

Research Article

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Association between Urinary Prostaglandin E₂ Metabolite and Breast Cancer Risk: A Prospective, Case–Cohort Study of Postmenopausal Women

Sangmi Kim^{1,2}, Jack A. Taylor^{2,3}, Ginger L. Milne⁴, and Dale P. Sandler²

Abstract

Overweight or obese women are at increased risk of developing and dying from breast cancer. Obesity-driven inflammation may stimulate prostaglandin E₂ (PGE₂)-mediated aromatase activation and estrogen biosynthesis in breast tissues. We hypothesized that increased production of PGE₂ would contribute to elevated breast cancer risk in postmenopausal women. We carried out a case–cohort study with 307 incident breast cancer cases and 300 subcohort members from the Sister Study cohort. HRs and 95% confidence intervals (CI) were estimated for the association between urinary levels of a major PGE₂ metabolite (PGE-M) and breast cancer risk using Prentice's pseudo-likelihood approach. Several lifestyle factors were associated with urinary levels of PGE-M: smoking, high-saturated fat diet, and obesity increased urinary PGE-M, and use of nonsteroidal antiinflammatory drugs (NSAID) decreased urinary PGE-M. Although there was no association between urinary PGE-M and postmenopausal breast cancer risk in the overall analysis or among regular users of NSAIDs, there was a positive association among postmenopausal women who did not regularly use NSAIDs with HRs of 2.1 [95% confidence interval (CI): 1.0–4.3]; 2.0 (95% CI: 1.0–3.9); and 2.2 (95% CI: 1.1–4.3) for the second, third, and highest quartiles of PGE-M. Our findings suggest a link between systemic PGE₂ formation and postmenopausal breast cancer, and a possible modification of the association by lifestyle and pharmacologic interventions. If confirmed in larger studies, these results may have useful implications for the development of preventive strategies. *Cancer Prev Res*; 6(6); 511–8. ©2013 AACR.

Introduction

Estrogens have been considered a key factor in mammary carcinogenesis (1). Evidence from both animal model and breast cancer cell line studies shows that estrogens directly and indirectly induce cell proliferation (2–4). In postmenopausal women, however, estrogens are no longer directly secreted from the ovaries; instead, adipose tissue becomes a primary source of peripheral production of estrogens (5). The observed higher levels of circulating estrogens in obese women (6) may underlie the association between obesity and breast cancer in postmenopausal women.

Obesity is also a state of chronic inflammation, with increased circulating levels of proinflammatory cytokines and chemokines (7, 8). Emerging data indicates that obesity

may promote inflammation in breast adipose tissues, stimulating prostaglandin E₂ (PGE₂)-mediated aromatase activation and estrogen biosynthesis (9–11). PGE₂ is a key mediator of inflammation that is derived from cyclooxygenase-2 (COX-2; ref. 12) and is also an important regulator of aromatase expression in mammary adipose tissue via a cyclic AMP (cAMP)-dependent signal transduction (13).

We hypothesized that increased production of PGE₂ would contribute to elevated breast cancer risk in postmenopausal women. We conducted a case–cohort study including 607 postmenopausal women enrolled in the prospective Sister Study cohort to examine the association between a major urinary metabolite of PGE₂ (PGE-M) and breast cancer risk. Nonsteroidal antiinflammatory drugs (NSAID) directly target COX-2 and suppress PGE₂ production (14). Therefore, we further evaluated whether use of NSAIDs would modify the association between urinary levels of PGE-M and postmenopausal breast cancer risk.

Materials and Methods

Study design

We carried out a case–cohort analysis within the Sister Study, a prospective cohort of U.S. women ages 35 to 74 years who had a sister with breast cancer but who did not have breast cancer themselves (15, 16). At the time of enrollment (2003–2009), participants completed questionnaires on lifetime exposures to potential risk factors

Authors' Affiliations: ¹Georgia Regents University Cancer Center, Medical College of Georgia, Section of Hematology/Oncology, Department of Medicine, Augusta, Georgia; ²Epidemiology Branch; ³Laboratory of Molecular Carcinogenesis, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, North Carolina; and ⁴Eicosanoid Core Laboratory, Division of Clinical Pharmacology, Vanderbilt University, Nashville, Tennessee

Corresponding Author: Sangmi Kim, Georgia Regents University Cancer Center, Medical College of Georgia, Section of Hematology/Oncology, Department of Medicine, 1410 Laney Walker Blvd., Augusta, GA 30912. Phone: 706-721-8039; Fax: 706-721-5566; E-mail: sankim@gru.edu

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including medication use, medical and reproductive history, and diet. Participants also provided a self-collected first morning urine sample that was refrigerated and retrieved the same day by the study staff during a home visit. Samples were shipped overnight to the Sister Study lab, and were aliquoted and stored at -80°C before analyses. Information on incident breast cancer is collected via annual and biennial follow-up questionnaires or from participant-initiated reports to the Sister Study Helpline. Six months after the self-reported diagnosis date, women were contacted for information regarding their diagnosis and treatment and asked to authorize release of pertinent medical records. The study was approved by the Institutional Review Board of the National Institute of Environmental Health Sciences, NIH, and the Copernicus Group.

Within the Sister Study, 25,800 subjects who completed baseline activities by June 1, 2007 and had first morning urine samples were eligible for selection for the present case-cohort analysis. Participants were further required to be postmenopausal, age 50 or older, and not currently using hormone replacement therapies at the time of baseline data collection ($N = 11,338$).

As of September 2010, 352 eligible women reported incident breast cancer and 307 were included in the present study. Excluded were 22 women who refused to authorize access to pathology reports or medical records, 12 women who were later found to have compromised urine samples, and 11 women whose breast cancer diagnosis date was unknown or within 1 month of the completion of baseline activity. Subcohort members ($N = 300$) were randomly selected from those meeting the aforementioned eligibility criteria ($N = 11,338$) by frequency matching to the age distribution of the 352 initially eligible breast cancer cases. Four women in the subcohort were also diagnosed with breast cancer since the selection for the present analysis.

At the time of the current analysis, breast cancer diagnosis was confirmed by pathology reports or medical records for 92% of cases ($N = 282$). Records were not yet available for 25 cases, but these cases were retained in the analysis because the accuracy of a self-report of cancer was high among the cases whose medical records were already available (98%).

Quantification of urinary PGE-M

As an index of systemic PGE₂ production, a major urinary metabolite of PGE₂ (11- α -hydroxy-9,15-dioxo-2,3,4,5-tetranor-prostane-1,20-dioic acid; PGE-M; ref. 17) was quantified using liquid chromatography/tandem mass spectrometry (LC/MS/MS) in the Eicosanoid Laboratory at Vanderbilt University School of Medicine using methods as previously described (18, 19). Twenty samples were run in a single batch and 20% of the total samples ($N = 127$) were run in duplicate. The coefficient of variation (%CV) of PGE-M was 6% within a batch and 14% between batches. Urinary PGE-M levels were normalized to the creatinine level of the sample to account for differences arising from variations in urine concentrations. Urinary creatinine levels were measured in duplicate using the Creatinine Color-

metric Detection Kit supplied by Enzo Life Sciences (P/N ADI1907030A) at the Vanderbilt University. The within- and between-batch%CV were 4% and 8%, respectively.

The short-term reliability of PGE-M measurements was also estimated using 100 samples from a separate set of 12 individuals who provided urine samples at 3 time points across 9 months. After controlling for batch effects, 64% of the total variance in PGE-M measurements was attributed to between-subject variability. PGE-M levels were below the detection limit for 3 subjects (2 cases and 1 subcohort member) even after reanalysis, leaving a total of 305 cases and 299 subcohort members in the final study sample.

Statistical analysis

Prentice's pseudo-likelihood approach with Barlow's weighting scheme (weight of one for cases and the inverse of the sampling fraction for subcohort members) was used to estimate HRs and 95% confidence intervals (95% CI) for breast cancer in relation to urinary levels of PGE-M in the case-cohort design (20, 21). Age was used as the primary time scale, and women were left-censored beginning on the day when they completed enrollment with follow-up continuing until their date of diagnosis of breast cancer for cases and the completion date of their last health update for noncases. Women who did not respond to their most recent eligible health update were censored at the midpoint of the interval between the last completed health update and the end of the window of eligibility for responding to their first skipped health update.

Urinary levels of PGE-M were positively skewed; therefore, crude and adjusted geometric means were used to evaluate PGE-M associations among the subcohort members with the following potential factors that may influence endogenous PGE₂ production: body mass index (<25, 25–29.9, 30–34.9, or ≥ 35 kg/m²), regular use of NSAIDs (use of NSAIDs at least 3 times per week for 3 months or longer), smoking status (never, past, or current smoking), physical activity (quartiles), type of menopause (natural or surgical), years of hormone replacement therapy use (never, <3, 3–9, or ≥ 10 years), age at last menstrual period (<40, 40–49, 50–52, or ≥ 53 years), self-reported health status (excellent, very good, good or fair/poor), history of cardiovascular disease, dietary and total vitamin E intake (quartiles), dietary omega 3 fatty acids intake (quartiles) and supplement use, total energy intake (quartiles), and percent of energy intake from saturated fat (quartiles). For the analyses of the association between quartiles of the urinary PGE-M and breast cancer risk, PGE-M levels were categorized into 4 groups based on the distribution among the subcohort. To identify a minimal adjustment set of covariates that are sufficient to control confounding, a directed acyclic diagram (DAG) was drawn using DAGitty v. 1.1 (<http://www.dagitty.net>; ref. 22). Final multivariable models included regular use of NSAIDs, percent of energy intake from saturated fat, BMI, and family history of breast cancer as covariates.

Tests for linear trend were carried out by treating an ordered categorical variable as a continuous variable. A likelihood ratio test was used to test whether the association

between urinary PGE-M and breast cancer risk was modified by regular use of NSAIDs. Significance tests were 2 sided with the level of significance at 0.05. Stata 12.0 was used for all the analyses.

Results

Subject characteristics

The average age of the women included in the present analysis was approximately 61 years and more than 90% of them were non-Hispanic whites (Table 1). Compared with the subcohort, women who developed breast cancer during the follow-up tended to have additional family members with breast cancer (39% vs. 29%, $P = 0.02$). Regular use of selective COX-2 inhibitors (COXib) was more commonly reported by cases (14% vs. 8%, $P = 0.02$) but there were no significant differences in regular use of aspirin or other types of NSAIDs between cases and the subcohort.

Lifestyle and reproductive factors associated with urinary PGE-M

Several known pro- and antiinflammatory factors were associated with urinary PGE-M in postmenopausal women (Fig. 1). Urinary PGE-M was increased with current smok-

ing, poor self-reported health status, and high-saturated fat diet and decreased with NSAID use 1 day before urine collection. In general, increasing lifetime use of NSAIDs was also associated with reduced levels of urinary PGE-M but the association was not strictly linear and no inverse association with urinary PGE-M was observed for lifetime use of COXibs. Urinary PGE-M was higher with increasing BMI ($P_{trend} < 0.01$). Other measures of obesity such as waist and hip circumferences, and waist-to-hip ratio were also positively associated with urinary PGE-M but the dose-dependent relationship with urinary PGE-M was less evident. We also found no significant relationships between PGE-M levels and age and other lifestyle factors such as intake of omega 3 fatty acids and vitamin E, or physical activity (data not shown).

Urinary PGE-M and postmenopausal breast cancer risk

Overall, we observed a slight, nonsignificant, increase in postmenopausal breast cancer risk in relation to increasing urinary PGE-M ($P_{trend} = 0.13$; Table 2). However, addition of the interaction terms between regular use of NSAIDs and quartiles of urinary PGE-M to the model indicated that the association between urinary PGE-M and postmenopausal

Table 1. Baseline characteristics of Sister Study breast cancer cases and subcohort members

	Breast cancer cases (N = 305)	Subcohort (N = 299)	P
Mean (SD) age, y	61.5 (6.0)	61.2 (5.9)	0.48
Non-Hispanic whites, N (%)	276 (90.5)	279 (93.3)	0.21
Having ≥ 2 first degree family members with breast cancer, N (%)	118 (38.7)	88 (29.4)	0.02
Natural menopause, N (%)	213 (70.3)	210 (70.5)	0.96
Mean (SD) age at last menstrual period, y	48.2 (7.0)	48.5 (6.7)	0.56
Ever use of HRT, N (%)	186 (61)	198 (66)	0.18
Mean (SD) years of HRT use among ever users	8.4 (7.1)	7.6 (6.8)	0.25
Poor or fair self-reported health status, N (%)	17 (5.6)	20 (6.7)	0.95
Current smokers, N (%)	29 (9.5)	17 (5.7)	0.14
Normal BMI (<25 kg/m ²), N (%)	99 (32.5)	108 (36.1)	0.4
Mean (SD) total current activities, MET-hours/week	51.2 (30.3)	51.2 (27.8)	0.97
Mean (SD) daily energy intake, kcal/d	1662.7 (561)	1573.6 (578.6)	0.06
Mean (SD) percent energy intake from saturated fat, %	10.8 (2.6)	10.4 (2.5)	0.1
Mean (SD) long chain omega 3 fatty acids intake, mg/day	130.8 (124.4)	138.9 (154.5)	0.48
Mean (SD) intake of vitamin E, mg/day	9.1 (4.3)	8.5 (4.2)	0.12
Regular use ^a of NSAIDs, N (%)	186 (61)	161 (53.9)	0.08
Mean (SD) pill-years of NSAID use among regular users	54.2 (84.7)	57.6 (79.2)	0.69
Regular use ^a —aspirin only, N (%)	131 (43)	106 (35.5)	0.06
Mean (SD) pill-years of aspirin use among regular aspirin users	44.8 (86.2)	48.7 (73.1)	0.71
Regular use ^a —COXibs only, N (%)	42 (13.8)	24 (8)	0.02
Mean (SD) pill-years of COXib use among regular COXibs users	22.5 (34.5)	19.2 (23.8)	0.68
Regular use ^a —other NSAIDs only, N (%)	84 (27.5)	80 (26.8)	0.83
Mean (SD) pill-years of other NSAID use among regular users of other NSAIDs	38.9 (44.9)	45.6 (67.9)	0.45
NSAID use in the past 24 hours, N (%)	124 (40.7)	118 (39.5)	0.77

Abbreviations: HRT, hormone replacement therapy; BMI, body mass index; MET, metabolic equivalents; NSAID, nonsteroidal antiinflammatory drug; COXibs, selective COX-2 inhibitors.

^aRegular use was defined as taking one pill for at least 3 times per week for 3 months or longer, which is equivalent to 0.75 pill-year.

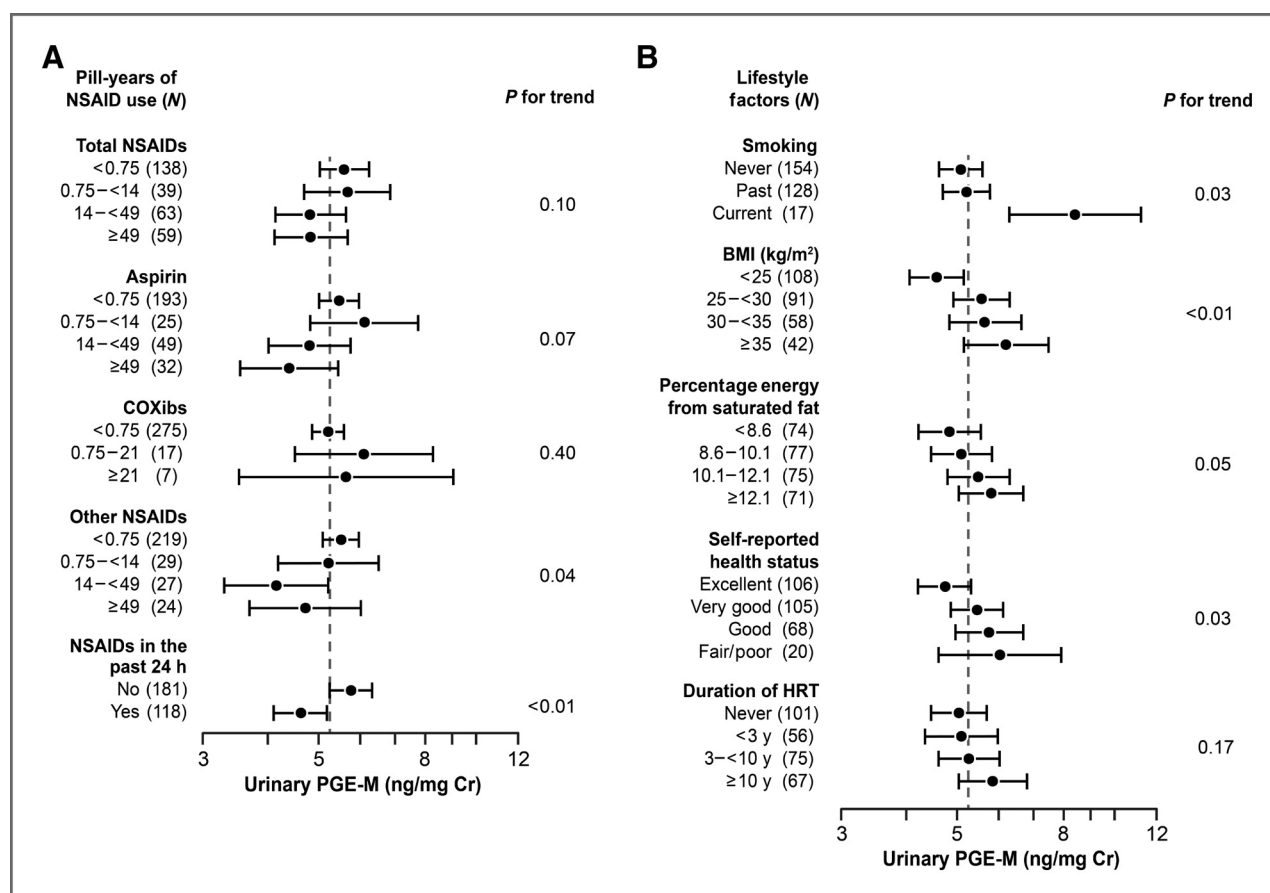


Figure 1. Geometric mean of urinary PGE-M concentrations (ng/mg Cr) by (A) pill-years of NSAID use and (B) selected lifestyle factors in the Sister Study subcohort members. Mean values (●) and 95% CIs (–) were plotted on log scale, adjusted for pill-years of NSAID use, percent energy intake from saturated fat, BMI, smoking status, years of HRT use, type of menopause, self-reported health status, and use of NSAIDs in the past 24 hours of urine collection. Estimates associated with specific types of NSAID use were additionally adjusted for other types of NSAID use. A reference line indicates the overall mean of urinary PGE-M concentrations in the subcohort members. Abbreviations: NSAID, nonsteroidal antiinflammatory drug; COXibs, selective COX-2 inhibitors; BMI, body mass index; HRT, hormone replacement therapy.

breast cancer was largely attributed to the relationship among women who did not regularly use NSAIDs, with HRs of 2.1 (95% CI: 1.0–4.3); 2.0 (95% CI: 1.0–3.9); and 2.2 (95% CI: 1.1–4.3) for the second, third, and highest quartiles of urinary PGE-M (likelihood ratio test comparing the models with and without the interaction terms $P = 0.09$). No association was found between urinary PGE-M and postmenopausal breast cancer among regular users of NSAIDs. After adjusting for the potential confounders identified through the DAG analysis, the estimates were slightly attenuated but an increased risk of breast cancer associated with high levels of urinary PGE-M remained among those who did not report regular use of NSAIDs (likelihood ratio test $P = 0.08$).

Sensitivity analysis

Because use of NSAIDs 1 day before urine collection was found to be a significant correlate of urinary PGE-M in the present study, we conducted additional analyses to take this into account. First, use of NSAIDs 1 day before urine collection was additionally included in the multi-

variable model. But the results produced were almost identical to the estimates from the previous model. Next, we identified women whose NSAID use at the time of urine collection was different from their lifetime pattern of NSAID use. This included (i) women who were defined as regular lifetime NSAID users but did not use NSAIDs 1 day before urine collection ($N = 142$) and (ii) those who were not defined as regular lifetime NSAID users but used NSAIDs 1 day before urine collection ($N = 38$). However, excluding these 180 women from the analysis did not change observed relationships between urinary PGE-M and breast cancer risk among regular or nonregular users of NSAIDs (data not shown).

Discussion

In this prospective analysis of postmenopausal women, high levels of urinary PGE-M were not associated with increased breast cancer risk. However, when we stratified by NSAID use, urinary PGE-M was positively associated with breast cancer risk among those who did not use NSAIDs regularly.

Table 2. HRs (95% CIs) for the association between the quartiles of urinary PGE-M and postmenopausal breast cancer in the Sister Study, overall and by regular use of NSAIDs^a

	Overall population		No regular use of NSAIDs		Regular use of NSAIDs	
	Unadjusted HR (95% CI)	Adjusted ^b HR (95% CI)	Unadjusted HR (95% CI)	Adjusted ^b HR (95% CI)	Unadjusted HR (95% CI)	Adjusted ^b HR (95% CI)
Quartiles of PGE-M (ng/mg Cr)						
Q1 (<3.4)	1. (Ref.)	1. (Ref.)	1. (Ref.)	1. (Ref.)	1. (Ref.)	1. (Ref.)
Q2 (3.4–<5.2)	1.0 (0.8–1.5)	1.0 (0.7–1.4)	2.1 (1.0–4.3)	2.1 (1.0–4.2)	0.8 (0.5–1.2)	0.8 (0.5–1.2)
Q3 (5.2–<8.1)	1.2 (0.9–1.7)	1.1 (0.8–1.6)	2.0 (1.0–3.9)	1.8 (0.9–3.6)	1.1 (0.7–1.6)	1.0 (0.7–1.5)
Q4 (≥ 8.1)	1.2 (0.9–1.7)	1.1 (0.8–1.6)	2.2 (1.1–4.3)	2.0 (1.0–4.0)	1.0 (0.7–1.5)	0.9 (0.6–1.4)
P for linear trend	0.13	0.34	0.06	0.12	0.65	0.97

Abbreviations: NSAID, nonsteroidal antiinflammatory drug; BMI, body mass index.

^aRegular use was defined as use of NSAIDs for at least 3 times per week for 3 months or longer, which is equivalent to 0.75 pill-year.

^bMultivariable models were adjusted for percent energy intake from saturated fat, BMI, smoking status, and number of first-degree relatives with breast cancer.

There is a wealth of evidence from experimental and clinical studies suggesting that PGE₂ plays a key role in influencing cancer development. PGE₂ and COX-2 are over-expressed in premalignant and malignant tissues compared with normal tissues (23, 24), whereas expression of 15-hydroxyprostaglandin dehydrogenase (15-PGDH), which catabolizes PGE₂ into an inactive form, is upregulated in normal tissues (25). More directly, it has been shown that treatment with PGE₂ increases both size and number of intestinal tumors in experimental animals by promoting cell proliferation and stimulating angiogenesis (23, 26, 27).

PGE₂ may also promote estrogen-dependent carcinogenic processes by stimulating estrogen biosynthesis. PGE₂ has been identified as a potent activator of aromatase gene expression via c-AMP-dependent pathway (13). Further studies have shown that there was a strong correlation between aromatase *CYP19* gene and cyclooxygenase *PTGS2* gene expression, and NSAIDs inhibited aromatase gene expression and enzyme activity in a dose-dependent manner (28, 29). As estrogens are predominantly derived via aromatization of androgens in postmenopausal women (5), the significance of PGE₂ in estrogen-dependent cancers is likely to be greater in postmenopausal women. It is also plausible that the association of urinary PGE-M with breast cancer risk is more evident for estrogen-positive tumors. However, our study included too few ER-negative cases to compare associations by molecular phenotype of breast cancer.

We are unaware of any previous study of urinary PGE-M and breast cancer risk, but several studies have evaluated urinary PGE-M as a promising biomarker for colorectal neoplasia. In a case series study, urinary levels of PGE-M were higher in patients with colorectal cancer and large adenomas as well as large Crohn disease compared with patients with small or no polyps (30). Similarly, there was a 25% increase in urinary PGE-M among patients with advanced or multiple adenomas compared with adenoma-free subjects in a case-control study of modest sample

size (31). High urinary levels of PGE-M were also associated with increased risk of colorectal (32) and gastric cancers (33) in a prospective cohort of Chinese women. Contrary to our findings, however, use of NSAIDs did not appear to be an important effect modifier of the association between urinary PGE-M and cancer risk in that study population where use of NSAIDs was relatively uncommon. On the other hand, a recent study of patients with breast cancer reported higher urinary PGE-M in relation to lung metastases, and the difference in urinary PGE-M associated with lung metastases tended to be greater among patients who did not report the use of NSAIDs within 7 days of urine collection (34).

NSAIDs are a class of drugs that block COX enzyme activity and thus suppress PGE₂ synthesis (35). Different types and dosages of NSAIDs have been shown to reduce levels of PGE₂ at both target tissues and urinary excretion (36, 37). In the present study, we assessed not only lifetime use of NSAIDs but also use of NSAIDs 1 day before urine collection, and found that urinary PGE-M was more strongly associated with immediate NSAID use rather than lifetime use. Although compared with nonregular users, lifetime regular users were more likely to use NSAIDs 1 day before urine collection as well, there was no perfect agreement between lifetime NSAID use and NSAID use 1 day before urine collection. Excluding women who had inconsistent NSAID use patterns from the analysis, however, did not change the association between urinary PGE-M and postmenopausal breast cancer risk in regular users of NSAIDs.

Consistent with the existing literature, we observed increased levels of urinary PGE-M in relation to several lifestyle factors. Smoking is known to induce the transcription of *PTGS2*, ultimately leading to an increased production of PGE₂. Previous studies have shown a dose-response relationship between pack-years of smoking and urinary PGE-M (38). While the present study included only a few current smokers, our data also suggested that

urinary PGE-M was higher in current smokers who smoke more than 1 pack per day compared with those who smoke less than 1 pack a day. Obesity is recognized as an inflammatory condition (7); increasing adiposity leads to the recruitment of macrophages that produce and release proinflammatory cytokines, thus upregulating *PTGS2* expression (39). Several reports including ours have reported a dose-dependent relationship between increasing obesity and higher circulating levels of inflammatory cytokines (39, 40). The present finding of the relationship between various obesity indices and urinary PGE-M suggests that obesity-driven inflammation leads to an increase in an inflammatory mediator which may influence estrogen bioavailability (5, 10, 11).

Low-fat diet enriched with long-chain omega 3 fatty acids has been associated with a reduced level of PGE₂ (41). Omega 3 fatty acids, particularly fish-derived, long-chain omega 3 fatty acids, suppress PGE₂ synthesis by reducing the relative composition of arachidonic acids in cell membrane phospholipids (42), whereas saturated fatty acids upregulate the expression of COX-2 and other inflammatory mediators (43). Our data partially supports these previous findings: there was a dose-response increase in urinary PGE-M in relation to higher relative intake of saturated fat, but no association was found between dietary intake of long-chain omega 3 fatty acids and urinary levels of PGE-M. Overall, low dietary intake of marine-derived, omega 3 fatty acids in our study population could partly explain the lack of the association with urinary PGE-M. But taking into account supplement use did not make a difference. We also did not observe increased urinary PGE-M with increasing chronological age. But the age range in our study (50–75 years) may not have been sufficiently wide to see an effect. Alternatively, self-reported health status as well as several health behavior-related factors that were significantly associated with urinary PGE-M may suggest that aging-related increases in inflammation are modifiable.

Measurements of urinary PGE-M were shown to have relatively good reliability over a 1-year period, with intraclass correlation coefficient (ICC) of 0.67 (44). We also examined intra and interindividual variability of urinary PGE-M over a 9-month-period in a small set of samples and obtained a similar ICC of 0.64. The observed association of urinary PGE-M with obesity and smoking in the present analysis suggests that a single measurement of urinary PGE-M is a robust index against relatively constant individual characteristics. On the other hand, urinary PGE-M measurements tended to be more sensitive to immediate exposure to NSAIDs than lifetime use of NSAIDs, posing a challenge to use urinary PGE-M as a long-term indicator of endogenous PGE₂ production among those who have used NSAIDs over time with varying dose intensities. Conversely, the findings also support that urinary PGE-M may have a utility as a biomarker or a surrogate endpoint to assess the effectiveness of preventive interventions.

Several limitations of the present study require further discussion. First, a relatively small sample size is a major

limitation, especially with regard to drawing a firm conclusion from subgroup findings for women who did not regularly use NSAIDs. Although the sample size was determined *a priori* to provide sufficient power to detect a relative risk of 1.3 or more, this study was not powered to examine the specific subgroup effect. Second, some health conditions that involve inflammation in their pathogenesis may lead to an increase in urinary PGE-M. Excess production of PGE₂ has been reported in serum and synovial fluids of rheumatoid arthritic and osteoarthritic patients (45). Increased levels of PGE₂ were also observed in relation to amyotrophic lateral sclerosis (46, 47), periodontitis (48), and HIV infection (19). However, these conditions are relatively uncommon or not strongly associated with breast cancer risk and thus, are unlikely to have introduced a significant bias in the present study. Finally, we arbitrarily defined regular users of NSAIDs as those who used NSAIDs at least 3 times per week for 3 months or longer. Using different cumulative dosages as a cut-off point to define regular users did not change the overall conclusion, but intensity and indication of NSAID use likely vary within the regular users defined in our analysis.

In summary, well-known risk factors for cancer such as high fat diet, smoking, and obesity increased urinary PGE-M, and high levels of urinary PGE-M were associated with an increased risk for postmenopausal breast cancer among women who did not regularly use NSAIDs. Our results are supported by laboratory data suggesting that PGE₂ can stimulate estrogen biosynthesis, which in turn could promote breast carcinogenesis (28, 29). Additional studies are warranted to confirm our findings.

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
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): G.L. Milne, D.P. Sandler
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): S. Kim, J.A. Taylor
Writing, review, and/or revision of the manuscript: S. Kim, J.A. Taylor, G.L. Milne, 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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