

Research Article

Sex Steroid Hormone Metabolism in Relation to Risk of Aggressive Prostate Cancer

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Abstract

Background: The combined action of androgens and estrogens—specifically their balance—may play a role in prostate carcinogenesis, but existing evidence is sparse and inconsistent. We investigated associations between serum sex steroid hormones, including estrogen metabolites, and risk of aggressive prostate cancer.

Methods: In a case–control study nested within the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial cohort, we measured serum estrone, estradiol, and 13 estrogen metabolites, in the 2-, 4-, or 16-hydroxylation pathways, using an LC/MS-MS assay. Cases ($n = 195$) were non-Hispanic white men ages 55 to 70 years when diagnosed with aggressive prostate cancer (stage III or IV and/or Gleason ≥ 7). Controls ($n = 195$) were non-Hispanic white men without prostate cancer who were frequency matched to cases by age and year at blood draw, and time since baseline screen. Only men with serum testosterone and sex hormone-binding globulin measured previously were eligible. Logistic regression models were used to estimate ORs and 95% confidence intervals (95% CI).

Results: Risk of aggressive prostate cancer was strongly inversely associated with estradiol:testosterone ratio (OR_{4th quartile vs. 1st} = 0.27; 95% CI, 0.12–0.59, $P_{\text{trend}} = 0.003$) and positively associated with 2:16 α -hydroxyestrone ratio (OR_{4th quartile vs. 1st} = 2.44; 95% CI, 1.34–4.45, $P_{\text{trend}} = 0.001$). Individual estrogen metabolites were unrelated to risk.

Conclusions: Our findings suggest that sex steroid hormones, specifically the estrogen-androgen balance, may be important in the development of aggressive prostate cancer.

Impact: Improved understanding of the hormonal etiology of prostate cancer is critical for prevention and therapeutic interventions. *Cancer Epidemiol Biomarkers Prev*; 23(11); 2374–82. ©2014 AACR.

Introduction

Despite extensive evidence of the essential role of sex steroid hormones in the growth, development, and maintenance of healthy prostate epithelium, a pooled analysis of the largest epidemiologic dataset to date, including 3,886 cases, demonstrated no evidence of an association between circulating androgens and

prostate cancer risk (1). Animal studies also suggest that androgen alone is insufficient to induce prostate carcinogenesis (2, 3).

Interest has recently turned to evaluating a possible role for other hormones, specifically estrogens and the balance of androgens and estrogens. It has been difficult to accurately measure the relatively low levels of circulating estrogen in men, and studies that have assessed estrogen in relation to prostate cancer have provided inconsistent findings (1, 4–9). Recently, the development of liquid chromatography-mass spectrometry (LC-MS/MS) methods has resulted in highly sensitive and accurate measurement of estrogens (10–12). This methodology has also made it possible to assess levels of multiple metabolites of estrogen allowing, for the first time, reliable explorations of these metabolites with disease risk.

To shed further light on the relationship of serum estrogens, androgens, and their balance on risk of prostate cancer, and to screen a number of estrogen metabolites for any relationship to prostate cancer risk, we utilized data from the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (PLCO) cohort, focusing on a population

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with clinically relevant disease, namely younger men who developed aggressive prostate cancers.

Materials and Methods

Study design and population

PLCO is a randomized, controlled trial designed to assess the effectiveness of screening on disease-specific mortality (13). Between 1993 and 2001, 76,683 men ages 55 to 74 years, were recruited to one of 10 screening centers across the United States. At baseline, all participants were requested to complete a questionnaire that asked about demographics, personal and family medical history, and health-related behaviors. Men randomized to the intervention arm underwent a PSA blood test and digital rectal exam (DRE) at baseline and annually for a further 3 years, and a PSA test alone for an additional 2 years. Blood samples were collected and processed according to a standardized protocol (14). Incident prostate cancers were ascertained through routine follow-up of positive screens (PSA > 4 ng/mL and/or a positive DRE) and an annual study update form that inquired about all cancer diagnoses. Cancers were confirmed with medical records. This trial was approved by the Institutional Review Boards at the National Cancer Institute (Rockville, MD) and individual screening centers.

The subjects for this analysis were drawn from a set of 727 cases and 889 controls with androgen data, measured by radioimmunoassay (RIA), in a previous PLCO analysis (15). Men were eligible for inclusion in this previous analysis if they had completed a baseline questionnaire, provided a blood sample at baseline, provided informed consent for use of their biospecimens, were self-reported to be non-Hispanic, and were not diagnosed with any cancer during the first year of follow-up.

Overdiagnosis of prostate cancer (i.e., a cancer that would not have become clinically apparent or symptomatic in the absence of screening) is a common concern and recent evidence suggests the likelihood of overdiagnosis increases with age (16). Therefore, we defined cases as aggressive cancer (stage III or IV and/or Gleason ≥ 7) diagnosed in younger men (≤ 70 years) because such a population is enriched for "clinically relevant" disease. Controls were defined as men free from prostate cancer for the duration of follow-up who were frequency matched, with a case-control ratio of 1:1, by age at blood draw (5-year intervals), time since baseline screen (1-year time windows), and year of blood draw. To minimize the potential effect of undetected preclinical prostate cancer on serum estrogens, control subjects from the original selection were deemed ineligible if they had subsequently been diagnosed with nonaggressive prostate cancer (through December 31, 2009; $n = 36$). Controls in the previous selection who were subsequently diagnosed with aggressive prostate cancer ($n = 10$) were eligible for inclusion in our study as cases. The final analytic sample included 195 cases ≤ 70 years of age at diagnosis of

histologically confirmed aggressive prostate cancer and 195 frequency-matched controls.

Laboratory assays

Blood samples collected at baseline were aliquoted to 1.8 mL vials within 2 hours of collection and frozen and stored at -70°C . Serum samples were thawed at 4°C for measurement of parent estrogens and estrogen metabolites (14).

A total of 15 estrogen and estrogen metabolites (EM) were quantitated concurrently using stable isotope dilution LC/MS-MS, including the parent estrogens (estrone and estradiol) and 13 metabolites in the 2-, 4-, and 16-hydroxylation pathways (Fig. 1). This method has been described previously (10, 11). In this analysis, we aimed to quantify the total amount of each estrogen metabolite (i.e., the sum of unconjugated and conjugated forms). Therefore, samples were hydrolyzed, extracted, and derivatized before analysis using LC/MS-MS, which was performed using a TSQ Quantum Ultra triple quadrupole mass spectrometer coupled with a Surveyor HPLC system (ThermoFinnigan). Serum estrogen metabolites were quantified using the Xcalibur Quan Browser (ThermoFinnigan). Calibration curves for each estrogen metabolite were constructed by plotting EM-dansyl/SI-EM-dansyl peak area ratios obtained from EM calibration standards versus amounts of EM and fitting these data using linear regression with $1/X$ weighting. The assay specificity and quantitative analysis were enhanced by the inclusion of carbon-13 labeled stable isotope-labeled internal standards for the estrogen metabolites.

Cases and controls were included in each assay batch of approximately 40 samples. Serum samples from 4 healthy men, ages 55 to 70 years, were included as quality control samples; two aliquots from each of three of these subjects were randomly included with each batch. Laboratory personnel were blinded to both case-control status and quality control samples. The overall coefficients of variation (CV) ranged from 9.7% for 4-hydroxyestrone to 26.5% for 16-epiestriol. The overall CVs were 11.77% for estrone and 11.88% for estradiol.

Testosterone was measured by direct RIA (Immuno-technique; CV = 14%) and sex hormone binding globulin (SHBG) by a sandwich immunoradiometric assay (CIS-Bio; CV = 18%) by methods that have been previously described (15). Although testosterone and estrogens are measured using different methods (RIA vs. LC/MS-MS), a previous comparison of the two assays showed that—although the absolute values differed—there was a very strong correlation (17).

Statistical analysis

We compared baseline characteristics of cases and controls using the χ^2 test for categorical variables and the two-sided Wilcoxon rank-sum test for continuous variables. To evaluate correlations between hormone levels, we calculated Spearman correlation coefficients using continuous measures of estrogen, estrogen metabolites,

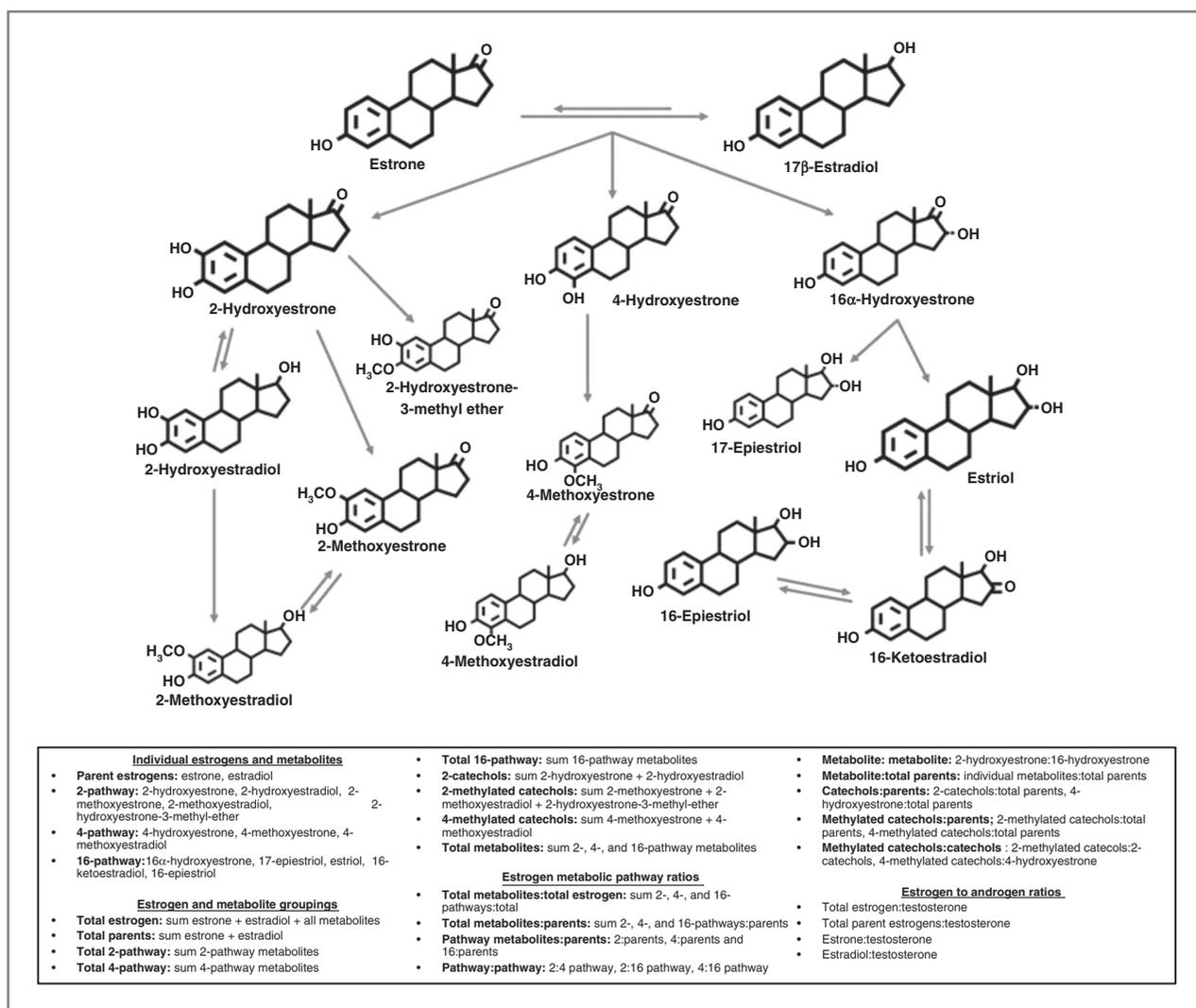


Figure 1. Endogenous estrogen metabolism pathways and list of estrogens, estrogen metabolites, and hormone ratios explored.

and testosterone in control subjects. Wilcoxon rank-sum tests were used for univariate comparisons of hormone levels in cases versus controls.

Estrogens and estrogen metabolites were analyzed individually, as groups classified by their metabolic pathway, and in ratios of individual hormones and metabolic pathway groups (Fig. 1). Individual and grouped estrogens, estrogen metabolites, and ratios were log-transformed and assigned to quartiles using the distribution of hormone levels in control subjects. We examined *a priori* associations of risk with estrone, estradiol, and the ratio of each to testosterone and then explored a total of 54 estrogen metabolites, combinations of metabolites, and ratios (Fig. 1).

ORs and 95% confidence intervals (95% CI) were calculated using logistic regression models, treating the lowest quartile as the reference group. All models presented are adjusted for age at blood draw, body mass index (BMI), and SHBG. Further adjustment for potential

confounders, including family history of prostate cancer, diabetes, and smoking status, did not materially influence the results. We tested for trend across quartiles by treating the median value of each quartile as an ordinal variable in the logistic regression model.

Results

Characteristics of the cases and controls at baseline are presented in Table 1. The mean age at baseline blood draw was 62.8 years and the mean duration of follow-up between blood draw and diagnosis was 2.9 years (range: 1.0–8.9 years).

Median concentrations of estrogens, estrogen metabolites, estrogen metabolism pathways, testosterone, and SHBG were generally similar between cases and controls (Supplementary Table S1). The estrogen and estrogen metabolite concentrations among controls were moderately to highly correlated (Table 2). For example,

Table 1. Baseline characteristics of prostate cancer cases and controls

Characteristics	Cases, <i>n</i> (%)	Controls, <i>n</i> (%)	<i>P</i> ^a
Age at baseline			0.99
55–59	33 (16.9)	33 (16.9)	
60–64	94 (48.2)	95 (48.7)	
65–70	68 (34.9)	67 (34.4)	
Year at blood draw			1.00
94–95	99 (50.7)	99 (50.7)	
96–97	75 (38.5)	75 (38.5)	
98–99	21 (10.8)	21 (10.8)	
Family history of prostate cancer	20 (10.3)	9 (4.6)	0.03
Diabetes	7 (3.6)	15 (7.8)	0.08
BMI (kg/m ²)			0.64
<25	49 (25.4)	53 (27.3)	
25–29.99	103 (53.4)	95 (49)	
≥30	41 (21.2)	46 (23.7)	
Smoking status			0.02
Never	98 (50.2)	64 (32.8)	
Former	85 (43.8)	112 (57.4)	
Current	11 (5.7)	19 (9.7)	
Median (IQR) PSA at blood draw (ng/mL)	3.45 (2.54)	0.96 (1.01)	<0.0001

^a χ^2 test for categorical variables and the two-sided Wilcoxon rank sum test for PSA as a continuous variable.

correlation coefficients ranged from 0.50 ($P < 0.001$), for 2- and 4-hydroxyestrone, to 0.98 ($P < 0.001$), for estradiol and 2-hydroxyestradiol. Testosterone showed low correlations with estrone and estradiol ($r = 0.20$ and 0.34 , respectively), whereas estradiol and estrone were moderately correlated ($r = 0.65$). SHBG was moderately correlated with testosterone ($r = 0.67$), but only weakly correlated with estradiol ($r = 0.19$).

Table 3 shows the adjusted ORs and 95% CIs for the relationships between estradiol, estrone, testosterone, and the ratio of the estrogens to testosterone (quartiles) with respect to risk. Consistent with a previous analysis (of most of the same cases), we saw an increasing risk with increasing testosterone levels (OR_{4th quartile vs. 1st} = 2.96; 95% CI, 1.25–7.00). Although we observed no association of risk with estrone or estradiol, the estradiol:testosterone ratio was statistically significantly associated with a decreased risk (OR_{4th quartile vs. 1st} = 0.27; 95% CI, 0.12–0.59, $P_{\text{trend}} = 0.003$). We mutually adjusted testosterone and estradiol:testosterone ratio to explore whether the observed protective effect of the ratio was being driven by the increased risk associated with testosterone. This resulted in a marked attenuation of the association between testosterone and aggressive prostate cancer while the association between estradiol:testosterone ratio and risk was minimally affected (Table 3). Although the ratio is the appropriate metric for this analysis, we also modeled cross-classified tertiles of estradiol and tertiles of testosterone in relation to aggressive prostate cancer risk to provide a fuller picture of the combined effects (Supplementary

Table S2), results of which support our interpretation that increased risk was largely driven by estradiol:testosterone ratio and no individual component of such.

We observed no significant trends between any individual estrogen metabolites with respect to risk (Table 4). The ratio of 2:16 α -hydroxyestrone was statistically significantly associated with an increased risk of aggressive prostate cancer (OR_{4th quartile vs. 1st} = 2.44; 95% CI, 1.34–4.45, $P_{\text{trend}} = 0.001$).

Discussion

In this analysis of sex steroid hormones in relation to risk of aggressive prostate cancer in men ages ≤ 70 years, we found that a higher estradiol:testosterone ratio was strongly associated with reduced risk, and that controlling for this ratio markedly reduced the previously reported positive association with testosterone. Our exploration of estrogen metabolites revealed only one statistically significant association—a higher ratio of 2:16 α -hydroxyestrone associated with an increased risk.

Parent estrogens

There is circumstantial evidence to support both the idea that higher circulating levels of estrogens increase prostate cancer risk and the idea that they play a protective role (2, 18–24). However, there has been no consistent pattern from epidemiologic studies of pre-diagnostic serum estrogens and prostate cancer risk (1, 5–9). In a large pooled analysis in 2008, which

Table 2. Spearman correlation coefficients of sex steroid hormones and SHBG in control subjects

	T	SHBG	E1	E2	2-OHE1	2-OHE2	2-MEOE1	2-MEOE2	3-MEOE1	16-OHE1	E3	17-EPIE3	1-KETOE2	16-EPIE3	4-OHE1	4-MEOE1	4-MEOE2
SHBG	0.67	1.00															
E1	0.20 ^a	0.05 ^b	1.00														
E2	0.34	0.19 ^a	0.65	1.00													
2-OHE1	0.21 ^a	0.10 ^b	0.89	0.57	1.00												
2-OHE2	0.23 ^a	0.13 ^b	0.80	0.50	0.92	1.00											
2-MEOE1	0.08 ^b	0.01 ^b	0.80	0.54	0.72	0.61	1.00										
2-MEOE2	0.17 ^c	0.06 ^b	0.74	0.65	0.70	0.60	0.80	1.00									
3-MEOE1	0.09 ^b	0.01 ^b	0.74	0.45	0.68	0.56	0.88	0.76	1.00								
16-OHE1	0.21 ^a	0.09 ^b	0.90	0.58	0.99	0.91	0.75	0.72	0.71	1.00							
E3	0.17 ^c	0.05 ^b	0.90	0.56	0.91	0.83	0.85	0.79	0.83	0.93	1.00						
17-EPIE3	0.10 ^b	-0.01	0.79	0.50	0.81	0.66	0.81	0.84	0.83	0.83	0.88	1.00					
1-KETOE2	0.20 ^a	0.08 ^b	0.90	0.59	0.98	0.90	0.75	0.74	0.73	0.99	0.93	0.84	1.00				
16-EPIE3	0.14 ^c	0.04 ^b	0.84	0.54	0.83	0.73	0.87	0.83	0.86	0.86	0.93	0.91	0.88	1.00			
4-OHE1	0.20 ^a	0.08 ^b	0.87	0.54	0.98	0.93	0.70	0.68	0.66	0.97	0.90	0.78	0.96	0.81	1.00		
4-MEOE1	0.10 ^b	-0.01	0.81	0.59	0.74	0.61	0.95	0.84	0.84	0.76	0.84	0.85	0.76	0.85	0.70	1.00	
4-MEOE2	0.05 ^b	-0.08	0.73	0.61	0.65	0.49	0.84	0.94	0.80	0.68	0.77	0.84	0.70	0.84	0.63	0.82	1

NOTE: All correlations are at the *P* value of <0.0001, except where noted.Abbreviations: 16-EPIE3, 16-epiestriol; 16-OHE1, 16 α -hydroxyestrone; 17-EPIE3, 17-epiestriol; 1-KETOE2, 16-ketoestradiol; 2-MEOE1, 2-methoxyestrone; 2-OHE1, 2-hydroxyestrone; 2-OHE2, 2-hydroxyestradiol; 3-MEOE1, 2-hydroxyestrone-3-methyl ether; 4-MEOE1, 4-methoxyestrone; 4-MEOE2, 4-methoxyestradiol; 4-OHE1, 4-hydroxyestrone; E1, estrone; E2, estradiol; E3, estriol; T, testosterone.^a*P* < 0.01.^bN/S.^c*P* < 0.05.

Table 3. Associations (OR; 95% CI) between circulating estrogens and testosterone concentrations and aggressive prostate cancer

Hormone measure	Quartile				<i>P</i> _{trend}
	1	2	3	4	
Parent estrogens	1.00	1.39 (0.79–2.46)	1.21 (0.68–2.16)	0.92 (0.50–1.68)	0.70
Estrone	1.00	1.39 (0.79–2.47)	1.36 (0.77–2.42)	0.86 (0.47–1.60)	0.55
Estradiol	1.00	1.52 (0.87–2.68)	1.13 (0.63–2.02)	0.80 (0.43–1.48)	0.30
Testosterone	1.00	1.83 (0.88–3.76)	1.82 (0.88–3.76)	2.96 (1.25–7.00)	0.018
Estrogen-to-testosterone ratios					
Total parents:testosterone	1.00	0.81 (0.45–1.46)	0.66 (0.35–1.24)	0.62 (0.31–1.26)	0.16
Estrone:testosterone	1.00	0.79 (0.44–1.41)	0.74 (0.40–1.39)	0.64 (0.32–1.27)	0.21
Estradiol:testosterone	1.00	0.48 (0.26–0.89)	0.50 (0.26–0.97)	0.27 (0.12–0.59)	0.003
Mutually adjusted model ^a					
Estradiol:testosterone	1.00	0.52 (0.28–0.99)	0.59 (0.28–1.25)	0.35 (0.14–0.89)	0.016
Testosterone	1.00	1.54 (0.80–2.98)	1.30 (0.59–2.85)	1.64 (0.60–4.47)	0.47

NOTE: Adjusted for age at blood draw, BMI, and SHBG. Boldface indicates findings that are statistically significant.

^aEstimates derived from the same model that is mutually adjusted for testosterone and estradiol:testosterone ratio.

included 18 prospective studies, 3,886 incident prostate cancer cases, and 6,438 control subjects, no association was found between estradiol and prostate cancer (1), but exposure misclassification may have been introduced through use of study-specific categorization of hormone distributions. Furthermore, the included studies all used RIAs from various manufacturers to assess hormone levels, a method often limited by cross-reactivity, which may have contributed to inconsistent findings. Nevertheless, using the gold standard method of LC/MS-MS to quantitate serum estrogens and estrogen metabolites, we also found no associations between estradiol or estrone in relation to aggressive prostate cancer risk. Although a recent study using gas chromatography mass spectrometry assays reported a strong, positive association between serum estrone and incident prostate cancer risk (HR = 3.93; 95% CI, 1.61–9.57), the possibility of extant disease (by inclusion of men diagnosed within one year of blood draw) might explain this association (4). Consistent with our findings, the authors observed no association between estradiol—the most potent estrogen—and prostate cancer risk.

Estrogen-to-testosterone ratio

In men, testosterone is predominantly converted to dihydrotestosterone (DHT) by 5 α reductase but may also be converted to parent estrogens via the action of the aromatase (CYP19) enzyme. It has been suggested that men with higher levels of intraprostatic DHT (as indicated by higher circulating levels of androstenediol glucuronide) may be at increased prostate cancer risk (25). It is plausible that if 5 α reductase activity is inhibited, the resultant excess testosterone that is prevented from being converted to DHT may be metabolized to estradiol. Nota-

bly, in Prostate Cancer Prevention Trial, serum estrogen levels were found to increase in men taking Finasteride (a 5 α reductase inhibitor; ref. 9). Moreover, aberrant aromatase activity is seen in some prostate cancer cells (26) and a study recently identified an association between functional polymorphisms in genes related to estrogen metabolism (CYP1B1 and CYP19A1) and prostate cancer risk (27), further supporting a role for estrogen and androgen metabolites, or the estrogen-androgen balance, in prostate carcinogenesis.

The few epidemiologic studies that have explored the estrogen:androgen ratio in prostate cancer risk have produced inconsistent results (4, 7, 28–30). In a case-control study of men who were diagnosed in the pre-PSA era (a population presumably enriched for "clinically relevant" disease), Gann and colleagues found a weak inverse association between estradiol:testosterone ratio and total prostate cancer risk (OR_{4th quartile vs. 1st} = 0.77; 95% CI, 0.47–1.27; OR_{3rd quartile vs. 1st} = 0.51; 95% CI, 0.30–0.85; ref. 7). Tsai and colleagues reported a strong, inverse association with prostate cancer in Caucasians (OR_{4th quartile vs. 1st} = 0.33; 95% CI, 0.16–0.70); this association was reported to be stronger, albeit non-statistically significant, when the analysis was restricted to aggressive cases (those diagnosed at regional or distant stages or with poorly differentiated or undifferentiated grades; ref. 28). In contrast, Platz and colleagues reported an increased risk of high-grade (Gleason \geq 7) prostate cancer in men with higher estradiol:testosterone ratio levels (OR_{4th quartile vs. 1st} = 3.02; 95% CI, 1.29–7.04), and a decreased risk for low-grade disease (OR_{4th quartile vs. 1st} = 0.47; 95% CI, 0.23–0.96; ref. 29). The 2008 Endogenous Hormones Prostate Cancer Collaborative Group pooled analysis did not report an analysis of estradiol:testosterone ratio. Of note, however, is their observation of an

Table 4. Associations (OR; 95% CI) between circulating sex steroid hormone concentrations and aggressive prostate cancer

Estrogen and estrogen metabolism measures	Quartile				<i>P</i> _{trend}
	1	2	3	4	
All estrogens and estrogen metabolites	1.00	1.27 (0.72–2.23)	1.28 (0.72–2.27)	0.84 (0.46–1.54)	0.65
2-Hydroxylation pathway	1.00	1.53 (0.87–2.70)	1.27 (0.71–2.28)	0.94 (0.51–1.72)	0.69
2-Hydroxylation pathway catechols	1.00	1.35 (0.75–2.41)	1.63 (0.92–2.87)	0.87 (0.47–1.62)	0.95
2-Hydroxyestrone	1.00	1.48 (0.82–2.65)	1.49 (0.84–2.64)	0.91 (0.49–1.68)	0.89
2-Hydroxyestradiol	1.00	1.23 (0.70–2.16)	0.94 (0.52–1.68)	0.92 (0.52–1.65)	0.79
2-Hydroxylation pathway methylated catechols	1.00	0.54 (0.30–0.98)	0.71 (0.41–1.24)	0.59 (0.33–1.06)	0.14
2-Methoxyestrone	1.00	0.47 (0.26–0.85)	0.65 (0.37–1.13)	0.53 (0.29–0.94)	0.06
2-Methoxyestradiol	1.00	1.07 (0.61–1.88)	0.96 (0.54–1.70)	0.80 (0.44–1.45)	0.38
2-Hydroxyestrone-3-methyl ether	1.00	0.75 (0.42–1.33)	0.65 (0.36–1.15)	0.82 (0.47–1.44)	0.34
4-Hydroxylation pathway	1.00	0.82 (0.46–1.45)	1.13 (0.65–1.97)	0.63 (0.34–1.14)	0.33
4-Hydroxyestrone	1.00	1.85 (1.05–3.28)	1.20 (0.66–2.17)	1.07 (0.58–1.97)	0.89
4-Hydroxylation pathway methylated catechols	1.00	0.63 (0.32–1.27)	0.60 (0.30–1.20)	0.54 (0.27–1.10)	0.09
4-Methoxyestrone	1.00	0.48 (0.24–0.97)	0.67 (0.34–1.30)	0.45 (0.22–0.92)	0.08
4-Methoxyestradiol	1.00	0.58 (0.29–1.17)	0.70 (0.36–1.37)	0.52 (0.25–1.06)	0.12
16-Hydroxylation pathway	1.00	1.02 (0.58–1.81)	1.04 (0.59–1.83)	0.76 (0.42–1.38)	0.43
16 α -Hydroxyestrone	1.00	1.54 (0.86–1.77)	1.73 (0.97–3.07)	0.84 (0.44–1.58)	0.87
Estrinol	1.00	1.00 (0.57–1.75)	0.77 (0.43–1.38)	0.82 (0.46–1.49)	0.33
17-Epiestriol	1.00	0.73 (0.41–1.30)	0.85 (0.48–1.50)	0.83 (0.47–1.47)	0.72
16-Ketoestradiol	1.00	1.23 (0.69–2.19)	1.41 (0.80–2.48)	0.87 (0.47–1.60)	0.80
16-Epiestriol	1.00	0.75 (0.42–1.34)	0.75 (0.42–1.34)	0.72 (0.41–1.28)	0.30
Estrogen metabolic pathway ratios					
2-Hydroxylation pathway:parent estrogens	1.00	1.54 (0.86–2.76)	0.85 (0.45–1.60)	1.69 (0.95–3.02)	0.24
4-Hydroxylation pathway:parent estrogens	1.00	1.11 (0.62–2.01)	1.40 (0.79–2.47)	1.14 (0.63–2.05)	0.51
16-Hydroxylation pathway:parent estrogens	1.00	1.32 (0.74–2.35)	0.98 (0.55–1.77)	0.99 (0.55–1.78)	0.71
2-Hydroxylation pathway:16-hydroxylation pathway	1.00	1.31 (0.71–2.42)	1.73 (0.96–3.12)	1.53 (0.84–2.79)	0.10
2-Hydroxyestrone:16-hydroxyestrone	1.00	1.24 (0.65–2.37)	1.87 (1.01–3.44)	2.44 (1.34–4.45)	0.001
2-Hydroxylation pathway:4-hydroxylation pathway	1.00	1.41 (0.78–2.52)	1.09 (0.61–1.98)	1.29 (0.72–2.31)	0.62
4-Hydroxylation pathway:16-hydroxylation pathway	1.00	1.14 (0.63–2.03)	1.18 (0.66–2.10)	1.27 (0.71–2.27)	0.42
2-Hydroxylation pathway methylated catechols:catechols	1.00	1.12 (0.65–1.94)	0.56 (0.31–1.02)	0.73 (0.41–1.30)	0.08
4-Hydroxylation pathway methylated catechols:catechols	1.00	0.60 (0.30–1.22)	1.01 (0.52–1.94)	0.37 (0.17–0.80)	0.08

NOTE: Adjusted for age at blood draw, BMI, and SHBG. Boldface indicates findings that are statistically significant.

increased risk of prostate cancer with higher free testosterone when analyses were restricted to prostate cancers diagnosed pre-1990 (approximately the pre-PSA era, enriching for "clinically relevant" disease; ref. 1). Similar to the prior PLCO sub-analysis restricted to aggressive disease and adjusted for SHBG, we observed a strong increased risk of aggressive prostate cancer with higher levels of testosterone. Furthermore, the association between estradiol-to-testosterone ratio and aggressive prostate cancer risk was somewhat strengthened when SHBG was included in our analyses. SHBG can bind to both testosterone and estradiol, with a slightly stronger affinity for the former. As such, SHBG levels are directly related to the active estrogen to androgen balance and may indicate a potential mechanism for the previously observed, small but statistically significant, inverse association between SHBG and prostate cancer risk (1). There-

fore, the inconclusive findings to date may be explained, in part, by key differences between studies such as heterogeneous case mixes (e.g., total incident cancer, clinical disease, PSA-detected disease), varying definitions of aggressive disease, lack of adjustment for SHBG, differing ages of the subjects at blood draw and cancer diagnosis, variable follow-up time, variable potential for extant disease in controls, and use of RIAs that are less accurate than mass spectrometry and are variable in quantitative parameters by manufacturer.

Estrogen metabolites

One proposed mechanism of estrogen action in carcinogenesis involves the conversion of parent estrogens to estrogen metabolites that can form DNA adducts or produce oxidative DNA damage (31). Specifically, catechols in the 2- and 4-hydroxylation pathways can be oxidized to

form quinones that can then react with DNA to form a variety of adducts. This study is the first to evaluate prediagnostic serum estrogen metabolites in relation to (aggressive) prostate cancer risk but urinary estrogen metabolites have been assessed in a few studies (32–34). A recent meta-analysis showed an increased risk of total prostate cancer with higher urinary levels of 16 α -hydroxyestrone (OR_{3rd tertile vs. 1st} = 1.82; 95% CI, 1.09–3.05), whereas we observed no association between this metabolite and aggressive prostate cancer (34). The authors also reported a protective effect of higher urinary excretion of 2:16 α -hydroxyestrone ratio (OR_{3rd tertile vs. 1st} = 0.53; 95% CI, 0.31–0.90), whereas we observed the converse. Our finding of a positive association between serum 2:16 α -hydroxyestrone ratio and aggressive prostate cancer may indicate a potential role for estrogen metabolism in the development of aggressive prostate cancer; yet mechanistic studies in prostate cancer model systems are lacking. In breast cancer model systems, 16 α -hydroxyestrone has been shown to be a potent estrogen whereas 2-hydroxyestrone has been shown to have weak binding affinity with estrogen receptors and presumed antiestrogenic capabilities (35–37). Given the number of associations explored in this metabolite analysis of prostate cancer, it is also possible that this finding could be due to chance and thus requires replication.

Strengths and limitations

There are several limitations of note. We measured serum hormones but the extent to which circulating levels reflect intraprostatic levels of hormones is unclear. Furthermore, we measured hormones using nonfasting blood drawn at a single time-point that may not reflect cumulative lifetime exposure to hormones or levels at a time when they are most etiologically relevant. It is noteworthy that a recent study showed a decreased risk of total prostate cancer in men with higher levels of estrogen relative to testosterone measured in blood drawn in younger men (median age 34 years) followed for a median of 32 years (28). We investigated many associations resulting in the potential for false discovery. However, we did not account for multiple comparisons because the metabolites were all moderately to highly correlated. It is plausible that the influence of the estrogen-androgen balance is differentially associated with localized and advanced prostate disease, but we did not measure estrogens in men with nonaggressive disease and thus cannot directly address this question. We cannot exclude the possibility of reverse causation; in analyses restricted to cancers diagnosed 1 to 3 years from blood draw, we observed a strongly reduced risk of disease associated with estradiol to testosterone ratio (data not shown). For cases diagnosed more than 3 years from blood draw, the risk of disease remained markedly reduced with a higher estradiol to testosterone ratio but the results were no longer statistically significant.

Strengths include the moderate size of the current study and use of a sensitive and reliable assay. The associations we observed were not explained by confounding due to age, cigarette smoking, diabetes, family history of prostate cancer, or obesity. It is unlikely that our findings can be explained by an effect of prevalent but undiagnosed cancer on hormone levels; while contamination of the control group with extant disease would be expected to attenuate any true associations, none of our control subjects, who underwent annual PSA screening for 6 years, were diagnosed with prostate cancer during the 17 years of follow-up.

Conclusion

In this analysis of prediagnostic serum sex steroid hormones in relation to aggressive prostate cancer, diagnosed at or before the age of 70 years, we present evidence of a substantially reduced risk of disease in men with higher estradiol:testosterone ratios. We also observed an increased risk with higher ratios of 2:16 α -hydroxyestrone. Considered together, these associations suggest that strong estrogenic effects (relative to androgen or antagonistic metabolites of estrogen) may offer protection against aggressive prostate cancer. It is conceivable that the numerous previous attempts to identify the independent effects of androgens (and estrogens) on prostate cancer risk have provided equivocal and inconclusive results because hormones do not act independently in relation to prostate cancer risk but rather elicit their effects as part of a complex pathway. Future studies should explore the estrogen-androgen balance in relation to such prostate cancer endpoints as symptomatic, aggressive, and/or fatal disease.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): A. Black, P.F. Pinsky, R.T. Falk, L. Chu, X. Xu, K. Yu, R.N. Hoover, M.B. Cook

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References

1. Roddam AW, Allen NE, Appleby P, Key TJ. Endogenous sex hormones and prostate cancer: a collaborative analysis of 18 prospective studies. *J Natl Cancer Inst* 2008;100:170–83.
2. Nelles JL, Hu WY, Prins GS. Estrogen action and prostate cancer. *Expert Rev Endocrinol Metab* 2011;6:437–51.
3. Bosland MC. A perspective on the role of estrogen in hormone-induced prostate carcinogenesis. *Cancer Lett* 2013;334:28–33.
4. Daniels NA, Nielson CM, Hoffman AR, Bauer DC. Sex hormones and the risk of incident prostate cancer. *Urology* 2010;76:1034–40.
5. Chen C, Weiss NS, Stanczyk FZ, Lewis SK, DiTommaso D, Etzioni R, et al. Endogenous sex hormones and prostate cancer risk: a case-control study nested within the Carotene and Retinol Efficacy Trial. *Cancer Epidemiol Biomarkers Prev* 2003;12:1410–6.
6. Dorgan JF, Albanes D, Virtamo J, Heinonen OP, Chandler DW, Galmarini M, et al. Relationships of serum androgens and estrogens to prostate cancer risk: results from a prospective study in Finland. *Cancer Epidemiol Biomarkers Prev* 1998;7:1069–74.
7. Gann PH, Hennekens CH, Ma J, Longcope C, Stampfer MJ. Prospective study of sex hormone levels and risk of prostate cancer. *J Natl Cancer Inst* 1996;88:1118–26.
8. Kristal AR, Till C, Tangen CM, Goodman PJ, Neuhauser ML, Stanczyk FZ, et al. Associations of serum sex steroid hormone and 17 α -androstane-3 α ,17 β -diol glucuronide concentrations with prostate cancer risk among men treated with finasteride. *Cancer Epidemiol Biomarkers Prev* 2012;21:1823–32.
9. Yao S, Till C, Kristal AR, Goodman PJ, Hsing AW, Tangen CM, et al. Serum estrogen levels and prostate cancer risk in the prostate cancer prevention trial: a nested case-control study. *Cancer Causes Control* 2011;22:1121–31.
10. Ziegler RG, Faupel-Badger JM, Sue LY, Fuhrman BJ, Falk RT, Boyd-Morin J, et al. A new approach to measuring estrogen exposure and metabolism in epidemiologic studies. *J Steroid Biochem Mol Biol* 2010;121:538–45.
11. Xu X, Roman JM, Issaq HJ, Keefer LK, Veenstra TD, Ziegler RG. Quantitative measurement of endogenous estrogens and estrogen metabolites in human serum by liquid chromatography-tandem mass spectrometry. *Anal Chem* 2007;79:7813–21.
12. Fuhrman BJ, Schairer C, Gail MH, Boyd-Morin J, Xu X, Sue LY, et al. Estrogen metabolism and risk of breast cancer in postmenopausal women. *J Natl Cancer Inst* 2012;104:326–39.
13. Prorok PC, Andriole GL, Bresalier RS, Buys SS, Chia D, Crawford ED, et al. Design of the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial. *Control Clin Trials* 2000;21(6 Suppl):273S–309S.
14. Hayes RB, Reding D, Kopp W, Subar AF, Bhat N, Rothman N, et al. Etiologic and early marker studies in the prostate, lung, colorectal and ovarian (PLCO) cancer screening trial. *Control Clin Trials* 2000;21(6 Suppl):349S–55S.
15. Weiss JM, Huang WY, Rinaldi S, Fears TR, Chatterjee N, Hsing AW, et al. Endogenous sex hormones and the risk of prostate cancer: a prospective study. *Int J Cancer* 2008;122:2345–50.
16. Gulati R, Inoue LY, Gore JL, Katcher J, Etzioni R. Individualized estimates of overdiagnosis in screen-detected prostate cancer. *J Natl Cancer Inst* 2014;106:djt367.
17. Hsing AW, Stanczyk FZ, Belanger A, Schroeder P, Chang L, Falk RT, et al. Reproducibility of serum sex steroid assays in men by RIA and mass spectrometry. *Cancer Epidemiol Biomarkers Prev* 2007;16:1004–8.
18. Glantz GM. Cirrhosis and carcinoma of the prostate gland. *J Urol* 1964;91:291–3.
19. Leav I, Merk FB, Kwan PW, Ho SM. Androgen-supported estrogen-enhanced epithelial proliferation in the prostates of intact Noble rats. *Prostate* 1989;15:23–40.
20. Nguewa PA, Calvo A. Use of transgenic mice as models for prostate cancer chemoprevention. *Curr Mol Med* 2010;10:705–18.
21. Ricke WA, Ishii K, Ricke EA, Simko J, Wang Y, Hayward SW, et al. Steroid hormones stimulate human prostate cancer progression and metastasis. *Int J Cancer* 2006;118:2123–31.
22. Rohrmann S, Nelson WG, Rifai N, Brown TR, Dobs A, Kanarek N, et al. Serum estrogen, but not testosterone, levels differ between black and white men in a nationally representative sample of Americans. *J Clin Endocrinol Metab* 2007;92:2519–25.
23. Swerdlow AJ, Schoemaker MJ, Higgins CD, Wright AF, Jacobs PA. Cancer incidence and mortality in men with Klinefelter syndrome: a cohort study. *J Natl Cancer Inst* 2005;97:1204–10.
24. Yan L, Spitznagel EL. Soy consumption and prostate cancer risk in men: a revisit of a meta-analysis. *Am J Clin Nutr* 2009;89:1155–63.
25. Platz EA, Giovannucci E. The epidemiology of sex steroid hormones and their signaling and metabolic pathways in the etiology of prostate cancer. *J Steroid Biochem Mol Biol* 2004;92:237–53.
26. Ellem SJ, Schmitt JF, Pedersen JS, Frydenberg M, Risbridger GP. Local aromatase expression in human prostate is altered in malignancy. *J Clin Endocrinol Metab* 2004;89:2434–41.
27. Cussenot O, Azzouzi AR, Nicolaiew N, Fromont G, Mangin P, Cormier L, et al. Combination of polymorphisms from genes related to estrogen metabolism and risk of prostate cancers: the hidden face of estrogens. *J Clin Oncol* 2007;25:3596–602.
28. Tsai CJ, Cohn BA, Cirillo PM, Feldman D, Stanczyk FZ, Whittemore AS. Sex steroid hormones in young manhood and the risk of subsequent prostate cancer: a longitudinal study in African-Americans and Caucasians (United States). *Cancer Causes Control* 2006;17:1237–44.
29. Platz EA, Leitzmann MF, Rifai N, Kantoff PW, Chen YC, Stampfer MJ, et al. Sex steroid hormones and the androgen receptor gene CAG repeat and subsequent risk of prostate cancer in the prostate-specific antigen era. *Cancer Epidemiol Biomarkers Prev* 2005;14:1262–9.
30. Severi G, Morris HA, MacInnis RJ, English DR, Tilley W, Hopper JL, et al. Circulating steroid hormones and the risk of prostate cancer. *Cancer Epidemiol Biomarkers Prev* 2006;15:86–91.
31. Cavalieri E, Frenkel K, Liehr JG, Rogan E, Roy D. Estrogens as endogenous genotoxic agents—DNA adducts and mutations. *J Natl Cancer Inst Monogr* 2000:75–93.
32. Muti P, Westerlind K, Wu T, Grimaldi T, De Berry J III, Schunemann H, et al. Urinary estrogen metabolites and prostate cancer: a case-control study in the United States. *Cancer Causes Control* 2002;13:947–55.
33. Yang L, Gaikwad NW, Meza J, Cavalieri EL, Muti P, Trock B, et al. Novel biomarkers for risk of prostate cancer: results from a case-control study. *Prostate* 2009;69:41–8.
34. Barba M, Yang L, Schunemann HJ, Sperati F, Grioni S, Stranges S, et al. Urinary estrogen metabolites and prostate cancer: a case-control study and meta-analysis. *J Exp Clin Cancer Res* 2009;28:135.
35. Lewis JS, Thomas TJ, Klinge CM, Gallo MA, Thomas T. Regulation of cell cycle and cyclins by 16 α -hydroxyestrogen in MCF-7 breast cancer cells. *J Mol Endocrinol* 2001;27:293–307.
36. Bradlow HL, Hershcopf RJ, Martucci CP, Fishman J. Estradiol 16 α -hydroxylation in the mouse correlates with mammary tumor incidence and presence of murine mammary tumor virus: a possible model for the hormonal etiology of breast cancer in humans. *Proc Natl Acad Sci U S A* 1985;82:6295–9.
37. Bradlow HL, Telang NT, Sepkovic DW, Osborne MP. 2-hydroxyestrogens: the 'good' estrogen. *J Endocrinol* 1996;150 Suppl:S259–65.

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