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The Steroid Metabolome and Breast Cancer Risk in Women with a Family History of Breast Cancer: The Novel Role of Adrenal Androgens and Glucocorticoids

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ABSTRACT

Background: No study has comprehensively examined how the steroid metabolome is associated with breast cancer risk in women with familial risk.

Methods: We examined 36 steroid metabolites across the spectrum of familial risk (5-year risk ranged from 0.14% to 23.8%) in pre- and postmenopausal women participating in the New York site of the Breast Cancer Family Registry (BCFR). We conducted a nested case–control study with 62 cases/124 controls individually matched on menopausal status, age, and race. We measured metabolites using GC-MS in urine samples collected at baseline before the onset of prospectively ascertained cases. We used conditional logistic regression to estimate odds ratios (OR) and 95% confidence intervals (CI) per doubling in hormone levels.

Results: The average proportion of total steroid metabolites in the study sample were glucocorticoids (61%), androgens (26%), progestogens (11%), and estrogens (2%). A doubling in glucocorticoids (aOR = 2.7; 95% CI = 1.3–5.3) and androgens (aOR = 1.6; 95% CI = 1.0–2.7) was associated with increased breast cancer risk. Specific glucocorticoids (THE, THF αTHF, 6β-OH-F, THA, and α-THB) were associated with 49% to 161% increased risk. Two androgen metabolites (AN and 11-OH-AN) were associated with 70% (aOR = 1.7; 95% CI = 1.1–2.7) and 90% (aOR = 1.9; 95% CI = 1.2–3.1) increased risk, respectively. One intermediate metabolite of a cortisol precursor (THS) was associated with 65% (OR = 1.65; 95% CI = 1.0–2.7) increased risk. E1 and E2 estrogens were associated with 20% and 27% decreased risk, respectively.

Conclusions: Results suggest that glucocorticoids and 11-oxygenated androgens are positively associated with breast cancer risk across the familial risk spectrum.

Impact: If replicated, our findings suggest great potential of including steroids into existing breast cancer risk assessment tools.

Introduction

The dominant paradigm for breast cancer etiology focuses on estrogens. However, estrogens are the products of a complex metabolic pathway that begins with cholesterol and produces glucocorticoids, androgens, progestogens, and estrogens, all of which have varying carcinogenic and genotoxic activities. Both androgens and progestogens play independent roles in increasing breast cancer risk (1–3), but have received less attention than estrogens. Moreover, although glucocorticoids have been associated with breast cancer recurrence (4, 5), only one prior study has investigated the role of glucocorticoids in breast cancer etiology and yielded null results (6).

Although synthesizing results about individual metabolites and risk across individual studies can provide clues regarding the role of steroids, no study has comprehensively examined how the steroid metabolome is associated with breast cancer in women across the range of familial breast cancer risk. As steroid hormones, in general, are important modulators of vital biological processes, taking a metabolomics approach provides an essential integrated picture of the steroid hormonal status of an individual. It enables characterization of steroid hormone actions not only for estrogens, as the classic female sex hormones, but also for adrenal and gonadal androgens as well as for the adrenal stress hormone cortisol and its precursors. Moreover, few studies have measured concentrations in urine, which capture different metabolic processes than circulating levels (7). Steroid hormones undergo mostly reductive catabolic reactions and after conjugation either as glucuronides or sulfates are excreted predominantly into urine (8, 9). Thus, the urinary steroid metabolome mirrors the integrated steroid hormonal status of an individual and accordingly reflects multiple sources including diet and exogenous factors, whereas circulating levels of metabolites reflect liver metabolism (7). Urine and blood measure different aspects of steroid metabolism, as is evident by the negligible to moderate correlations of individual metabolites measured in concurrently collected blood and urine samples (7). Urine samples are much easier to collect than serum, making urine measurement a less invasive and more feasible and comprehensive option for population-level screening.

Using a robust, highly reproducible assay, we examined the association between 36 urinary metabolites (see **Fig. 1**; Supplementary Table S1), including glucocorticoids, androgens, progestogens (including other intermediates), and estrogens, with breast cancer risk in pre- and

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Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

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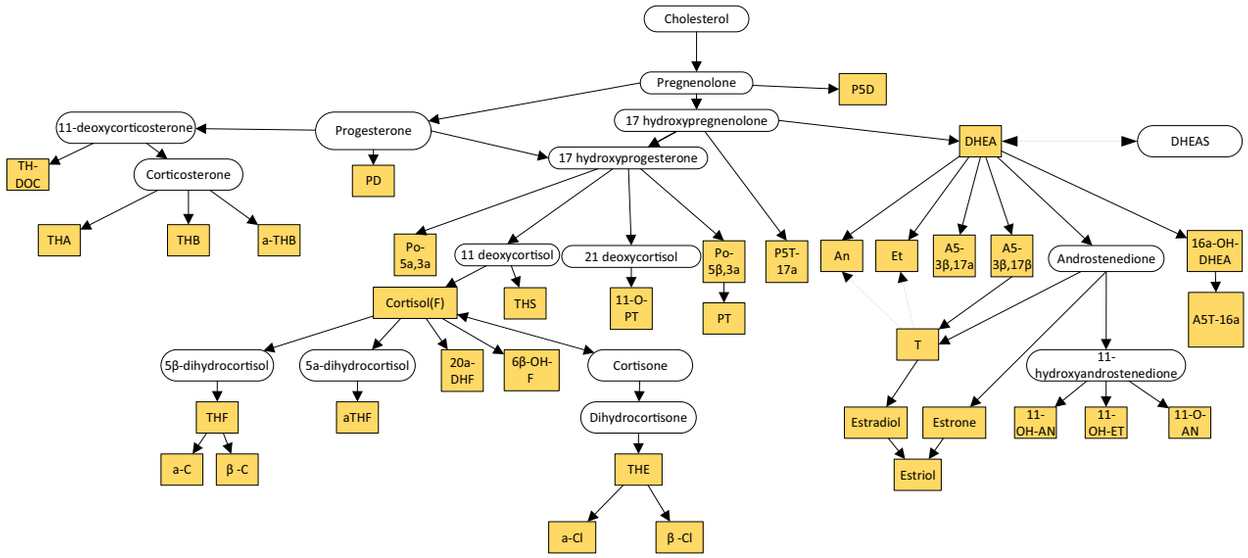

Figure 1.

The steroid metabolome. The steroid metabolome was measured simultaneously in urine using GC-MS for 36 steroid hormone metabolites (in yellow boxes), including 14 glucocorticoids (including mineralcorticoids), 10 androgens, 9 intermediates of progestogens and other steroids (referred to as progestogens), and 3 estrogens in the Laboratory for Translational Hormone Analytics in Pediatric Endocrinology at Justus Liebig University in Germany under Dr. Stefan Wudy. Other metabolites not measured by the assay (in circles) illustrate the pathways of metabolism.

postmenopausal women at varying familial risk for breast cancer in a nested, case–control study (10). We also assessed whether including the urinary steroid metabolome improved the discrimination of risk assessment models.

Materials and Methods

Population

We used data from the New York site of the Breast Cancer Family Registry (BCFR; $n = 2{,}136$), a family-based cohort started in 1998 that includes women at average risk (5-year risk <1.67%) and high risk of cancer (≥1.67; refs. 10–12). Excluding cases that occurred within 2 months of baseline ($n = 64$), 65% of the cohort provided a urine sample at baseline ($n = 1{,}357$), and of those, 74% were not taking exogenous hormones ($n = 1{,}008$). After additionally excluding women who were breastfeeding ($n = 12$) or pregnant ($n = 5$) at the time of sample collection, we were left with 991 women. We compared urinary metabolites between breast cancer cases ($n = 62$) and controls matched on menopausal status, race/ethnicity, and age using incidence density sampling ($n = 124$; ref. 13). Columbia University Institutional Review Board granted ethical approval.

Invasive breast cancer

Prospectively ascertained (incident) cases were pathologically confirmed for at least 80% of cases (10). Tumor characteristics were available on 76% of our sample, and 62% of cases were ER^+, 49% were PR^+, and 46% were $HER2^+$; however, given the small overall number of cases, we did not stratify by subtype in analyses.

Steroid metabolites

Baseline spot urines were assayed by GC-MS for 36 steroid hormone metabolites including 14 glucocorticoids (including mineralcorticoids), 10 androgens, 9 intermediates of progestogens and other steroids (hereby referred to as progestogens), and 3 estrogens in the Laboratory for Translational Hormone Analytics in Pediatric Endocrinology under Dr. Stefan Wudy (14–16). Briefly, free and conjugated urinary steroids were extracted by solid phase extraction, and conjugates were enzymatically hydrolyzed. After recovery of hydrolyzed steroids by solid phase extraction, known amounts of internal standards (5α-androstane-3α, 17α-diol, stigmasterol) were added to each extract before formation of methyloxime-trimethylsilyl ethers. GC was performed using an Optima-1 fused silica column (Macherey–Nagel) housed in an Agilent Technologies 6890 series GC that was directly interfaced to an Agilent Technologies 5975 inert XL mass selective detector. The MS was run in the selected ion-monitoring mode. Quantification was performed using the peak area ratios between analyte and internal standard. We also measured creatinine [DVIA Chemistry Enzymatic Creatinine_2; SIEMENS; coefficients of variation (CV) < 1.5%] in each sample.

We batched samples so that masked case–control sets were together. We placed blinded, replicate quality-control samples in each batch to assess the intra- and interassay CVs (<12% and 15%, respectively). For concentrations below the limit of detection, we assigned half the limit of detection (Supplementary Table S1). We also explored other methods to handle samples with concentrations below the limit of detection, such as including an indicator variable, and our results were robust regardless of the method. We $\log_2$-transformed each concentration to improve normality and then divided by creatinine. We also further examined whether specific metabolites had a nonlinear association by comparing models with and without a squared hormone term and these models differed for four metabolites (AN, E2, P5D, and THB). We examined each metabolite individually and summed into androgens (A), estrogens (E), progestogens (P), and glucocorticoids (G), as others have previously done (8, 9, 17). Due to correlation

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between steroids, we also created metabolite ratios (E:A, P:A, and G:A), so that we could investigate the associations of each summed grouping independent of androgens, especially in the case of estrogens. Furthermore, others have shown the G:A ratio to be a marker of stress response (18).

Cancer family history

Family history of cancer included age at diagnosis of all cancers (except nonmelanoma skin cancer) and deaths for first- and second-degree relatives. These pedigree data, in addition to *BRCA1* and *BRCA2* mutations where available (9% of current sample), were used to calculate a continuous risk score using the BOADICEA (Breast and Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm) risk model (19–21). BOADICEA produces an absolute risk of breast cancer over a fixed-time interval (21).

Covariates

At baseline, self-administered questionnaires ascertained established risk factors for breast cancer including behavioral factors [alcohol intake (never, former, current), cigarette smoking (never, former, current), body mass index (BMI), and physical activity (average metabolic equivalents (MET), continuous)] and reproductive factors [age at menarche (continuous), parity nulliparous/parous, breastfeeding (yes/no), exogenous hormone use (yes/no), and menopausal status (premenopausal/postmenopausal)]. Menopausal status was based on self-report of last menstrual period (LMP >12 months signified menopause) and surgical information for individuals who had reported an oophorectomy. For women whose LMP was less than 12 months or who were missing data on LMP, we also applied an algorithm based on age, HRT use, and recency of pregnancy to infer menopausal status. Additional covariates included year of birth and education. Data on menstrual day and confirmation of ovulatory cycles were not collected at the time of sample collection.

Statistical analysis

Due to the matched case–control design, we used conditional logistic regression (22, 23) to investigate the association between metabolites and breast cancer risk. We screened a list of *a priori* confounders, including BMI, year of birth, education, physical activity, smoking, alcohol consumption, age at menarche, parity, breastfeeding, and hormone use. To keep the model parsimonious, we included covariates into the final model only if they changed the beta coefficient for the main effect of each metabolite by more than 10% and improved model fit using the likelihood ratio test. Final models for estrogens and progestogens included no covariates, whereas final models for androgens included year of birth, education, physical activity, and age at menarche. Final models for glucocorticoids included education, physical activity, and age at menarche.

We investigated the potential interactive effect between metabolite and BOADICEA risk in multiple ways. We tested for multiplicative interactions by including the cross-product terms between each metabolite and the continuous BOADICEA 1-year risk score, and tested its statistical significance using the Wald Chi-square test. We also tested for additive interaction using dichotomous variables by cutting the metabolite and risk score at the median. For significant multiplicative interaction terms, we estimated the effect of each metabolite at the 10th, 50th, 75th, and 90th percentiles of the risk score. We assessed whether women with extreme BOADICEA risk scores influenced estimates through visual examination of Pearson residual scores, deviance residuals, and Pregibon leverage (24).

To assess improvement in risk prediction, we first calculated the areas under the receiver operator curves (AUC) from unconditional logistic regression models using the 5-year Gail (25), IBIS (26), or BOADICEA (27) risk score as the predictor. Using DeLong's test, we then compared the AUC from the models with risk score only to the AUC with the risk score plus each metabolite or sum of metabolites (28). We also compared the models with and without the metabolites using the c-statistic, and results were consistent with DeLong's test. We compared our estimates to estimates derived from models using inverse probability weighting, but there was minimal difference between the estimates suggesting no bias due to the nonrandom sampling in our matched study. Instead of testing in a separate validation set, we used k-fold cross-validation (using 10 subsets), and the results remained unchanged. Lastly, to consider the possibility of Type I error, we used the Benjamini–Hochberg procedure to adjust the individual *P* values to control the false discovery rate (FDR) at 0.05 (29). We also applied the newly developed Cauchy combination statistical method to determine the group-wise significance (30).

Results

Cases and controls were similar across all covariates as shown in **Table 1**. The majority of women were premenopausal (66%).

On average, the largest proportion of steroid metabolites were glucocorticoids (61%), followed by androgens (26%), progestogens (11%), and estrogens (2%). Of the individual metabolites, THE was the most abundant (21% of the total steroid metabolites), followed by THF (11%), ACL (9%), and ET (8%). E2 was the least abundant metabolite (0.06%).

A doubling in glucocorticoids was associated with increased breast cancer risk (aOR = 2.7; 95% CI = 1.3–5.3). Higher individual glucocorticoid metabolites were associated with increased breast cancer risk; 6 of 14 were significant (**Fig. 2**). Specifically, a doubling in concentration of THE, THF, αTHF, 6β-OH-F, THA, and α-THB was associated with 161%, 116%, 75%, 49%, 83%, and 52% increased risk, respectively (**Fig. 2**). There was a significant negative multiplicative interaction between predicted familial risk and α-THB ($P = 0.032$; Supplementary Table S2). In women at the 10th and 50th percentiles of familial risk, a doubling in α-THB was associated with 2.5 (1.3–4.7) and 1.9 (1.2–3.0) increased risk, respectively (**Table 2**).

A doubling in androgens was associated with increased breast cancer risk (aOR = 1.6; 95% CI = 1.0–2.7). Higher concentrations of all individual androgen metabolites were associated with increased breast cancer risk; two were statistically significant (**Fig. 2**). Specifically, a doubling in AN and 11-OH-AN was associated with 72% (aOR = 1.72; 95% CI = 1.1–2.7) and 91% (aOR = 1.9; 95% CI = 1.2–3.1) increased risk, respectively (**Fig. 2**). There was a significant negative multiplicative interaction between predicted familial risk and AN, A5-3β,17α, and 11-O-AN (**Table 2**). For example, when stratifying women by the 10th, 50th, and 75th percentiles of familial risk, a doubling in AN was associated with 2.6 (1.4–4.8), 2.2 (1.3–3.7), and 1.9 (1.2–3.1) increased breast cancer risk, respectively.

The positive associations between progestogens and breast cancer risk were less robust than the other metabolite groupings. A doubling in progestogens was associated with increased breast cancer risk but was not statistically significant (OR = 1.2; 95% CI = 0.8–1.7). Only a doubling of THS was associated with a significant 65% increase (OR = 1.65; 95% CI = 1.0–2.7) in breast cancer risk (**Fig. 2**). There were no statistically significant multiplicative interactions with familial risk but, unlike the interactions for androgen and glucocorticoid

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Table 1. Characteristics of cases and controls from prospective, nested case–control of pre- and postmenopausal women participating in the New York site of the BCFR[a].

	Cases ($n = 62$)		Controls ($n = 124$)	
	N	**%**	***N***	**%**
Parity				
0	18	29	32	26
1	5	8	13	10
2	26	42	48	39
3	11	18	20	16
4+	2	3	11	9
Education				
High school graduation/GED	6	10	16	13
Vocational or technical school/some college or university	12	19	35	28
Bachelor's/graduate degree	44	71	73	59
Cigarette use				
Missing	0	0	1	1
Never	32	52	69	56
Former	25	40	46	37
Current	5	8	8	6
Alcohol use				
Missing	0	0	1	1
Never	29	47	56	45
Former	11	18	27	22
Current	22	35	40	32
Hormone replacement therapy use				
Missing	0	0	1	1
Never	54	87	111	90
Ever	8	13	12	10
Oral contraceptive use				
Never	24	39	55	44
Ever	38	61	69	56
Breastfeeding				
No	31	50	59	48
Yes	31	50	65	52
Menopause				
Pre	41	66	82	66
Post	21	34	42	34
	Mean (SD)		Mean (SD)	
Age at entry	48.8 (10.0)		48.7 (10.1)	
Age at menarche	12.5 (1.4)		12.4 (1.2)	
Average METs	36.4 (28.2)		32.4 (28.5)	
BMI (kg/m^2)	25.1 (4.1)		24.9 (5.8)	
Follow-up time (years)	15.9 (3.5)		7.3 (5.0)	

Abbreviation: GED, general equivalency diploma.
[a]All cases and controls are matched on race/ethnicity, menopausal status, and age.

metabolites, which were negative, the interaction was positive in direction for 5 of 8 intermediate metabolites (Supplementary Table S2).

Unlike all the other steroid groupings, estrogens were associated with decreased breast cancer risk, but the association with the total sum was not statistically significant (RR = 0.78; 95% CI = 0.60–1.00). The associations were statistically significant for two of the three estrogen metabolites. Specifically, a doubling in E1 and E2 was associated with 20% and 27% decreased risk, respectively (**Fig. 2**).

To compare steroid metabolites in different metabolic pathways, we investigated several ratios. Both the E:A and P:A ratios were negatively associated with breast cancer risk, and the G:A ratio was positively associated with risk, but only the association with the E:A ratio was statistically significant (OR = 0.70; 95% CI = 0.5–0.9; Supplementary Table S2). There were no statistically significant multiplicative interactions between the ratios and familial risk. There were no statistically significant interactions on the additive scale for any individual metabolite or ratio.

In our sample, the IBIS had the highest discriminatory performance (AUC = 0.66) compared with BOADICEA (AUC = 0.61) and Gail (AUC = 0.52; Supplementary Table S3). Across all three prediction models, adding glucocorticoids or androgens led to the largest improvements in discrimination. This improvement was most marked in the Gail model (11-point improvement), compared with IBIS (5-point) and BOADICEA (6-point) models. BMI was only weakly correlated with the metabolites ($r < 0.17$), so adding BMI before or after adding the metabolites to the model did not alter the AUC estimates.

Regarding our statistical methods to address Type I error, most metabolites remained statistically significant after the FDR adjustment. The FDR adjusted P values for AN, OHAN, THE, THF, and a-THF are 0.04, 0.04, 0.02, 0.03, and 0.02, respectively. The adjusted

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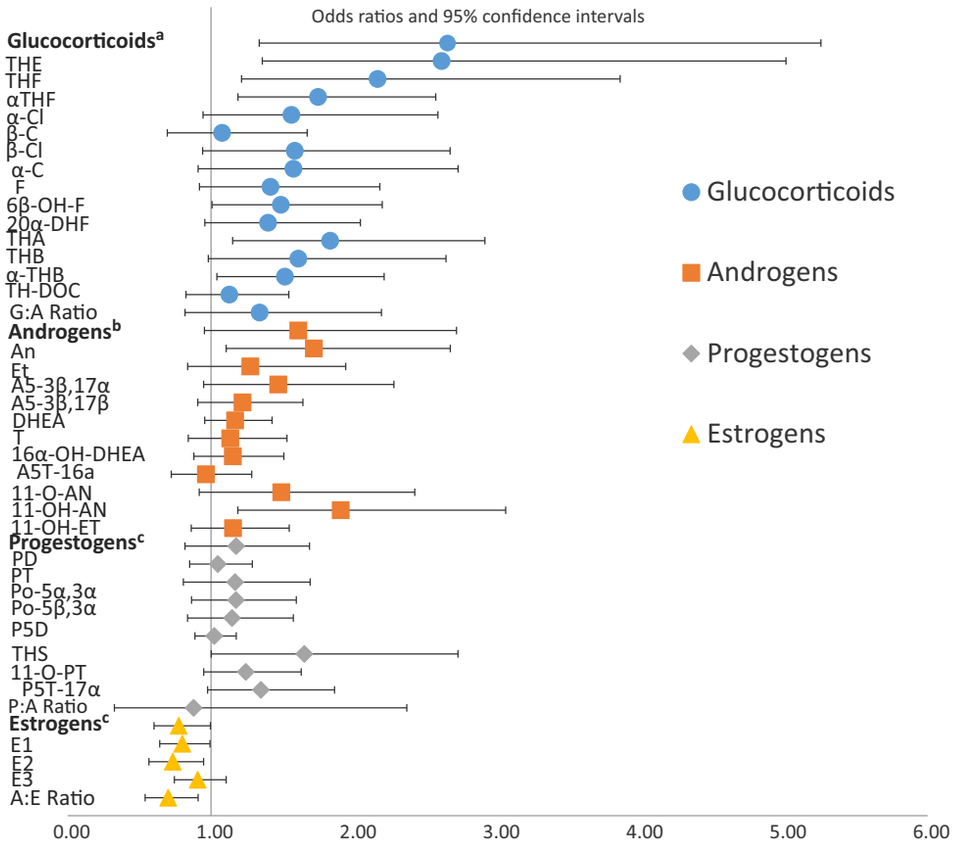

Figure 2.
Steroid metabolites and breast cancer risk (OR and 95% CI) in the New York site of the BCFR. All cases and controls are matched on race/ethnicity, menopausal status, and age. [a]Glucocorticoids are adjusted for education, physical activity, and age at menarche. [b]Androgens are also adjusted for year of birth. [c]Progestogens and estrogen models are unadjusted.

P values for the grouping of hormones were 0.01, 0.03, and 0.04 for glucocorticoids, androgens, and estrogens, respectively. Both analyses confirm that our findings on the metabolite effect are beyond a random chance. We also ran sensitivity analyses excluding cases diagnosed within 2 years of sample collection, and our results remained of similar magnitude.

Discussion

We found that urinary glucocorticoid and androgen metabolites were associated with an increased risk of breast cancer, and that this effect was seen across the range of predicted familial risk but was strongest among women below the 75th percentile. Although the effect was smaller for women above the 75th percentile of risk (i.e., with a 5-year risk of 3.5% or higher), glucocorticoids and androgens may still be important in women with high familial risk given their higher absolute risk. We also found that progestogens were positively, while nonsignificantly, associated with breast cancer risk. Unlike the other steroid groupings, but consistent with another study of premenopausal women (31), estrogen metabolites were negatively associated with risk.

The majority of previous studies have investigated single steroids, recurrence rather than first cancer in relation to glucocorticoids (5, 32, 33), or serum steroid concentrations (1, 34, 35), making direct comparison with our study difficult. There are, however, some studies that have investigated breast cancer risk associated with urinary estrogen, particularly the ratio of the 2 to 16 pathway (37–43) among pre- and postmenopausal women (44, 45). Although serum estrogens are generally positively associated with breast cancer, Eliassen and colleagues found that higher mid-luteal urinary estrogen, specifically estrone and estradiol, were associated with lower breast cancer risk among premenopausal women (31). Although their results are similar to ours, less than 15% of women in their study had a breast cancer family history compared with 100% in our sample. In two cross-sectional studies comparing mean urinary estrogens in premenopausal women with and without breast cancer family history, Fishman and colleagues found lower urinary estrone and estradiol excretion (46) in those with a family history whereas Ursine and colleagues found no association (47). The consistency between previous studies and our findings regarding estrogens lends credibility to the positive associations we observed with androgens and glucocorticoids.

The positive associations between all glucocorticoid metabolites and breast cancer risk, ranging from 1.4 to 2.6 higher risk, are provocative. Previously, a cross-sectional study ($n = 135$) found higher 24-hour cortisol excretion in pre-and postmenopausal women with a family history of breast cancer compared with those without (48). Another smaller cross-sectional study ($n = 60$) found no difference in 12-hour urinary glucocorticoids between premenopausal women with and without a breast cancer family history (46). In a case–control study of pre- and postmenopausal women from the Shanghai Breast Cancer Study, Zheng and colleagues found a positive association between the ratio of urinary 6β-OHC to cortisol and breast cancer risk [OR = 3.7 (1.9–7.4) for the top vs. bottom quartile; ref. 49]. However, this retrospective study used this ratio as a proxy for estrogen metabolism rather than as a glucocorticoid biomarker (49). Therefore, our study represents the first prospective case–control to examine the relationship between the full range of glucocorticoid metabolites and incident breast cancer.

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Table 2. Doubling of steroid metabolite concentration breast cancer risk (OR and 95% CI) in women stratified at the 10th, 50th, 75th, and 90th percentiles of familial risk in the New York site of the BCFR.[a,b]

OR	Estimate	95% confidence limits
AN[c]		
10th percentile	2.6	(1.4–4.8)
50th percentile	2.2	(1.3–3.7)
75th percentile	1.9	(1.2–3.1)
90th percentile	1.5	(0.9–2.4)
A5-3β,17α[c]		
10th percentile	2.8	(1.4–5.4)
50th percentile	2.1	(1.2–3.6)
75th percentile	1.6	(1.0–2.7)
90th percentile	1.1	(0.6–1.8)
11-O-AN[c]		
10th percentile	2.4	(1.3–4.7)
50th percentile	1.7	(1.0–2.9)
75th percentile	1.3	(0.7–2.2)
90th percentile	0.8	(0.4–1.6)
α-THB[d]		
10th percentile	2.5	(1.3–4.7)
50th percentile	1.9	(1.2–3.0)
75th percentile	1.5	(1.0–2.2)
90th percentile	1.0	(0.6–1.7)

[a]All cases and controls are matched on race/ethnicity, menopausal status, and age.
[b]This table stratifies by percentile of familial risk for those metabolites that had statistically significant interactions. For tests of interactions for all metabolites, see Supplementary Table S2.
[c]Androgens are also adjusted for year of birth.
[d]Glucocorticoids are adjusted for education, physical activity, and age at menarche.

The significant associations observed with higher androgen levels are consistent with previous studies of women at average risk (1, 50, 51). In cross-sectional studies of high-risk pre-and postmenopausal women, urinary androgen levels were lower in sisters but not in daughters of women with breast cancer (52), but in another study there was no difference (53) between women with and without a family history. Other case–control studies have found positive associations between specific androgen metabolites in urine and breast cancer risk, including testosterone (54–55), 5alpha-androstane-3alpha, 17beta-diol (56), androstanediol (55), androsterone, and etiocholanolone (57). In the latter study, there was an inverse association in premenopausal women. We lack the numbers to stratify by menopausal status because the majority of our sample (66%) was premenopausal, but by design we matched on menopausal status. Our findings extend those of previous studies by examining a larger panel of androgens and by examining the strength of the association among women with breast cancer family history. In particular, we found that 11-oxygenated androgens were associated with the highest increase in breast cancer risk.

Androgens also improved risk prediction models by 5 to 11 points, though this finding was not statistically significant. A similar improvement in risk prediction was reported by Tworoger and colleagues using nested case–control data from postmenopausal women in the Nurses' Health Study (58). In particular, they observed 5.9 and 3.4 point improvements in the Gail and Rosner–Colditz risk models, respectively, after including serum concentrations of estrone sulfate, testosterone, and prolactin (58). Adding testosterone and DHEAS alone yielded a 2.3 and 2.7 change in the AUC in that study.

Biologically, glucocorticoids, androgens, progestogens, and estrogens have varying carcinogenic and genotoxic activities, as well as independent and synergistic mechanisms in breast carcinogenesis. For example, estrogens stimulate, whereas androgens inhibit mammary cell proliferation in both normal and cancer tissues (59). Androgens function by binding to the intracellular androgen receptor, which, like the estrogen receptor, is present in mammary epithelial cells. Interestingly, the *BRCA1* protein binds to the androgen receptor, suggesting it may blunt androgen effects (60), which may explain the weaker association we observed in women above the 75th percentile of familial risk. Recent *in vitro* and *in vivo* studies demonstrate that glucocorticoids promote the transition of ductal carcinoma *in situ* to invasive ductal carcinoma via myoepithelial cell apoptosis (61). Specific metabolites within each of the major steroid groupings may be more carcinogenic. Of the androgen metabolites, we found stronger effects for the 11-oxygenated metabolites. These are highly potent androgens of adrenal origin stemming DHEA after conversion to 4-androstenedione, 11-hydroxylation, and 11-oxidation. Although our positive findings were only statistically significant for 11-OH-AN (∼10% of which is also a metabolite of glucocorticoid; ref. 62), the magnitude of the association was similar for 11-O-AN as well. 11-oxygenated metabolites have been implicated in 21-hydroxylase deficiency and in polycystic ovary syndrome, but have not been studied in relation to breast cancer (63).

It is intriguing that A5-3β,17α, a metabolite of the adrenal androgen DHEA, was significantly associated with increased breast cancer risk after stratification in women at the 10th and 50th percentiles of familial risk, given that this particular hormone has been coined a hermaphrodiol, in that it acts estrogenic in highly androgenic states and androgenic in estrogenic states (64). The fact that we observed significant associations with particular metabolites even in our small sample justifies further investigation. Unlike androgens and estrogens, glucocorticoids are all derived from cortisol and do not have other precursors, thus the consistent association we observed across all glucocorticoids makes sense metabolically and also begs for more research given the current paucity in the literature. Our novel observation that typical adrenal steroids, such as glucocorticoids and DHEA-derived androgens, play a significant role in the genesis of breast cancer points to an underappreciated role of the adrenals in carcinogenesis.

Our study had several limitations. First, our assessment of the steroid metabolites was collected from a single urine sample and may not accurately capture long-term exposure or reflect what is happening in the breast tissue. That being said, studies have demonstrated high intraclass correlation coefficients for urinary steroids (5–59), suggesting single measurements may be valid. Furthermore, urine may reflect metabolic processes distinct from what is measured in plasma—both of which may have implications on breast cancer etiology. Second, our findings could be confounded by menstrual and ovulatory cycle, which was not available in the study, but may influence hormone levels, particularly estrogens and progestogens in premenopausal women, at the time of measurement. However, there is relatively minimal variation in glucocorticoids and androgens across the menstrual cycle (65, 66), where we found statistically significant results. Third, there may be important variation in the effect of steroids by breast cancer subtype (e.g., ER^+) but our sample size was not large enough to stratify. Overall, our sample size was small, and our findings need to be replicated in larger studies with greater power to rule out chance findings due to multi-comparisons and to assess nonlinear

relationships. However, we found consistent patterns within the major groupings of metabolites, and our results were robust after using multiple testing correction methods.

Conclusion

In this prospective, nested case–control study covering a range of familial risk, we analyzed steroid metabolism using a highly sensitive and reproducible assay that measures 36 metabolites in urine. We identified patterns of statistically significant associations with glucocorticoid and adrenal androgen metabolites, which—in this context—are largely understudied hormones. Our findings, if replicated in larger studies, provide direction for new research into the hormonal etiology of breast cancer and identify new adrenal-derived biomarkers for cancer risk, particularly 11-oxygenated androgens and glucocorticoids. Further research into what modifiable factors alter glucocorticoids and androgens, such as stress, is warranted.

Authors' Disclosures

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Authors' Contributions

L.C. Houghton: Conceptualization, formal analysis, funding acquisition, visualization, methodology, writing–original draft, project administration, writing–review and editing. **R.E. Howland:** Formal analysis, and writing–original draft. **Y. Wei:** Formal analysis, supervision, funding acquisition, writing–review and editing. **X. Ma:** Data curation, formal analysis, writing–review and editing. **R.D. Kehm:** Data curation, formal analysis, writing–review and editing. **W.K. Chung:** Resources, funding acquisition, writing–review and editing. **J.M. Genkinger:** Resources, project administration, writing–review and editing. **R.M. Santella:** Resources, data curation, writing–review and editing. **M.F. Hartmann:** Formal analysis, writing–review and editing. **S.A. Wudy:** Formal analysis, writing–review and editing. **M.B. Terry:** Conceptualization, resources, data curation, supervision, funding acquisition, project administration, writing–review and editing.

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