.

The current study has several important strengths. The WHI-OS cohort is a large prospective study with standardized pre-diagnostic specimen collection. The androgens and androgen metabolites measured in circulation provide a novel phenotypic characterization of individual patterns of androgen metabolism—including 5α-reduced androgen metabolites and better markers of androgenic activity in postmenopausal women than measuring DHEA, testosterone, or androstenedione alone. We were able to measure a wide range of androgens in our study population, including those with relatively low levels, using an LC-MS/MS assay with high sensitivity[16]. Limitations of our study include the low power, which affected our ability to evaluate specific subtypes of non-serous tumors but our results for the commonly measured androgens align with those previously reported[11]. Additional investigation in a larger prospective study is needed to clarify the risk of individual ovarian cancer subtypes with improved markers of androgenic activity, namely ADT-G and total

glucuronide metabolites. We also measured circulating androgens in a single baseline serum sample, which may not accurately reflect long-term androgen levels. Ongoing research by our group suggests that among postmenopausal women, temporal stability was moderate-to-high for most of the androgens and androgen metabolites; 2-year ICCs across the androgens/androgen metabolites averaged 0.78 (ranging from 0.34 for 5α-A to >0.96 for ADT-G, 3αdiol-17G, and the measure of total glucuronide metabolites) (unpublished data).

Our work shows that sex steroid metabolism is important in the etiology of non-serous ovarian cancers and supports a heterogeneous hormonal etiology across histologic subtypes of ovarian cancer. We observed increased risks for these tumors with both relatively high levels of estrogens[17] and in the present analysis, with relatively high levels of biomarkers of increased androgenic activity in tissues. Our study provides novel molecular data that support a role for one such marker, ADT-G, in the development of non-serous ovarian cancer. Combined with other accumulating evidence that there is substantial etiologic heterogeneity across subtypes of ovarian cancer--particularly for hormonally-related risk factors--the subtype-specific associations we observed for ADT-G as well as for estrogen metabolites measured in our prior study[17], support the evaluation of other circulating sex steroid hormones by ovarian cancer subtypes. Such work will further clarify the hormonal mechanisms that underlie the development of ovarian cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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For a list of all the investigators who have contributed to WHI science, please visit: https://www.whi.org/researchers/Documents%20%20Write%20a%20Paper/WHI%20Investigator%20Long%20List.pdf

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Abbreviations

WHI	Women's Health Initiative
OS	Observational Study
ADT	androsterone
ADT-G	androsterone-glucuronide
DHEA	dehydroepiandrosterone
DHEAS	metabolite dehydroepiandrosterone sulfate
DHT	dihydrotestosterone
DHTS	dihydrotestosterone sulfate
3α-diol-3G	5α-androstane-3α,17β diol-3-glucuronide
3α-diol-17G	5α-androstane-3α,17β diol-17-glucuronide
5α-androstanedione	5α-androstane-3,17-dione
LC-MS/MS	liquid chromatography-tandem mass spectrometry
OR	odds ratio
CI	confidence interval
CV	coefficient of variation
ICC	intraclass correlation coefficient
BMI	body mass index

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Novetly and Impact:

The circulating androgen metabolites measured in the current study provide a novel phenotypic characterization of individual patterns of androgen metabolism associated with ovarian cancer risk—including 5α-reduced androgen metabolites (e.g., androsterone-glucuronide (ADT-G)) that have been shown to reflect tissue level androgen activity. ADT-G was associated with increased risk of non-serous ovarian cancer while a null association was indicated for serous tumors; as ADT-G cannot be converted to estrogen, this data suggests a unique role for androgen metabolism in non-serous tumors.

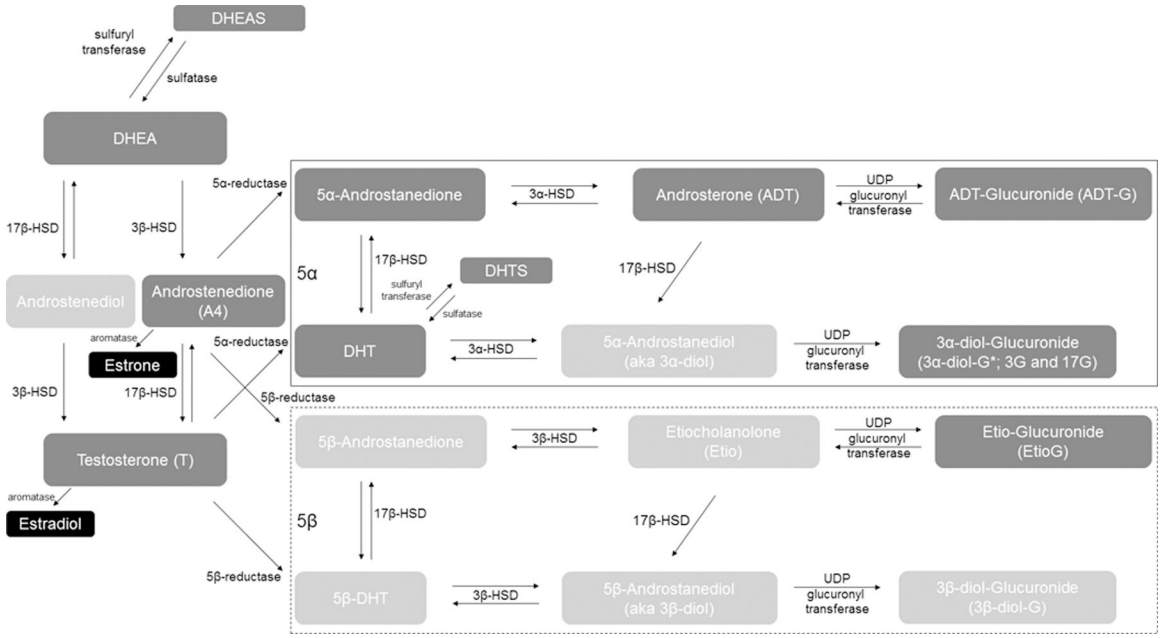

Figure 1.

The synthesis of androgens and estrogens occurs from the adrenal androgenic precursor, dehydroepiandrosterone (DHEA). Androgen metabolites are formed from androstenedione and testosterone via 5α-reductase (top pathway framed by solid outline) or 5β-reductase (bottom pathway framed by dotted outline). The current assay measures the 12 androgens/androgen metabolites in dark grey[16]; those in light grey were not measured. Alternatively, androstenedione and testosterone can be converted to estrone and estradiol (in black) via aromatase. Estrogens were measured previously, using an independent assay[17].

Table 1.

Demographic and health characteristics of controls and ovarian cancer cases overall and by serous/non-serous subtype, nested case-control study within the Women's Health Initiative-Observational Study.

	Controls (n=410)		Ovarian Cancer Cases (n=169)		Serous cases (n=102)		Non-serous cases (n=67)	
Characteristics	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Age	64.3	7.2	64.1	7.2	64.7	7.2	63.2	7.1
Year of blood draw	n	%	n	%	n	%	n	%
1993–1996	253	61.7	108	63.9	62	60.8	46	68.7
1997–1998	157	38.3	61	36.1	40	39.2	21	31.3
Race/ethnicity								
White	369	90.0	151	89.3	96	94.1	55	82.1
Black	17	4.1	8	4.7	3	2.9	5	7.5
Hispanic	12	2.9	6	3.6	2	2.0	4	6.0
Other	12	2.9	4	2.4	1	1.0	3	4.5
Hysterectomy (baseline or follow-up prior to event)								
No	338	82.4	140	82.8	83	81.4	57	85.1
Yes	72	17.6	29	17.2	19	18.6	10	14.9
Time since last menopausal hormone therapy use								
>1 year	398	97.1	163	96.4	97	95.1	66	98.5
≤1 year	12	2.9	6	3.6	5	4.9	1	1.5
Smoking status								
Never	203	49.5	81	47.9	51	50.0	30	44.8
Former	166	40.5	76	45.0	43	42.2	33	49.3
Current	38	9.3	12	7.1	8	7.8	4	6.0
BMI (kg/m2)								
<25	179	43.7	66	39.1	43	42.2	23	34.3
25–29.9	127	31.0	52	30.8	31	30.4	21	31.3
30+	103	25.1	51	30.2	28	27.5	23	34.3
Age at menarche								
<12	100	24.4	37	21.9	24	23.5	13	19.4
12–13	219	53.4	100	59.2	59	57.8	41	61.2
14+	88	21.5	32	18.9	19	18.6	13	19.4
Ever pregnant								
No	54	13.2	25	14.8	13	12.7	12	17.9
Yes	356	86.8	144	85.2	89	87.3	55	82.1
Duration oral contraceptive use (years)								
Never	254	62.0	106	62.7	65	63.7	41	61.2
<5	83	20.2	36	21.3	23	22.5	13	19.4
5-<10	39	9.5	14	8.3	5	4.9	9	13.4
10+	34	8.3	13	7.7	9	8.8	4	6.0
History of tubal ligation								

	Controls (n=410)		Ovarian Cancer Cases (n=169)		Serous cases (n=102)		Non-serous cases (n=67)	
Characteristics	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
No	338	82.4	151	89.3	89	87.3	62	92.5
Yes	71	17.3	18	10.7	13	12.7	5	7.5
Age at menopause								
<40	17	4.1	4	2.4	3	2.9	1	1.5
40–44	33	8.0	14	8.3	11	10.8	3	4.5
45–49	89	21.7	34	20.1	18	17.6	16	23.9
50–54	183	44.6	83	49.1	52	51.0	31	46.3
55+	66	16.1	22	13.0	14	13.7	8	11.9
Menopausal hormone therapy use								
Never	242	59.0	129	76.3	74	72.5	55	82.1
Former	170	41.4	40	23.7	28	27.5	12	17.9

Table 2.

Median concentrations and interdecile ranges (IDR) of androgens, androgen metabolites, and parent estrogens (and ratios of relevant hormone concentrations) among controls and ovarian cancer cases, overall and by serous/non-serous subtype.

	Controls (n=410)		All ovarian cancer cases (n=169)			Serous cases (n=102)		Non-serous cases (n=67)		
Pro-androgens/androgens	**Median**	**(IDR)**	**Median**	**(IDR)**	**P[a]**	**Median**	**(IDR)**	**Median**	**(IDR)**	**P[b]**
dehydroepiandrosterone (DHEA), nmol/L	4.9	(2.0–10.4)	4.9	(1.9–10.3)	0.94	4.7	(1.7–8.3)	5.4	(2.4–11.1)	0.10
DHEA sulfate (DHEAS), nmol/L	1113	(411–2511)	1118	(448–2398)	0.60	1044	(428–2119)	1221	(564–2792)	0.05
Androstenedione, nmol/L	1.3	(0.7–2.3)	1.3	(0.8–2.3)	0.74	1.2	(0.8–2.0)	1.4	(0.8–2.6)	0.08
Testosterone, nmol/L	0.6	(0.3–1.0)	0.6	(0.3–1.1)	0.17	0.6	(0.3–1.0)	0.6	(0.3–1.1)	0.26
5α-reduced androgens										
5α-androstanedione, nmol/L	1.2	(0.7–2.3)	1.3	(0.7–2.3)	0.55	1.3	(0.8–2.1)	1.3	(0.7–2.6)	0.78
Dihydrotestosterone (DHT), nmol/L	0.2	(0.1–0.3)	0.2	(0.1–0.3)	0.77	0.2	(0.1–0.3)	0.2	(0.1–0.3)	0.55
DHT sulfate (DHTS), nmol/L	1.0	(0.4–2.1)	1.1	(0.5–2.2)	0.45	1.0	(0.5–2.1)	1.2	(0.5–2.3)	0.24
Androsterone (ADT), nmol/L	0.5	(0.3–0.9)	0.5	(0.3–0.8)	0.42	0.5	(0.3–0.8)	0.5	(0.4–0.8)	0.68
ADT glucuronide (ADT-G), nmol/L	19.3	(7.0–52.3)	19.4	(8.1–60.0)	0.59	16.5	(6.4–45.6)	22.1	(13.3–69.4)	0.01
5α-androstane-3α,17β diol-3-glucuronide (3α-diol-3G), nmol/L	1.3	(0.5–3.6)	1.2	(0.5–3.7)	0.69	1.2	(0.5–3.6)	1.5	(0.6–4.1)	0.16
3α-diol-17-glucuronide (3α-diol-17G), nmol/L	1.2	(0.4–3.0)	1.2	(0.5–3.0)	0.80	1.2	(0.5–2.3)	1.4	(0.5–4.3)	0.14
Total 5α-reduced glucuronide metabolites										
Σ(ADT-G, 3α-diol-3G, 3α-diol-17G), nmol/L	22.1	(8.3–57.7)	21.9	(9.3–64.2)	0.70	19.2	(8.0–52.5)	25.2	(14.8–77.9)	0.01
5β-reduced androgen										
Etiocholanolone-glucuronide, nmol/L	32.1	(11.9–84.9)	30.4	(13.6–80.2)	0.72	28.5	(11.9–71.4)	34.1	(16.8–85.6)	0.16
Parent estrogens										
Unconjugated Estrone, pmol/L	55.4	(29.0–112)	58.9	(29.6–154)	0.20	52.0	(30.7–133)	65.5	(28.6–174)	0.03
Unconjugated estradiol, pmol/L	11.6	(4.0–37.4)	12.2	(4.2–42.7)	0.19	10.4	(4.3–38.3)	15.8	(4.2–70.1)	0.01
Ratios of parent estrogens to androgens										
Unconjugated estradiol/Androstenedione	8.6	(3.3–29.0)	9.4	(3.9–33.1)	0.24	8.3	(4.0–27.0)	10.3	(3.9–47.2)	0.10
Unconjugated estradiol/Testosterone	19.8	(7.6–68.7)	21.2	(8.2–82.9)	0.61	18.6	(7.8–68.3)	26.0	(9.6–117)	0.06

[a] *P* value from Wilcoxon test comparing two groups (controls and all ovarian cancer cases)

[b] *P* value from Kruskal-Wallis test comparing three groups (controls, serous cases, and non-serous cases)

Table 3.

Odds Ratios (ORs) and 95% Confidence Intervals (CIs) for risk of epithelial ovarian cancer overall and by subtypes for individual androgens/androgen metabolites and relevant ratios, nested case-control study within the WHI-OS.

	Tertile Median	**Controls**	**Cases**	**All ovarian cancer cases**		**Serous**			**Non-Serous**			
DHEA		n (%)	n (%)	OR[a]	95% CI	n (%)	OR[b]	95% CI	n (%)	OR[b]	95% CI	P het[c]
Tertile (T)1	2.4	135 (32.8)	50 (29.6)	1.00		35 (34.3)	1.00		15 (22.4)	1.00		0.14
T2	4.8	135 (32.8)	65 (38.5)	1.22	(0.78, 1.91)	41 (40.2)	1.14	(0.67, 1.92)	24 (35.8)	1.40	(0.69, 2.83)	
T3	8.7	140 (34.0)	54 (32.0)	1.00	(0.62, 1.62)	26 (25.5)	0.70	(0.39, 1.26)	28 (41.8)	1.56	(0.78, 3.14)	
DHEAS												
T1	518	135 (32.8)	48 (28.4)	1.00		37 (36.3)	1.00		11 (16.4)	1.00		0.05
T2	1109	135 (32.8)	67 (39.6)	1.32	(0.84, 2.08)	37 (36.3)	1.08	(0.64, 1.84)	30 (44.8)	2.78	(1.32, 5.87)	
T3	2045	140 (34.0)	54 (32.0)	1.00	(0.62, 1.62)	28 (27.5)	0.74	(0.42, 1.31)	26 (38.8)	1.97	(0.91, 4.27)	
Androstenedione												
T1	0.8	134 (32.5)	49 (29.0)	1.00		36 (35.3)	1.00		13 (19.4)	1.00		0.09
T2	1.3	136 (33.0)	63 (37.3)	1.29	(0.82, 2.03)	37 (36.3)	1.08	(0.64, 1.85)	26 (38.8)	1.91	(0.92, 3.94)	
T3	2.0	140 (34.0)	57 (33.7)	1.12	(0.70, 1.79)	29 (28.4)	0.78	(0.45, 1.38)	28 (41.8)	1.86	(0.91, 3.82)	
Testosterone												
T1	0.3	134 (32.5)	47 (27.8)	1.00		28 (27.5)	1.00		19 (28.4)	1.00		0.09
T2	0.5	134 (32.5)	57 (33.7)	1.17	(0.74, 1.84)	40 (39.2)	1.48	(0.85, 2.57)	17 (25.4)	0.86	(0.42, 1.75)	
T3	0.9	142 (34.5)	65 (38.5)	1.32	(0.84, 2.07)	34 (33.3)	1.12	(0.63, 1.99)	31 (46.3)	1.47	(0.77, 2.79)	
5α-androstanedione												
T1	0.8	135 (32.8)	56 (33.1)	1.00		33 (32.4)	1.00		23 (34.3)	1.00		0.57
T2	1.2	134 (32.5)	46 (27.2)	0.83	(0.52, 1.32)	32 (31.4)	0.97	(0.56, 1.68)	14 (20.9)	0.60	(0.29, 1.22)	
T3	2.0	141 (34.2)	67 (39.6)	1.19	(0.77, 1.84)	37 (36.3)	1.18	(0.69, 2.02)	30 (44.8)	1.37	(0.74, 2.53)	
DHT												
T1	0.1	134 (32.5)	56 (33.1)	1.00		34 (33.3)	1.00		22 (32.8)	1.00		0.04
T2	0.2	133 (32.3)	59 (34.9)	1.05	(0.67, 1.63)	29 (28.4)	0.84	(0.48, 1.47)	30 (44.8)	1.42	(0.77, 2.63)	
T3	0.3	143 (34.7)	54 (32.0)	0.86	(0.55, 1.35)	39 (38.2)	1.08	(0.64, 1.83)	15 (22.4)	0.67	(0.33, 1.35)	
DHTS												

	Tertile Median	Controls	Cases	All ovarian cancer cases		Serous			Non-Serous			
T1	0.5	134 (32.5)	50 (29.6)	1.00		36 (35.3)	1.00		14 (20.9)	1.00		0.16
T2	1.0	136 (33.0)	54 (32.0)	1.12	(0.71, 1.77)	30 (29.4)	0.80	(0.46, 1.40)	24 (35.8)	1.59	(0.77, 3.25)	
T3	1.8	140 (34.0)	65 (38.5)	1.22	(0.78, 1.91)	36 (35.3)	0.88	(0.52, 1.51)	29 (43.3)	1.76	(0.87, 3.55)	
Androsterone (ADT)												
T1	0.4	131 (31.8)	62 (36.7)	1.00		41 (40.2)	1.00		21 (31.3)	1.00		0.08
T2	0.5	139 (33.7)	51 (30.2)	0.73	(0.46, 1.14)	25 (24.5)	0.59	(0.33, 1.03)	26 (38.8)	1.16	(0.61, 2.21)	
T3	0.8	140 (34.0)	56 (33.1)	0.81	(0.53, 1.25)	36 (35.3)	0.76	(0.45, 1.28)	20 (29.9)	0.80	(0.41, 1.57)	
ADT-G												
T1	8.8	135 (32.8)	43 (25.4)	1.00		37 (36.3)	1.00		6 (9)	1.00		0.002
T2	18.9	135 (32.8)	74 (43.8)	1.65	(1.04, 2.62)	40 (39.2)	1.15	(0.68, 1.96)	34 (50.7)	5.97	(2.37, 15.05)	
T3	42.6	140 (34.0)	52 (30.8)	1.06	(0.64, 1.76)	25 (24.5)	0.68	(0.37, 1.24)	27 (40.3)	4.36	(1.68, 11.32)	
3αdiol-3G											*P trend*[d] *= 0.08*	
T1	0.6	135 (32.8)	59 (34.9)	1.00		41 (40.2)	1.00		18 (26.9)	1.00		0.32
T2	1.3	135 (32.8)	55 (32.5)	0.86	(0.55, 1.35)	32 (31.4)	0.81	(0.47, 1.39)	23 (34.3)	1.24	(0.62, 2.47)	
T3	2.9	140 (34.0)	55 (32.5)	0.80	(0.50, 1.28)	29 (28.4)	0.70	(0.39, 1.23)	26 (38.8)	1.31	(0.66, 2.63)	
3αdiol-17G												
T1	0.6	135 (32.8)	50 (29.6)	1.00		31 (30.4)	1.00		19 (28.4)	1.00		0.05
T2	1.2	135 (32.8)	67 (39.6)	1.24	(0.78, 1.96)	46 (45.1)	1.49	(0.87, 2.56)	21 (31.3)	0.96	(0.48, 1.92)	
T3	2.3	140 (34.0)	52 (30.8)	0.89	(0.55, 1.45)	25 (24.5)	0.78	(0.42, 1.43)	27 (40.3)	1.17	(0.60, 2.29)	
Total 5α-reduced glucuronide metabolites (Σ(ADT-G, 3αdiol-3G, 3αdiol-17G))												
T1	10.4	135 (32.8)	45 (26.6)	1.00		38 (37.3)	1.00		7 (10.4)	1.00		0.002
T2	21.8	135 (32.8)	73 (43.2)	1.54	(0.98, 2.44)	40 (39.2)	1.09	(0.64, 1.85)	33 (49.3)	4.99	(2.07, 11.98)	
T3	47.6	140 (34.0)	51 (30.2)	1.00	(0.60, 1.65)	24 (23.5)	0.61	(0.34, 1.12)	27 (40.3)	3.63	(1.47, 8.95)	
Etiocholanolone-glucuronide												
T1	15.9	135 (32.8)	62 (36.7)	1.00		41 (40.2)	1.00		21 (31.3)	1.00		0.25
T2	31.6	135 (32.8)	50 (29.6)	0.75	(0.48, 1.17)	31 (30.4)	0.81	(0.47, 1.38)	19 (28.4)	0.93	(0.47, 1.84)	
T3	63.6	140 (34.0)	57 (33.7)	0.84	(0.54, 1.32)	30 (29.4)	0.77	(0.44, 1.32)	27 (40.3)	1.32	(0.69, 2.50)	
Unconjugated Estrone										*P trend*[d] *= 0.10*		
T1	35.2	135 (32.8)	51 (30.2)	1.00		35 (34.3)	1.00		16 (23.9)	1.00		0.04

	Tertile Median	Controls	Cases	All ovarian cancer cases		Serous			Non-Serous			
T2	55.0	136 (33.0)	53 (31.4)	0.95	(0.59, 1.52)	35 (34.3)	0.92	(0.54, 1.59)	18 (26.9)	0.96	(0.46, 2.00)	
T3	90.4	140 (34.0)	64 (37.9)	1.06	(0.65, 1.75)	31 (30.4)	0.71	(0.39, 1.29)	33 (49.3)	1.79	(0.88, 3.62)	
Unconjugated Estradiol												
T1	5.0	135 (32.8)	50 (29.6)	1.00		37 (36.3)	1.00		13 (19.4)	1.00		0.01
T2	11.6	136 (33.0)	52 (30.8)	0.96	(0.60, 1.55)	32 (31.4)	0.83	(0.47, 1.44)	20 (29.9)	1.54	(0.72, 3.31)	
T3	27.1	140 (34.0)	66 (39.1)	1.09	(0.65, 1.84)	32 (31.4)	0.68	(0.36, 1.27)	34 (50.7)	2.57	(1.18, 5.60)	
Unconjugated estradiol/Androstenedione												
T1	3.9	135 (32.8)	51 (30.2)	1.00		39 (38.2)	1.00		12 (17.9)	1.00		0.01
T2	8.5	135 (32.8)	54 (32.0)	0.99	(0.61, 1.59)	27 (26.5)	0.67	(0.38, 1.20)	27 (40.3)	2.41	(1.13, 5.13)	
T3	20.7	140 (34.0)	63 (37.3)	1.06	(0.64, 1.76)	35 (34.3)	0.78	(0.43, 1.42)	28 (41.8)	2.30	(1.03, 5.13)	
Unconjugated estradiol/Testosterone												
T1	9.9	135 (32.8)	58 (34.3)	1.00		43 (42.2)	1.00		15 (22.4)	1.00		0.02
T2	19.7	135 (32.8)	50 (29.6)	0.76	(0.47, 1.23)	25 (24.5)	0.54	(0.30, 0.96)	25 (37.3)	1.55	(0.75, 3.18)	
T3	46.3	140 (34.0)	60 (35.5)	0.77	(0.45, 1.32)	33 (32.4)	0.60	(0.32, 1.13)	27 (40.3)	1.66	(0.75, 3.66)	

[a] OR from conditional logistic model, conditioned on matching factors (age at baseline, year of blood draw, race/ethnicity, hysterectomy status, and time since last menopausal hormone use) and additionally adjusted for body mass index, smoking status, gravidity, and duration of oral contraceptive use.

[b] OR from model adjusting for matching factors (age at baseline, year of blood draw, race/ethnicity, hysterectomy status, and time since last menopausal hormone use), and body mass index, smoking status, gravidity, and duration of oral contraceptive use.

[c] *P* heterogeneity

[d] *P* values for trend across tertile (median value of category), all other *P* trend>0.10