Research Article

Cancer Epidemiology, Biomarkers & Prevention

Systemic Levels of Estrogens and PGE_2 Synthesis in Relation to Postmenopausal Breast Cancer Risk

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Abstract

Background: Prostaglandin E_2 (PGE_2) induces aromatase expression in adipose tissue, leading to increased estrogen production that may promote the development and progression of breast cancer. However, few studies have simultaneously investigated systemic levels of PGE_2 and estrogen in relation to postmenopausal breast cancer risk.

Methods: Here, we determined urinary estrogen metabolites (EM) using mass spectrometry in a case–cohort study (295 incident breast cancer cases and 294 subcohort members), and using linear regression estimated the effect of urinary levels of a major PGE_2 metabolite (PGE-M) on EMs. HRs for the risk of developing breast cancer in relation to PGE-M and EMs were compared between Cox regression models with and without mutual adjustment.

Results: PGE-M was a significant predictor of estrone (E1), but not estradiol (E2) levels in multivariable analysis. Elevated E2 levels were associated with an increased risk of developing breast cancer [$HR_{Q5vs.Q1}$, 1.54; 95% confidence interval (CI), 1.01–2.35], and this association remained unchanged after adjustment for PGE-M ($HR_{Q5vs.Q1}$, 1.52; 95% CI, 0.99–2.33). Similarly, elevated levels of PGE-M were associated with increased risk of developing breast cancer ($HR_{Q4vs.Q1}$, 2.01; 95% CI, 1.01–4.29), and this association was only nominally changed after consideration of E1 or E2 levels.

Conclusions: Urinary levels of PGE-M and estrogens were independently associated with future risk of developing breast cancer among these postmenopausal women.

Impact: Increased breast cancer risk associated with PGE-M might not be fully explained by the estrogens–breast cancer association alone but also by additional effects related to inflammation.
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Introduction

Use of aspirin and other NSAIDs has been associated with reduced risk of several cancers including colon, stomach, lung, and breast (1). The chemopreventive actions of NSAIDs are mainly mediated by blocking cyclooxygenase-2 (COX-2) enzyme activity to suppress prostaglandin E_2 (PGE_2) synthesis (2). As a major COX-2–derived prostaglandin, PGE_2 plays a key role in the acute and chronic inflammatory responses that promote cell proliferation and angiogenesis, and inhibit apoptosis (3). PGE_2 can also induce aromatase expression, leading to increased estrogen production in mammary adipose stromal cells (4). After menopause, estrogens are predominantly produced in peripheral tissues by the aromatization of adrenal and ovarian androgens. By inhibiting PGE_2, and in turn aromatase induction, NSAIDs have been hypothesized to inhibit local estrogen biosynthesis and to decrease breast cancer risk particularly among postmenopausal women (4, 5).

Our previous case–cohort analysis showed that among postmenopausal women who did not regularly use NSAIDs, increased urinary levels of a major PGE_2 metabolite (PGE-M), a biomarker of systemic PGE_2 synthesis, were associated with increased risk of breast cancer (6). We used these same case–cohort samples to examine urinary profiles of 15 estrogens and estrogen metabolites (EM) via liquid chromatography-tandem mass spectrometry (LC-MS/MS) and examined the relationships among systemic levels of PGE_2 synthesis and EMs, and postmenopausal breast cancer risk.

Materials and Methods

The present study is a case–cohort study within the Sister Study that comprised 607 postmenopausal women aged 50 to 74 years who did not use exogenous hormones at the time of urine collection. Details of the study design and subject characteristics are found elsewhere (6, 7).

Urinary concentrations of 15 EMs [estrone (E1), estradiol (E2), estriol (E3), 16-epiestriol (16epiE3), 17-epiestriol (17epiE3), 16-ketoestradiol (16ketoE2), 16alpha-hydroxyestrone (16αOHE1), 2-methoxyestrone (2MeOE1), 4-methoxyestrone (4MeOE1), 2-hydroxyestrone-3-methyl ether (3MeOE1), 2-methoxyestradiol (2MeOE2), 4-methoxyestradiol (4MeOE2), 2-hydroxyestrone (2OHE1), 4-hydroxyestrone (4OHE1), and 2-hydroxyestradiol (2OHE2)] were determined using LC-MS/MS at Primera Analytical Solutions Corp. using established methods (8, 9). Briefly, 20 μL of an internal standard solution was added to each of 0.5 mL urine samples, followed by 0.5 mL of freshly prepared enzymatic

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hydrolysis buffer. The sample was then subjected to extraction with 8 mL dichloromethane. After extraction, the mixture was dried and the residue was reconstituted in 100 μL of 0.1 mol/L sodium bicarbonate buffer and 100 μL of dansyl chloride solution, and incubated at 60°C for 56 minute. A 20 μL of sample was injected on LC/MS/MS with flow rate of 200 μL/min. The instrument was equipped with Shimadzu HLPC system with two LC-10AD VP series pumps and a Shimadzu SIL HTc autosampler. Quantitation of each EM was carried out using Applied Biosystems/MDS Sciex Analyst Software. Calibration standards were prepared at the following concentrations: 0.04, 0.08, 0.4, 0.8, 4, 8, 30, and 40 ng/mL. Calibration curves were constructed by fitting a linear regression model to estimate the relation between EM/deuterium-labeled EM peak area ratios from the calibration standards versus amounts of the EMs. The linear curves were then used to estimate the amounts of each EM in a study sample. The median coefficient of variation (CV) of the assay was 5.2% (range, 1.2%–11.5%) within-batch and 10% (range, 10%–28%) between-batches, with higher %CVs found for methylated EMs. Urinary creatinine was determined as described (6) and used to adjust for EM levels. A total of 589 samples were measured for both PGE-M and EMs. Although many samples were below detection limit for 17-epiE3 (64%) and methylated EMs (23%–67%), the majority of the study samples were above the detection limit for E1, E2, E3 (>98%), 2-OHE1, 4-OHE1, 16-epiE3, 16-ketoE2, and 16α-OHE1 (90%–95%); and 2-OHE2 (87%). For analytical purpose, samples that fell below the detection limit were assigned values of half of the detection limit divided by square root of 2.

Statistical analysis

A total of 589 samples (295 cases and 294 subcohort women) were included in the final analysis. Descriptive statistics were reported using medians and interquartile ranges for continuous data and frequencies and proportions for categorical data. Characteristics for selected data were compared between cases and subcohort groups using Wilcoxon rank sum tests for continuous data and χ^2 statistics for categorical data. Normality of continuous data was examined with quantitative and graphical methods. Levels of EMs and PGE-M were log-transformed to examine influence of PGE-M and other factors on EM levels using univariate and multivariable linear regression models. Women were categorized in EM quintiles based on the distributions among the subcohort women. Cox regression models for the case–cohort analysis (10) were used to estimate the HRs for the association between quintiles of EMs and breast cancer risk after adjustment for age at enrollment (50–54; 55–59; 60–64; 65–69; 70–75 y), body mass index (BMI: <25, 25–29.9, 30–34.9, or $\geq$ 35 kg/m^2), smoking status (never, former, current), alcohol use status (never, social past; social current; regular past; regular current drinker), years of past hormone replacement therapy (HRT) use (continuous), history of breast biopsy (yes or no), number of first-degree family members with breast cancer (1 or $\geq$ 2), with and without additional adjustment for PGE-M (<3.4; 3.4–<5.24; 5.24–<8.34; $\geq$ 8.34 pg/mg Cr.), and an interaction term between PGE-M and regular use of NSAIDs (pill-years of NSAID use <0.75 or $\geq$ 0.75, equivalent to at least 3 pills of NSAID use per week for 3 months or longer). Tests for linear trend were performed by treating categorical variables as continuous variables. An exploratory path analysis was conducted to model the relationships among use of NSAIDs, BMI, and levels of PGE-M and EMs, with use of NSAIDs and BMI as predictor variables, PGE-M as a mediator variable, and EM as an outcome variable. As an extension of linear regression models, the path analysis assumes that all relations of path variables are linear and additive. Although it also assumes causal relationships of the modeled path diagram, it should be noted that the model does not prove causal effects (11). Path coefficients were estimated using structural equation models with full information maximum likelihood estimation. Goodness of fit was assessed using root mean squared error of approximation (RMSEA) and comparative fit index (CFI) where a RMSEA < 0.06 and a CFI $\geq$ 0.95 were considered as indicative of good fit (12). All statistical tests were two-sided at significance level of 0.05, and analyses were conducted using Stata 14.1.

Results

Urinary EMs and postmenopausal breast cancer risk

Total urinary estrogen concentrations were similar between cases and subcohort women, but cases had higher concentrations of parent estrogens, E1 and E2 combined, compared with the subcohort, with a median level of 1,536 versus 1,324 pg/mg Cr. (P = 0.04; Table 1). There was little difference in the concentrations of other EMs. In multivariable analysis, higher levels of parent estrogens were associated with increased risk of breast cancer [HR $_{Q5vs.Q1}$, 1.57; 95% CI, 1.05–2.34; Fig. 1; Supplementary Table S1]. In the analysis of individual parent estrogens, higher levels of E2, but not E1, were significantly associated with breast cancer risk. HRs associated with the highest versus lowest quintile of E1 were 1.30 (95% CI, 0.89–1.90) and for E2 were 1.54 (95% CI, 1.01–2.35). Although there was also a borderline significant association between levels of 2OHE1 and breast cancer risk (HR $_{Q5vs.Q1}$, 1.45; 95% CI, 1.00–2.12), concentrations of other specific EMs were not associated with breast cancer risk.

Parent estrogen levels in relation to PGE-M and other reproductive and lifestyle factors in postmenopausal women

PGE-M levels and BMI, but not other lifestyle or reproductive variables, were positively associated with E1 and E2 levels (Supplementary Table S2). PGE-M levels continued to be a significant predictor of E1 levels after adjustment for BMI. However, the effect of PGE-M on E1 levels was at most modest: A 10% increase in PGE-M levels was associated with a 1% increase in E1 ($1.1^{0.146}$ = 1.014; adjusted β = 0.146 for log-transformed PGE-M to predict log-transformed E1, P = 0.045). PGE-M levels were not significantly associated with concentrations of other individual EMs (data not shown).

An exploratory path analysis was based on the hypothesized path diagram (Supplementary Fig. S1). The hypothesized model provided adequate fit for the data (RMSEA = 0; CFI = 1), and supported that BMI was a significant contributor to E1 levels (estimated direct effect = 0.2, P = 0.001). The direct effect of PGE-M on E1 levels became borderline significant (estimated direct effect = 0.11, P = 0.06). We further postulated that PGE-M could also mediate the effects of BMI and use of NSAIDs on E1 levels. However, whereas the direct effect of BMI on PGE-M levels was significant (estimated direct effect = 0.18, P = 0.002), the indirect effects of BMI or use of NSAIDs on E1 levels via PGE-M were insignificant with the corresponding standardized effect of 0.01 (P = 0.10) and -0.01 (P = 0.20), respectively.

Table 1. Selected participant characteristics of a case–cohort analysis of PGE-M and EMs in the Sister Study

	Cases ($N = 295$)	Subcohort ($N = 294$)	P value[a]
No (%)			
Non-Hispanic white	268 (90.9%)	275 (93.5%)	0.224
Current smokers	29 (9.8%)	15 (5.1%)	0.048
Regular current drinkers	212 (71.9%)	204 (69.4%)	0.536
Regular NSAID users[b]	181 (61.4%)	154 (52.4%)	0.028
Median (25th–75th percentile)			
Age, y	61 (57–65)	61 (56–65)	0.519
Age at menarche, y	13 (12–13)	13 (12–14)	0.006
Age at menopause, y	50 (45–53)	50 (45–53)	0.896
Number of parity	2 (1–3)	2 (1–3)	0.594
Years of HRT use in the past	1 (0–9)	2 (0–9)	0.619
Number of first-degree relatives with breast cancer	1 (1–2)	1 (1–2)	0.007
BMI, kg/m^2	27.3 (24–32)	26.7 (23–31)	0.290
Urinary PGE-M, ng/mg Cr.	5.6 (3.8–8.5)	5.3 (3.4–8.2)	0.419
Urinary levels of estrogens and EMs, pg/mg Cr.			
Total estrogens and EMs	12,704 (7,462–21,302)	12,112 (7,826–17,926)	0.580
Parent estrogens	1,536 (869–2,522)	1,324 (829–2,168)	0.044
Estrone	1,205 (671–1,917)	1,053 (644–1,699)	0.057
Estradiol	313 (172–548)	276 (162–478)	0.057
C2-hydroxylation pathway	1,647 (885–2,964)	1,509 (911–2,785)	0.049
2-hydroxyestrone	1,026 (588–2,000)	935 (499–1,602)	0.063
2-hydroxyestradiol	174 (71–392)	177 (69–447)	0.361
2-methoxyestrone	146 (49–301)	134 (41–282)	0.757
2-methoxyestradiol	19 (0.35–67)	18 (0.55–75)	0.610
3-methoxyestrone	39 (16–96)	38 (12–79)	0.406
C4-hydroxylation pathway	492 (266–1,041)	562 (263–1,126)	0.603
4-hydroxyestrone	400 (192–869)	454 (207–916)	0.387
4-methoxyestrone	7.1 (0.02–47.7)	10.3 (0.01–58.4)	0.541
4-methoxyestradiol	6.2 (0.02–25.9)	5.7 (0.02–34.2)	0.912
C16-hydroxylation pathway	7596 (3,739–13,768)	7436 (4,064–12,372)	0.866
16α-hydroxyestrone	377 (163–742)	345 (145–721)	0.503
Estriol	5536 (2,508–11,390)	5847 (2,465–10,784)	0.807
16-epiestriol	215 (112–436)	196 (97–374)	0.154
17-epiestriol	37 (8–125)	32 (6–113)	0.293
16-ketoestradiol	398 (184–788)	415 (198–771)	0.958
C2-hydroxylation pathway EMs: C16α-hydroxylation pathway EMs	0.30 (0.15–0.68)	0.27 (0.73–0.71)	0.428

[a]χ^2 test for categorical variables and Wilcoxon rank-sum test for continuous variables.
[b]Regular use of NSAIDs defined as $\geq$ 0.75 pill-years of NSAID use (equivalent to at least 3 pills of NSAID use per week for 3 months or longer).

Parent estrogens, PGE-M, and breast cancer risk

Overall, additional adjustment for PGE-M made only a nominal change in the HRs for the association between parent estrogens and breast cancer risk, with the HR associated with the highest quintile of parent estrogens increasing from 1.57 (95% CI, 1.05–2.34) to 1.61 (95% CI, 1.07–2.42; Table 2). As previously reported, high levels of PGE-M were associated with breast cancer risk only among those who did not use NSAIDs regularly, and there was no association between PGE-M and breast cancer risk among regular NSAID users (P for interaction between PGE-M and NSAID use = 0.047; ref. 6). Although the statistical test for the interaction is no longer significant ($P = 0.06$) after adjusting for E1 or E2 levels, combined or individually, the association among nonregular NSAID users persisted. The HR for breast cancer associated with the highest versus lowest quartile of PGE-M was 2.01 (95% CI, 0.96–4.20) among nonregular NSAID users after additional adjustment for E1 and E2 levels combined.

Discussion

In the present case–cohort analysis of 598 postmenopausal women, we found that urinary levels of parent estrogens, particularly E2, were associated with increased risk of developing breast cancer. Although PGE-M levels were a significant independent correlate of E1 levels, additional adjustment for PGE-M levels did not modify the relationship of E1 or E2 levels with breast cancer risk. Among women who did not regularly use NSAIDs, high levels of PGE-M were associated with an increased risk of breast cancer independent of E1 or E2 levels.

Our finding of a positive association between E2 levels and breast cancer risk agrees with those from a pooled analysis of 9 prospective studies, where breast cancer risk was significantly increased across extreme quintiles of circulating levels of E2 (RR = 2.0; 95% CI, 1.5–2.7; ref. 13). A majority of previous studies, including the aforementioned cohort studies, have determined estrogen levels in serum or plasma using enzyme immunoassays (EIA; refs. 13, 14). We adopted a novel LC-MS/MS method (8), which is known to have higher sensitivity and specificity compared with EIAs (15). Three previous studies have examined the association between individual EMs in serum and postmenopausal breast cancer risk using the same LC-MS/MS method (16–18), all performed in the same laboratory. In addition to a significant positive association with

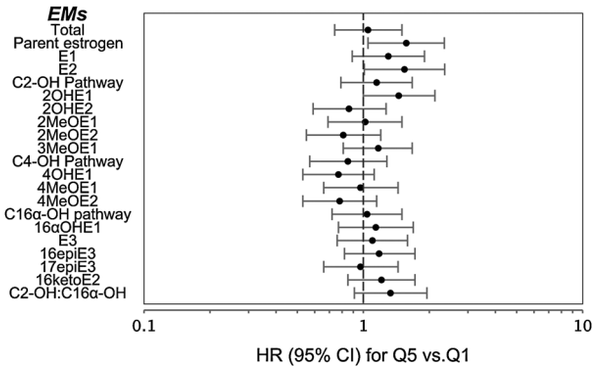

Figure 1.

Multivariable HRs and 95% CIs for breast cancer comparing the highest quintile (Q5) with the lowest quintile (Q1) of urinary EMs in postmenopausal women in the Sister Study. The models were adjusted for age at enrollment (50–54; 55–59; 60–64; 65–69; 70–75 y), BMI (<25, 25–29.9, 30–34.9, or $\geq$ 35 kg/m^2), smoking status (never, former, current), alcohol use status (never, social past; social current; regular past; regular current drinker), years of past HRT use (continuous), history of breast biopsy (yes or no); and number of first-degree family members with breast cancer (1 or $\geq$ 2). E1 (estrone); E2 (estradiol); C2-OH pathway (C2-hydroxylation pathway EMs); 2OHE1 (2-hydroxyestrone); 2OHE2 (2-hydroxyestradiol); 2MeOE1 (2-methoxyestrone); 2MeOE2 (2-methoxyestradiol); 3-MeOE1 (3-methoxyestrone); C4-OH pathway (C4-hydroxylation pathway EMs); 4OHE1 (4-hydroxyestrone); 4MeOE1 (4-methoxyestrone); 4-MeOE2 (4-methoxyestradiol); C16α-OH pathway (C16α-hydroxylation pathway EMs); 16αOHE1 (16α-hydroxyestrone); E3 (estriol); 16epiE3 (16epiestriol); 17epiE3 (17epiesetriol); 16ketoE2 (16ketoestriol). Figure was created using Forest Plot Viewer (41).

serum concentrations of parent estrogens, two of the previous studies have found that higher ratios of 2-OH pathway EMs relative to parent estrogens or 16-OH pathways were associated with reduced risk of breast cancer (16, 18). A similar protective association, however, was not found in a study by Falk and colleagues (17), or in our study, where there was also borderline significant association between urinary 2OHE1 levels and increased breast cancer risk. Recently, a nested case–control study of Shanghai women also examined urinary concentrations of 15 EMs and postmenopausal breast cancer risk, and reported an increased breast cancer risk in association with high concentrations of parent estrogens and with lower ratio of 2-OH pathway EMs to total EMs or parent estrogens. The latter associations, however, disappeared after adjustment for parent estrogens (19). The reasons for different results for individual EMs from the previous studies are not clear but could be partly attributed to different sample matrices (blood vs. urine). It has been speculated that blood might be more relevant to measure EMs than urine because parent estrogens were largely excreted without undergoing conversion into genotoxic metabolites (20, 21). Another explanation for the observed between-study variation may include differences in application of the relatively novel LC-MS/MS method. Varying sensitivity and specificity for individual EMs that can arise from sample collection, storage and processing, and assay calibration could have made results from different studies less comparable (22).

We previously found among postmenopausal women who did not regularly use NSAIDs that high levels of PGE-M were associated with an increased risk of developing breast cancer (6). PGE_2, a product of the metabolic activity of COX-2, can induce aromatase activity via the cAMP-mediated protein kinase A (PKA) signal transduction pathway (23, 24). Upregulation of COX-2 enzyme and increased PGE_2 concentration are associated with elevated aromatase expression and estrogen concentrations in adipose tissue adjacent to breast cancer (23, 25, 26) or inflamed breast tissue of obese women (24). Moreover, some epidemiologic studies have reported a stronger protection with use of NSAIDs for ER^+ breast cancer, supporting a role of PGE_2 in estrogen biosynthesis and estrogen-dependent breast carcinogenesis (27, 28). Our findings partially agree with the notion in that PGE-M levels are a significant, albeit modest, predictor of E1 concentration, but also raise the possibility that excess breast cancer risk associated with high levels of PGE-M may not be modulated through increased estrogens alone. This hypothesis is supported by findings from a recent randomized placebo-controlled study where a daily 325 mg aspirin did not lead to changes in serum estrogens in healthy postmenopausal women (29).

As a major prostaglandin of the inflammatory cascade, PGE_2 has an extensive role in promoting tumor growth (3, 30). Studies have described that PGE_2 activates EGFR and vascular endothelial growth factor (VEGF) to stimulate mitosis and angiogenesis, and favors *BAX* over *Bcl-2* genes to inhibit apoptosis (30–32). Increasingly, selective suppression of cytotoxic immunity by PGE_2 is also suggested as a mechanism by which PGE_2 supports breast cancer development and progression (33, 34). Although there is support for the biological plausibility of the association between PGE_2 and breast cancer risk independent of the estrogen pathway, only two epidemiologic studies including ours have examined this to date. The Shanghai Women's Health Study is the only other study that has reported association between urinary PGE-M and increased breast cancer risk among normal weight postmenopausal women ($RR_{Q4vs.Q1}$, 2.3; 95% CI, 1.2–4.4; ref. 35). In that study, use of NSAIDs was controlled at the study design stage by exclusion of women who used any NSAIDs in the 7 days before urine collection, but the role of endogenous estrogens was not simultaneously evaluated for the association between PGE-M and breast cancer risk. On the other hand, C-reactive protein (CRP), a biomarker of systemic inflammation, has been associated with a significantly increased risk of benign proliferative breast diseases and breast cancer independently of circulating levels of insulin and estrogens, suggesting a causal role of inflammation early in breast carcinogenesis (36, 37). Interestingly, in a case–control study of colorectal adenomas that jointly investigated CRP and PGE-M levels, the positive association between CRP levels and multiple or advanced adenoma risk was largely attributed to those with concurrently elevated PGE-M (38). However, the relationship and relative importance of CRP and PGE_2 to breast cancer risk and risk prediction remains to be determined.

The significance of the current study is that it is the first epidemiologic investigation of biomarkers of endogenous PGE_2 and estrogens and their prospective association with breast cancer risk. However, our study was limited by a relatively small sample size. The fact that we previously found the association between PGE-M and breast cancer only among those who did not regularly use NSAIDs further reduced the power of our study to examine the relationships among PGE-M, EM, and breast cancer risk in more detail. While not necessarily limitations of this study, two unique features of the present study are worth mentioning. First, it is important to emphasize that our investigation of PGE_2, EMs, and breast cancer risk is based on systemic levels of PGE_2 and EMs

Table 2. Multivariable HRs and 95% CI for breast cancer in relation to urinary levels of parent estrogens and PGE-M at baseline among postmenopausal women, with mutual adjustment

	Parent estrogens (Estrone plus estradiol; pg/mg Cr.)					
HR (95% CI)	**<680**	**680–<1,132**	**1,132–<1,612**	**1,612–<2,492**	**≥2,492**	***P* for trend**
Multivariable adjusted[a]	1	1.47 (0.99–2.18)	1.02 (0.67–1.57)	1.22 (0.82–1.82)	1.57 (1.05–2.34)	0.139
Additionally adjusted for PGE-M[a,b]	1	1.54 (1.02–2.31)	1.09 (0.70–1.70)	1.25 (0.82–1.90)	1.61 (1.07–2.42)	0.142
			Estrone (pg/mg Cr.)			
	<551	551–<871	871–<1,227	1,227–<1,911	≥1,911	*P* for trend
Multivariable adjusted[a]	1	1.10 (0.75–1.63)	0.88 (0.58–1.33)	1.13 (0.78–1.63)	1.30 (0.89–1.90)	0.183
Additionally adjusted for PGE-M[a,b]	1	1.13 (0.76–1.69)	0.91 (0.59–1.39)	1.14 (0.79–1.66)	1.34 (0.91–1.97)	0.158
			Estradiol (pg/mg Cr.)			
	<133	133–<222	222–<345	345–<589	≥589	*P* for trend
Multivariable adjusted[a]	1	1.39 (0.94–2.07)	1.33 (0.88–2.00)	1.30 (0.87–1.93)	1.54 (1.01–2.35)	0.120
Additionally adjusted for PGE-M[a,b]	1	1.43 (0.95–2.13)	1.36 (0.90–2.06)	1.31 (0.87–1.95)	1.52 (0.99–2.33)	0.152
			PGE-M (ng/mg Cr.)			
	<3.4	3.4–<5.24	5.24–<8.34	≥8.34	*p* for trend	*P for interaction*
Multivariable adjusted[a,b]						0.047
No regular use of NSAIDs	1	2.16 (1.03–4.56)	1.85 (0.90–3.81)	2.01 (1.01–4.29)	0.063	
Regular use of NSAIDs	1	0.90 (0.60–1.35)	1.00 (0.68–1.48)	0.99 (0.65–1.50)	0.851	
Additionally adjusted for parent estrogens[a,b,c]						0.066
No regular use of NSAIDs	1	2.14 (1.00–4.59)	1.81 (0.86–3.81)	2.01 (0.96–4.20)	0.065	
Regular use of NSAIDs	1	0.91 (0.60–1.37)	1.04 (0.70–1.52)	0.99 (0.65–1.51)	0.990	
Additionally adjusted for estrone[a,b,c]						0.064
No regular use of NSAIDs	1	2.09 (0.98–4.43)	1.80 (0.98–4.43)	1.97 (0.95–4.11)	0.072	
Regular use of NSAIDs	1	0.89 (0.59–1.34)	1.02 (0.69–1.50)	0.99 (0.65–1.51)	0.990	
Additionally adjusted for estradiol[a,b,c]						0.055
No regular use of NSAIDs	1	2.13 (1.01–4.48)	1.78 (0.86–3.69)	1.97 (0.95–4.10)	0.108	
Regular use of NSAIDs	1	0.87 (0.57–1.32)	1.00 (0.67–1.47)	0.97 (0.63–1.49)	0.915	

[a]Models included age at enrollment (50–54; 55–59; 60–64; 65–69; 70–75 y), BMI (<25, 25–29.9, 30–34.9, or ≥ 35 kg/m^2), smoking status (never, former, current), alcohol use status (never, social past; social current; regular past; regular current drinker), years of past HRT use (continuous), history of breast biopsy (yes or no); and number of first-degree family members with breast cancer (1 or ≥ 2).

[b]Models additionally included PGE-M (<3.4; 3.4–5.24; 5.24–<8.34; ≥ 8.34), lifetime pill-years of NSAID use (<0.75 vs. ≥ 0.75), and interaction terms between PGE-M and NSAID use; models were additionally adjusted for [c]parent estrogen levels (<680; 680-<1,132; 1,132-<1,612; >=1,612). E1 levels (<551; 551–<871; 871–<1,227; 1,227–<1,911; ≥ 1,911) or E2 levels (<133; 133–<222; 222–<345; 345–<589; ≥ 589).

determined in urine samples. Estrogens produced in peripheral adipose tissues can diffuse through the tissue to enter the breast duct (5). Serum concentrations of E1 and, to a lesser extent, E2 were highly correlated with the corresponding levels in breast adipose tissues of women with both hormone receptor positive and negative breast cancer (39). However, it remains to be validated whether urinary levels of EM and PGE-M are sufficiently strong proxies for their bioavailability in the normal breast tissues that lack constitutive *COX2* gene overexpression to sustain PGE_2 biosynthesis (30). Second, our study is also unique in that all women had at least one sister diagnosed with breast cancer at the time of study enrolment. Family history of breast cancer is a risk factor for breast cancer (40). The number of first-degree relatives with breast cancer was also associated with an increased risk of breast cancer in our cohort, but was not associated with either urinary PGE-M or EM levels. We thus expect that our findings are likely to reflect generalizable biological relationships among PGE-M, EM, and breast cancer risk.

In conclusion, we found that urinary levels of E2, but no other specific EMs, were associated with breast cancer risk, and that among women who did not regularly use NSAIDs, levels of PGE-M were associated with a higher risk of breast cancer independent of estrogen level. Our findings suggest that the increased risk associated with PGE-M might not be fully explained by the estrogens–breast cancer association alone but also by additional effects related to inflammation.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: S. Kim, J.A. Taylor, D.P. Sandler
Development of methodology: S. Kim
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): J.A. Taylor, D.P. Sandler
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): S. Kim, J. Campbell, W. Yoo, J.A. Taylor
Writing, review, and/or revision of the manuscript: S. Kim, J. Campbell, J.A. Taylor, D.P. Sandler
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): S. Kim
Study supervision: S. Kim, D.P. Sandler

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