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Research Article

Urinary Prostaglandin E_2 Metabolite and Breast Cancer Risk

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Abstract

Background: Levels of the cyclooxygenase 2 (COX2) enzyme are elevated in breast cancer tissue, and most COX2 effects are believed to be mediated through overproduction of prostaglandin E2 (PGE2). We evaluated associations between the primary urinary metabolite of PGE2 (PGE-M) and breast cancer risk.

Methods: A nested case–control study of 504 cases and 1,082 controls was conducted using data from the Shanghai Women's Health Study, a large population-based prospective cohort study of 74,941 Chinese women. Urinary PGE-M was measured using a liquid chromatography/tandem mass spectrometric method. Logistic regression estimated odds ratios (OR) and 95% confidence intervals (95% CI) with adjustment for potential confounders.

Results: Overall, no association between urinary PGE-M and breast cancer was detected. However, a suggestive positive association was found among postmenopausal women. In particular, a clear dose–response relationship between urinary PGE-M and breast cancer was observed among postmenopausal women with a body mass index (BMI) $< 25\ kg/m^2$ ($P_{linear\ trend} = 0.005$). Among these women, risk of breast cancer increased from 1.00 (reference) to 1.06 (95% CI, 0.56–1.99), 1.50 (95% CI, 0.79–2.83), and 2.32 (95% CI, 1.24–4.41) for the lowest to highest quartiles of PGE-M, and such associations were stronger among those who were diagnosed with cancer within the first four years of sample collection. No apparent association was observed among overweight postmenopausal women (BMI $\geq 25\ kg/m^2$).

Conclusion: High urinary PGE-M level was associated with elevated risk of breast cancer among normal weight, postmenopausal women.

Impact: Urinary PGE-M level may be useful for breast cancer risk assessment among normal weight, postmenopausal women. *Cancer Epidemiol Biomarkers Prev; 23(12); 2866–73. ©2014 AACR.*

Introduction

Breast cancer is the most common cancer and a leading cause of cancer-related mortality among women worldwide (1, 2). Cumulative evidence suggests that cyclooxygenase 2 (COX2) plays an important role in the tumorigenesis of several cancers, including breast cancer. COX2 is the rate-limiting enzyme of prostaglandin synthesis, and overexpression of the *COX2* gene can lead to increased prostaglandin E_2 (PGE_2) production. PGE_2 is a key mediator of inflammation and plays an important role in carcinogenesis (3–5). Experimental and animal model studies have found that overproduction of PGE_2 can induce epithelial cell proliferation and angiogenesis and inhibit immunosurveillance and cell apoptosis (6–10). In humans, COX2 overexpression has been observed in approximately 40% of cases of invasive breast carcinoma and at a higher frequency in preinvasive ductal carcinoma *in situ* (DCIS, stage 0) tumors, but not in normal breast tissue (11). Epidemiologic studies have shown that use of nonsteroidal anti-inflammatory drugs (NSAID) may be associated with reduced breast cancer risk (12–14). The protective effects of NSAIDs are thought to be mediated largely through COX2 inhibition, which, in turn, reduces PGE_2 production. Thus, COX2-derived PGE_2 may reflect inflammation status and related cancer risk. Because PGE_2 is an unstable compound that is rapidly metabolized *in vivo* to a stable metabolite, 11 α-hydroxy-9,15-dioxo-2,3,4,5-tetranorprostane-1,20-dioic acid (PGE-M), the measurement of excreted urinary PGE-M is used to quantify systemic PGE_2 production *in vivo* (15). It has been hypothesized that urinary PGE-M might serve as a promising biomarker for predicting cancer risk, including breast cancer risk (15–17).

Obesity is a known risk factor for postmenopausal breast cancer and is also considered a chronic inflammatory condition (18, 19). *In vitro* experiments and human studies have shown that excessive fat accumulation in

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breast adipose tissues may activate PGE_2-mediated aromatase and increase estrogen biosynthesis (20, 21). However, no study to date has investigated possible modifying effect of adiposity on the relationship between PGE-M and breast cancer risk. In this report, we use data from a prospective cohort study to evaluate the association of urinary PGE-M levels with breast cancer and further examine whether this association is modified by body mass index (BMI), a measure routinely used to quantify adiposity.

Materials and Methods

Study population

The Shanghai Women's Health Study (SWHS) is a large population-based prospective cohort study currently ongoing in Shanghai, China. The study was approved by the Institutional Review Boards of all collaborating institutions, and all participants provided written informed consent. The methodology for the SWHS has been described in detail previously (22). Briefly, from 1997 to 2000, 74,941 Chinese women of ages 40 to 70 years and residing in Shanghai were recruited into the study. At the time of enrollment, each woman completed an in-person survey conducted by trained interviewers. The participation rate for the baseline survey was 93%. Data collected at the baseline survey included sociodemographics, menstrual and reproductive history, usual dietary intakes and other lifestyle factors, medical history and medication use, and family history of cancers, including breast cancer, among first-degree relatives. For this analysis, we defined "regular users of aspirin" as individuals taking any aspirin three or more times a week for a minimum duration of 2 consecutive months, "ever regular smokers" as individuals who had smoked one or more cigarettes a day for a minimum duration of 6 consecutive months, "ever consumed alcohol regularly" as consuming alcohol at least three times a week over a duration of at least 6 consecutive months, and "regular physical activity" as doing physical exercise one or more times a week for at least 3 consecutive months. Body measurements were also taken. BMI was calculated as body weight in kilograms divided by height in meters squared (kg/m^2) and was categorized as underweight and normal weight (<25 kg/m^2), overweight (25–29.9 kg/m^2), and obese (≥ 30 kg/m^2) based on the World Health Organization's (WHO) definitions (23). We also used proposed BMI cutoff point for Asians to define overweight (≥ 23 kg/m^2; ref. 24). Of the study participants, 65,754 (88%) provided a spot urine sample. Urine samples were collected into a sterilized cup containing 125 mg ascorbic acid to prevent oxidation of labile metabolites. After collection, the samples were kept in a portable Styrofoam box with ice packs (at approximately 0°C to 4°C) and processed within 6 hours for long-term storage at −70°C. Each woman also filled out a biospecimen collection form at the time of sample collection, which included the date and time of sample collection, time of last meal, and day of last menstruation (for premenopausal women), as well as intake of selected foods, smoking habits, and use of any medications over the previous 24 hours and during the previous week.

The cohort has been followed using a combination of in-person surveys, record linkage to cancer incidence and mortality data collected by the Shanghai Cancer Registry, and death certificate data collected by the Shanghai Vital Statistics Unit. Follow-up surveys of all living cohort members or next of kin for deceased participants have been conducted by in-person interview with participation rates of 99.8% (2000–2002), 98.7% (2002–2004), 95.0% (2004–2006), and 91.9% (2007–2011). For cohort members who were diagnosed with cancer, medical charts were reviewed to verify the diagnosis, and detailed information on the pathology characteristics of the cancer was obtained.

This nested case–control study was conducted as part of a large project including breast and other cancers. Included in this study were 560 incident breast cancer cases identified during follow-up of the cohort through December 2009 who had provided a urine sample at baseline for PGE-M measurement. The median interval between urine sample collection and breast cancer diagnosis was 4.1 years. Using the incidence-density sampling method, we individually matched each case with 1 or 2 controls randomly selected from cohort members who were free of any cancer at the time the index case was diagnosed. A total of 645 controls were identified and cases and controls were matched on age (≤ 2 years), menopausal status (pre- or postmenopause), date (≤ 30 days), and time (morning or afternoon) at sample collection. To increase statistical power, we also included 563 controls selected for other cancers for the current analysis. We excluded all cases ($n = 56$) and controls ($n = 126$) who ever used NSAIDs regularly or used NSAIDs within 7 days before urine sample collection. Thus, data from 1,586 women (504 cases and 1,082 controls) were finally used for the analysis.

Urinary PGE-M measurement

Measurement of urinary PGE-M concentration was performed using a liquid chromatography/tandem mass spectrometric (LC/MS-MS) method described previously (16, 17). Briefly, 0.5-mL urine was acidified to pH 3 with 1 N HCl after addition of 10 ng internal standard, tetranor prostaglandin E metabolite-d_6 (tetranor-PGEM-d_6) containing six deuterium atoms at the 13, 13′, 14, 14′, 15, and 15′ positions (Cayman Chemical Co.). Endogenous PGE-M and the internal standard were then converted to the *O*-methyloxime derivative by treatment with methyloxime HCl at 37°C for 30 minutes. The methoximated PGE-M was purified by solid phase extraction on a C-18 Sep-Pak. LC was performed on a Kinetix C18 column (2.6 μm, 100 Å, 2.1 mm × 150 mm; Phenomenex) attached to an Accela UPLC Pump (Thermo Scientific). MS detection was carried out on a TSQ Vantage triple quadrupole mass spectrometer in the multiple reaction monitoring (MRM) mode. The transition from m/z 385.2 to 336.2 was used for PGE-M, and m/z 391.2 to 342.2 was used for the

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deuterated internal standard. The lower limit of detection of PGE-M was in the range of 40 pg, approximately 100-fold below levels in normal human urine. The coefficient of variation for samples analyzed in multiple batches was 3.13% and for intra batch samples was 2.86%. We used the Jaffe method to measure urinary creatinine concentration by using a kit from Nanjing Jiancheng. Laboratory staff is blinded to the case–control status of the urine samples and to the identity of the quality control samples included in the study.

Statistical analyses

Urinary PGE-M level in each sample was standardized using urinary creatinine level of the sample and expressed in ng/mg creatinine (ng/mg Cr). Data for PGE-M ratios were skewed to the high value, and thus log-transformation was used to improve normality. Geometric means of PGE-M levels were calculated, and the ANOVA test was applied to compare log-transformed PGE-M levels among different categories/subgroups of each variable of interest for difference. The distribution of PGE-M levels among controls was used to determine cutoff points for quartiles. Unconditional logistic regression models were used to estimate the risk of breast cancer associated with urinary PGE-M levels and to derive *P* values for linear trends by modeling the log-transformed urinary PGE-M levels as a continuous variable. Interaction terms were included in the models to test for interaction between PGE-M and variables of interest (BMI and menopausal status). All analyses were conducted using SAS version 9.1 (SAS Institute). All statistical tests were based on two-sided probability.

Results

Baseline characteristics of breast cancer cases and controls are presented in Table 1. On average, cases were slightly younger (53.4 vs. 55.0), less educated, younger at menarche, and older at first live birth. More cases than controls had a family history of breast cancer and a shorter duration of breastfeeding. Only a small number of study participants ever regularly drank alcohol, smoked cigarettes, or took hormone replacement therapy (HRT).

BMI was positively associated with urinary PGE-M among both pre- and postmenopausal women, while age and education showed a positive association with urinary PGE-M only among postmenopausal women (Table 2). Among postmenopausal women, urinary levels of PGE-M were higher among smokers than among nonsmokers, although the association was not statistically significant, possibly due to a small sample size. Family history of breast cancer, regular physical activity, age at menarche, age at first live birth, number of live births, and breastfeeding were not significantly associated with PGE-M level.

Overall, no association was observed between urinary levels of PGE-M and breast cancer (Table 3). However, in analyses stratified by menopausal status and BMI (<25 kg/m^2 or $\geq$25 kg/m^2), urinary PGE-M levels were positively associated with breast cancer risk among postmenopausal women with a BMI < 25 kg/m^2 in a dose–response manner ($P_{linear\ trend} = 0.005$). No such association was found among premenopausal women or postmenopausal women with BMI $\geq$ 25 kg/m^2, and interaction tests were statistically significant between menopausal status and urinary PGE-M ($P = 0.021$) and between BMI (<25 kg/m^2 or $\geq$25 kg/m^2) and urinary PGE-M among postmenopausal women ($P = 0.012$). The above findings remain unchanged when the quartile cutoff points (quartile cutoff points for all women) are same for pre- and postmenopausal women for analyses (data not shown in the Table 3). In addition, we performed conditional analyses restricting to individually matched cases and controls for

Table 1. Baseline characteristics of breast cancer cases and controls, SWHS (1997–2000)

Characteristics	Cases ($N = 504$)	Controls ($N = 1{,}082$)	$P_{difference}$[a]
Age (y, mean ± SD)	53.4 ± 9.0	55.0 ± 9.0	0.001
Education ≥ high school, %	48.8	61.8	<0.001
Postmenopausal, %	48.6	55.7	0.008
Family history of breast cancer, %	4.8	1.6	<0.001
BMI (among postmenopausal women, %)	24.9 ± 3.6	24.8 ± 3.7	0.618
Regular physical activity, %	34.7	38.1	0.198
Ever consumed alcohol regularly, %	1.8	2.5	0.377
Ever smoked cigarette regularly, %	1.4	3.1	0.050
Age at menarche (y, mean ± SD)	14.7 ± 1.7	15.0 ± 1.7	0.019
Age at first live birth (y, mean ± SD) among parous women	26.4 ± 4.2	25.4 ± 4.3	<0.001
Number of live births (mean ± SD) among parous women	1.7 ± 1.0	2.0 ± 1.2	<0.001
Breastfeeding (months, mean ± SD) among parous women	13.3 ± 14.9	18.5 ± 19.6	<0.001
Age at menopause (y, mean ± SD) among postmenopausal women	49.1 ± 4.8	49.2 ± 3.9	0.848
Use of HRT, %	2.4	1.9	0.749

[a]*P* values derived from *t* tests for continuous variables or χ^2 tests for categorical variables.

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Table 2. Influence of selected factors on urinary PGE-M level (ng/mg Cr) among controls by menopausal status

	Postmenopausal controls ($n = 603$)			Premenopausal controls ($n = 479$)		
Variables	**No.**	**Geometric mean (SD)**	***P*[a]**	**No.**	**Geometric mean (SD)**	***P*[a]**
Age at sample collection, y[b]						
≤45	4	6.46 (1.621)		188	8.31 (1.61)	
46–50	13	9.31 (1.45)		198	8.71 (1.54)	
51–55	77	10.38 (1.47)		88	9.69 (1.60)	
56–60	122	11.20 (1.65)	<0.001	5	8.73 (1.34)	0.081
61–65	169	11.25 (1.64)				
>65	218	13.14 (1.60)				
Education[c]						
<High school	215	10.72 (1.62)		198	8.40 (1.55)	
≥High school	388	12.26 (1.61)	0.048	281	8.95 (1.60)	0.121
BMI, kg/m^2[d]						
<25	333	11.34 (1.56)		339	8.35 (1.53)	
25–29.9	215	11.85 (1.64)	0.020	124	9.37 (1.69)	0.001
≥30	55	14.03 (1.84)		16	12.63 (1.76)	
Cigarette smoking status[d]						
Nonsmokers	580	11.59 (1.60)		469	8.68 (1.58)	
Ever-smokers	23	14.41 (1.89)	0.111	10	10.63 (1.77)	0.193
Regular physical activity[d]						
No	316	11.26 (1.58)		354	8.76 (1.58)	
Yes	287	12.15 (1.64)	0.113	125	8.58 (1.58)	0.638
Family history of breast cancer[d]						
No	592	11.65 (1.61)		473	8.90 (1.58)	
Yes	11	13.79 (1.79)	0.196	6	8.28 (1.96)	0.756
Age at menarche, y[d]						
≤12	33	10.82 (1.90)		28	7.85 (1.44)	
13–15	323	11.47 (1.59)	0.904	298	8.48 (1.57)	0.086
>15	247	12.09 (1.61)		152	9.37 (1.62)	
Age at first live birth, y[d]						
<25	377	12.22 (1.62)		89	9.39 (1.51)	
26–30	166	10.83 (1.60)	0.384	294	8.60 (1.62)	0.469
>30	45	10.71 (1.70)		84	8.32 (1.55)	
Number of live births[d]						
0	15	11.39 (1.35)		12	9.60 (1.43)	
1	93	10.17 (1.62)	0.561	405	8.47 (1.59)	0.049
≥2	495	12.0 (1.62)		62	10.36 (1.47)	
Breastfeeding, mo[e]						
≤Median	290	10.80 (1.60)		253	8.67 (1.60)	
>Median	298	12.60 (1.63)	0.095	214	8.73 (1.57)	0.633

[a]*P* values derived from ANOVA.
[b]Age at sample collection was not adjusted.
[c]Education was adjusted for age at sample collection.
[d]BMI, cigarette smoking status, regular physical activity, family history of breast cancer, age at menarche, age at first live birth, number of live births, and breastfeeding were adjusted for age at sample collection and education.
[e]Breastfeeding: among postmenopausal women, median = 23 months; among premenopausal women, median = 8 months.

the breast cancer study. Similar to the results from unconditional analyses using the larger sample size (Table 3), we found a positive association between urinary PGE-M levels and breast cancer risk among postmenopausal women with a BMI < 25 kg/m^2 in a dose–response manner ($P_{\text{linear trend}} = 0.048$), but not among those with a BMI < 25 kg/m^2 (data not shown in tables).

The association of breast cancer risk with BMI and urinary PGE-M levels among postmenopausal women was further evaluated using data from women with a

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Table 3. Association between urinary level of PGE-M and risk of breast cancer, stratified by menopausal status and BMI

	Overall		BMI < 25		BMI ≥ 25		
PGE-M, ng/mg Cr	Cases/controls	OR (95% CI)[a]	Cases/controls	OR (95% CI)[a]	Cases/controls	OR (95% CI)[a]	$P_{interaction}$[b]
All women							
Q1 (<1.99)	145/270	1.00 (ref.)	98/179	1.00 (ref.)	47/91	1.00 (ref.)	
Q2 (1.99–2.28)	118/271	0.88 (0.65–1.19)	82/190	0.85 (0.59–1.23)	36/81	1.01 (0.58–1.76)	
Q3 (2.29–2.63)	123/271	0.97 (0.71–1.33)	70/165	0.89 (0.60–1.34)	53/106	1.10 (0.66–1.84)	0.943
Q4 (>2.63)	118/270	1.07 (0.77–1.48)	59/138	1.13 (0.72–1.76)	59/132	0.99 (0.60–1.64)	
$P_{linear\ trend}$		0.609		0.691		0.977	
Postmenopausal women							
Q1 (<2.13)	60/150	1.00 (ref.)	26/88	1.00 (ref.)	34/62	1.00 (ref.)	
Q2 (2.13–2.42)	60/149	1.02 (0.66–1.58)	30/87	1.06 (0.56–1.99)	30/62	0.98 (0.52–1.85)	
Q3 (2.43–2.72)	53/153	1.03 (0.66–1.16)	31/85	1.50 (0.79–2.83)	22/68	0.72 (0.37–1.40)	0.012
Q4 (>2.72)	72/151	1.39 (0.90–2.15)	38/73	2.32 (1.24–4.41)	34/78	0.88 (0.47–1.63)	
$P_{linear\ trend}$		0.147		0.005		0.516	
Premenopausal women							
Q1 (<1.85)	71/119	1.00 (ref.)	58/85	1.00 (ref.)	21/34	1.00 (ref.)	
Q2 (1.85–2.11)	76/121	1.11 (0.73–1.69)	56/94	0.87 (0.54–1.41)	20/27	2.38 (0.94–6.01)	
Q3 (2.12–2.42)	48/118	0.73 (0.46–1.16)	37/92	0.61 (0.36–1.02)	11/26	1.70 (0.60–4.87)	0.266
Q4 (>2.42)	64/121	1.00 (0.65–1.55)	33/68	0.82 (0.47–1.42)	31/53	1.63 (0.70–3.69)	
$P_{linear\ trend}$		0.589		0.198		0.622	
$P_{interaction}$[c] = 0.021							

[a]Derived from unconditional logistic regression and adjusted for age (continuous variable), education (<high school or ≥high school), cigarette smoking status (nonsmokers or ever-smokers), number of live births (0, 1, or ≥2), and months of breastfeeding (four levels: <6.0, 6.0–11.9, 12.0–24.0, and >24.0).

[b]$P_{interaction}$ between urinary PGE-M (continuous variable) and BMI (<25 kg/m^2 or ≥25 kg/m^2).

[c]$P_{interaction}$ between urinary PGE-M (continuous variable) and menopausal status (premenopausal or postmenopausal).

BMI < 25 kg/m^2 and the lowest urinary PGE-M levels (first quartile) as the reference (Fig. 1). Overweight women (BMI ≥ 25 kg/m^2) had an elevated risk of breast cancer regardless of urinary level of PGE-M, whereas among women with a BMI < 25 kg/m^2, breast cancer risk increased with increasing urinary level of PGE-M in a dose–response fashion.

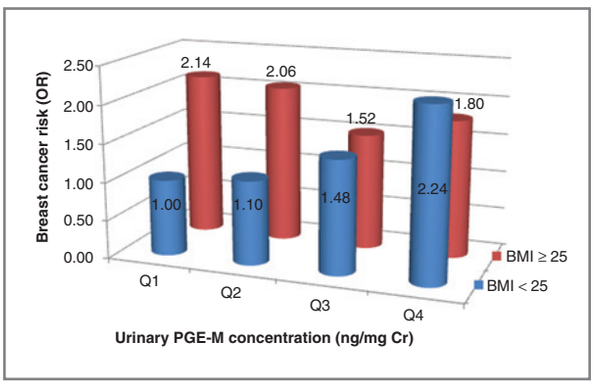

Figure 1. Associations between urinary PGE-M levels (ng/mg Cr; Q1, <2.12; Q2, 2.12–2.42; Q3, 2.43–2.72; Q4, >2.72), BMI (<25 kg/m^2 and ≥25 kg/m^2), and breast cancer risk (OR) in postmenopausal women. The regression model was adjusted for age (continuous variable), education (<high school or ≥high school), cigarette smoking status (nonsmokers or ever-smokers), number of live births (0, 1, or ≥2), and months of breastfeeding (four levels: <6.0, 6.0–11.9, 12.0–24.0, and >24.0).

Analyses were further conducted among postmenopausal women with a BMI < 25 kg/m^2, stratifying the median time interval between urine sample collection and cancer diagnosis (≤4 years or >4 years; Table 4). The association with urinary PGE-M was only seen among those who were diagnosed with breast cancer within the first 4 years after sample collection ($P_{trend} = 0.001$, with increasing quartile of PGE-M level). However, the association was attenuated among those who were diagnosed with breast cancer more than 4 years after sample collection.

We also repeated the analyses with the BMI cutoff point proposed for Asians to define overweight (BMI ≥ 23 kg/m^2) among postmenopausal women. Among those with a low BMI (<23 kg/m^2), a positive association between urinary PGE-M and breast cancer risk was observed ($P_{trend} = 0.087$), and adjusted odds ratios (OR) was 2.04 [95% confidence interval (CI), 0.87–4.83] for the highest quartile compared with the lowest quartile (data not shown in the tables). This association was more

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Table 4. Association of urinary PGE-M levels and risk of breast cancer stratified by median time interval between urine collection and cancer diagnosis among postmenopausal women with a BMI < 25

	Cancer diagnosed ≤ 4 years after sample collection		Cancer diagnosed > 4 years after sample collection	
PGE-M, ng/mg Cr	Cases/controls	OR (95% CI)[a]	Cases/controls	OR (95% CI)[a]
Q1 (<2.10)	10/88	1.00 (ref.)	16/88	1.00 (ref.)
Q2 (2.11–2.40)	15/87	1.30 (0.53–3.20)	15/87	0.79 (0.35–1.82)
Q3 (2.41–2.68)	21/85	2.55 (1.06–6.14)	10/85	1.02 (0.44–2.40)
Q4 (>2.68)	22/73	3.57 (1.51–8.43)	16/73	1.30 (0.58–2.91)
$P_{\text{linear trend}}$	<0.001		0.435	

[a]Derived from unconditional logistic regression and adjusted for age (continuous variable), education (<high school or ≥high school), cigarette smoking status (nonsmokers or ever-smokers), number of live births (0, 1, or ≥2), and months of breastfeeding (four levels: <6.0, 6.0–11.9, 12.0–24.0, and >24.0).

evident in the analysis restricting to case diagnosed within the first 4 years after sample collection [1.00 (ref.), 0.82 (0.25–2.62), 2.12 (0.73–6.23), 2.95 (0.96–9.13); $P_{\text{trend}} = 0.022$]. No apparent association was observed in the stratum with a BMI of >23 kg/m^2 (data not shown in the tables).

Discussion

In this study, we found a strong positive association between urinary PGE-M level and breast cancer among lean and normal weight (BMI < 25 kg/m^2), postmenopausal women. The association with PGE-M identified in this study is one of the strongest associations ever reported for established risk factors and biomarkers for breast cancer. Our finding is generally consistent with evidence from *in vitro* experiments and animal model studies indicating an important role of COX2 and PGE_2 in carcinogenesis (6–11).

Several lines of evidence suggest that that COX2 upregulation and, in turn, increased PGE_2 production is an early event in the development of breast cancer. In both transgenic mouse and carcinogen-dependent breast cancer models, overexpression of COX2 in mammary epithelial cells has been found to result in the development of mammary tumors (11, 25, 26). In human, COX2 is frequently overexpressed not only in invasive breast cancers, but also in adjacent ductal carcinoma *in situ* (DCIS; refs. 27–31). In a study, COX2 expression was detected in 85% of all DCIS specimens (31). Our finding for a stronger association of PGE-M with breast cancer risk among cases diagnosed within the first 4 years after sample collection than those diagnosed after 4 years of sample collection suggests that the elevated level of urinary PGE-M may be due to the overproduction of PGE_2 in breast cancer cells.

A recent case-cohort study nested within the Sister Study cohort reported a positive association between urinary PGE-M and breast cancer risk among postmenopausal women who did not regularly use NSAIDs (32). However, the potential effect of body weight on the association between urinary PGE-M and breast cancer risk was not evaluated. Our study demonstrated a modifying effect of body weight in the association of urinary PGE-M with breast cancer risk among postmenopausal women. The reasons for this modification are unclear. Studies have shown that both tumor cells and adipose tissue can produce inflammatory cytokines and PGE_2 (21). Overweight women have a chronic inflammatory condition and an increased PGE_2 production by adipose tissue, which results in a significantly higher level of urinary PGE-M than normal weight women, as demonstrated in our study and a previous study (33). In addition, multiple obesity-related pathways are also involved in and/or interact with COX2/PGE_2 signaling (20, 33–36). Future studies are needed to clarify the complicated interactions of these pathways in the etiology of breast cancer.

This study has several strengths, including a population-based, prospective cohort study design, and extremely high follow-up rates, which reduced the potential selection bias. It has been reported that NSAID use and cigarette smoking may influence urinary levels of PGE-M (17, 32, 37). In the SWHS cohort, only a small proportion of women (<5%) took aspirin regularly or smoked cigarettes. Women who used NSAIDs in the 7 days before urine sample collection and/or took aspirin regularly have been excluded, and cigarette smoking status was adjusted in this analysis. Thus, the influence of these potential confounding factors should be small. In addition, there may be a concern that the results from this study are based on a single measurement of a spot urine sample of PGE-M. However, previously we evaluated the specific interpersonal variations of urinary PGE-M levels in our laboratory and found that the intraclass correlation coefficient for urinary PGE-M was 0.67 and Spearman correlation coefficient, derived by using bootstrap analysis of single spot measurements and the average of the other three seasonal measurements, was 0.61 for urinary PGE-M (38). These results indicate that urinary level of PGE-M is stable and

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that measurement based on a single spot urine sample reflects well the PGE-M level over 1 year.

In summary, using data from the SWHS, we showed that high levels of urinary PGE-M were strongly associated with increased risk of breast cancer among lean and normal weight, postmenopausal women, but not among overweight postmenopausal women or premenopausal women. Our finding is consistent with the role of COX2 and PGE_2 in carcinogenesis and suggests that urinary PGE-M may serve as a promising biomarker to identify women likely to develop breast cancer in a relatively short period of time.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

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Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): Y. Cui, G. Yang, Y.-B. Xiang, W. Zheng
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Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): Y.-T. Gao, H.-L. Li, J. Wu, Y.-B. Xiang, W. Zheng
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