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Urinary PGE-M levels and risk of ovarian cancer

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Abstract

Background: Regular aspirin use may lower ovarian cancer risk by blocking the cyclooxygenase enzymes, resulting in lower expression of prostaglandins, including prostaglandin E2 (PGE2). We evaluated whether higher pre-diagnosis PGE-M (a urinary biomarker of PGE2) was associated with increased ovarian cancer risk in three prospective cohorts.

Methods: We conducted a case-control study nested in the Nurses' Health Study (NHS), NHSII and Shanghai Women's Health Study (SWHS). Our analyses included 304 cases of epithelial ovarian cancer diagnosed 1996–2015 and 600 matched controls. We measured urinary PGE-M

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using liquid chromatography-mass spectrometry (LC/MS) with normalization to creatinine. Measures from each study were recalibrated to a common standard. We estimated odds ratios (OR) and 95% confidence intervals (CI) using conditional logistic regression, with PGE-M levels modeled in quartiles. Multivariable models were adjusted for ovarian cancer risk factors.

Results: There was no evidence of an association between urinary PGE-M levels and ovarian cancer risk for women with PGE-M levels in the top versus bottom quartile (OR=0.80, 95%CI=0.51–1.27; p-trend=0.37). We did not observe heterogeneity by histotype (p=0.53), and there was no evidence of effect modification by BMI (p-interaction=0.82), aspirin use (p-interaction=0.59), or smoking (p-interaction=0.14).

Conclusion: Pre-diagnosis urinary PGE-M levels were not significantly associated with ovarian cancer risk. Larger sample sizes are needed to consider a more modest association, and evaluate associations for specific tumor subtypes.

Impact: Systemic prostaglandin levels do not appear strongly associated with ovarian cancer risk. Future research into aspirin use and ovarian cancer risk should consider local prostaglandins and prostaglandin-independent mechanisms.

Introduction

Chronic inflammation may contribute to the etiology of epithelial ovarian cancer. There is increasing evidence that regular use of non-steroidal anti-inflammatory drugs (NSAIDs) is associated with a modestly lower risk of ovarian cancer; however the underlying biologic mechanisms remain poorly understood (1–7). Aspirin and non-aspirin NSAIDs down-regulate the prostaglandin synthesis pathway via inhibition of the cyclooxygenase (COX) enzymes (8–11). COX1 and COX2 are overexpressed in ovarian tumor tissue relative to normal tissue (12–15), and greater COX1 and COX2 expression have been associated with poorer prognosis (15–19), suggesting that NSAIDs may influence risk through the prostaglandin pathway.

COX1 and COX2 promote the conversion of arachidonic acid into bioactive prostaglandins, the most abundant of which is prostaglandin E2 (PGE2) (20, 21). While endogenous levels of circulating PGE2 cannot be reliably measured in humans (22, 23), prior studies of prostaglandins and cancer have measured urinary 11 alpha-hydroxy,9,15-dioxo-2,3,4,5-tetranor-prostane-1,20-dioic acid (PGE-M), the primary urinary metabolite of PGE2, to approximate systemic levels of prostaglandins (22, 24). In these studies, PGE-M was associated with an increased risk of colorectal cancer (25, 26), colorectal adenoma (27), gastric cancer (28, 29), small cell lung cancer (30), pancreatic cancer (31, 32), and postmenopausal breast cancer (33, 34).

Here, we evaluated the association between pre-diagnosis urinary PGE-M levels and ovarian cancer risk in a case-control study nested in three prospective cohort studies, the Nurses' Health Study (NHS), NHSII, and the Shanghai Women's Health Study (SWHS). We hypothesized that higher PGE-M levels would be associated with increased ovarian cancer risk.

MATERIALS AND METHODS

Nurses' Health Studies

The NHS was established in 1976 with the enrollment of 121,700 female registered nurses, aged 30–55. The NHSII was established in 1989 with the enrollment of 116,429 female registered nurses, aged 25–42. NHS/NHSII participants completed a questionnaire on lifestyle factors, medication use and disease status at the time of enrollment, and provided updated lifestyle and health information biennially, by mailed questionnaire. Cases of epithelial ovarian cancer were identified on return of biennial questionnaires or via linkage to the National Death Index. An expert gynecologic pathologist confirmed cases by medical record review and, when records were not available, cases were confirmed by linkage to cancer registries. Tumor behavior and histopathology characteristics were abstracted for confirmed cases, and tumor histopathology was confirmed by slide review for all cases with available tumor blocks.

A subset of women in NHS/NHSII provided a urine sample (described in (35, 36)). In brief, 18,743 NHS participants aged 53–80 sent spot urine specimens by overnight mail (with an icepack) between 2000 and 2002 (93% first morning urine), where it was aliquoted without a preservative and stored in liquid nitrogen freezers at ≤-130°C. Similarly, 29,611 NHSII participants aged 32–54 years provided urine specimens between 1996 and 1999 (80% first morning urine). Of these, 18,521 premenopausal women provided a urine sample 7–9 days prior to the anticipated start of their next menstrual cycle (luteal phase). The other 11,090 participants provided an untimed specimen. All participants completed a biospecimen collection questionnaire asking about time of urine collection, whether it was a first morning urine, medication use, and body weight, among other characteristics. NHS/NHSII study protocols were approved by the institutional review boards of the Brigham and Women's Hospital and Harvard T.H. Chan School of Public Health, and those of participating registries as required. Research was conducted in accordance with the Belmont report. Participant return of self-administered questionnaires was accepted as informed consent.

Shanghai Women's Health Study

The SWHS conducted baseline interviews from 1996–2000, capturing data from 74,942 Chinese women, aged 40–70, living in urban communities in Shanghai. Women were approached by a trained interviewer and, after providing informed consent, completed a self-administered questionnaire and in-person interview to collect data on lifestyle factors, medication use, and disease outcomes. In-person follow-up surveys have been conducted every 2 to 6 years to obtain information on lifestyle factors and disease outcomes. Ovarian cancer cases were identified with a combination of record linkage to the Shanghai Cancer Registry or Shanghai Vital Statistics Unit and in-person follow-up surveys. Diagnoses were confirmed by medical record review and histologic type was assigned using International Classification of Diseases for Oncology codes.

Between 1997 and 2000, 65,754 SWHS women provided an untimed spot urine sample, as described previously (37). In brief, each participant answered questions related to urine collection and provided a urine sample using a sterilized 100mL cup with 125mg of ascorbic

acid. The sample was transported to the laboratory, with an ice pack, within 6 hours of collection and stored at ≤-70°C.

The Institutional Review Boards of all relevant institutions in the United States and the People's Republic of China approved the SWHS study protocol, and subjects provided written informed consent. Research was conducted in accordance with the Belmont report.

Study design

Two controls were matched to each case using incidence density sampling, within cohort, on year of birth (+/- 1 year for NHS/NHSII; +/- 2 years for SWHS), date (+/- 1 month) and time (+/- 2hr for NHS/NHSII; morning vs. afternoon for SWHS) of collection, and menopausal status (premenopausal, postmenopausal, unknown). NHS/NHSII additionally matched on menopausal status at diagnosis (premenopausal, postmenopausal, unknown), hormone therapy (HT) use at collection (yes/no), and luteal day (NHSII women only; +/- 1 day); NHS cases diagnosed before 2004 were not matched on time of day or hormone therapy. SWHS also matched on antibiotic use at the time of urine collection. Covariate data were assessed from questionnaires or interviews at or near the time of sample collection, including parity, oral contraceptive (OC) use, intrauterine device (IUD) use, tubal ligation, hysterectomy, family history of ovarian cancer, smoking, weight and height (for calculation of BMI in kg/m^2), and use of anti-inflammatory drugs.

Laboratory assays

The Eicosanoid Core Laboratory (PI: Ginger Milne) at Vanderbilt University measured PGE-M levels in the NHS/NHSII samples using a liquid chromatography-mass spectrometry (LC/MS) method that has been described previously (27, 30, 33, 38). Briefly, PGE-M in each 0.5mL urine specimen was stabilized by conversion to the *O*-methyloxime derivative and purified by C18 solid phase extraction with subsequent addition of the *O*-methyloxime derivatized deuterium-labeled internal standard (custom synthesis). Liquid chromatography (LC) was performed on an Acquity BEH C18 column (2.0 × 50 mm, 1.7μm particle, Waters Corporation, Milford, MA, USA) connected to a Waters Acquity I-Class UPLC system and delivered to a Waters Xevo TQ-S Micro triple quadrupole mass spectrometer (Waters Corporation, Milford, MA, USA). The lower limit of detection of PGE-M was 0.05 ng/mL, substantially lower than levels typically detected in human urine. Results from a pilot study of 40 NHS participants with urine collected two years apart, reported good within person stability of PGE-M (intraclass correlation=0.62).

For SWHS samples, levels of PGE-M were measured at the Shanghai Institutes for Biological Sciences (PI: Huiyong Yin) using a similar LC/MS method. Briefly, d6-PGEM internal standard (2 ng, Cayman Chemical, Ann Arbor, MI USA) was added to 0.75 ml urine and acidified to pH 3 with HCl; endogenous PGE-M was then converted to the *O*-methyloxime derivative by treatment with methoxyamine HCl. The methoximated PGE-M was extracted, applied to a C-18 Sep-Pak, and eluted with ethyl acetate. Liquid chromatography was performed on a Phenomenex Kinetex-C18 column (2.6 μm, 2.1 mm × 50.0 mm) attached to a CTC-HTS autosampler and Shimadzu LC-10 A VP system (Kyoto,

Japan). The lower limit of detection of PGE-M was 0.04 ng/mL, substantially lower than levels typically detected in human urine.

Case-control pairs were assayed in the same batch, and quality control (QC) samples were included to assess analytic error. Laboratory personnel were blinded to case, control, and QC status. The intra-assay coefficients of variation (CVs) were <4% and the inter-assay CVs were <9% in our NHS/NHSII samples and among prior PGE-M measures of SWHS samples at the Yin laboratory (33). Urinary creatinine was measured using a test kit from Enzo Life Sciences, Inc. (Farmingdale, NY USA) to standardize levels of PGE-M to account for variation in urine concentrations (ng PGE-M/mg creatinine). To account for differences between laboratories, we recalibrated all participant PGE-M measures to a common standard by re-assaying a batch of 30 samples (10 samples from each cohort) at the Milne laboratory (39).

Statistical analysis

We log-transformed ng PGE-M/mg creatinine and identified statistical outliers using the generalized extreme studentized deviate many-outlier procedure (40). One outlier with high PGE-M levels was detected in the SWHS dataset and excluded. We estimated odds ratios (OR) and 95% confidence intervals (CI) using conditional logistic regression. To avoid linearity assumptions we evaluated PGE-M in quartiles with cutpoints determined using the distribution of PGE-M values among controls, and we tested for trends using study-specific quartile medians. We first meta-analyzed the results from NHS/NHSII and SWHS and tested for heterogeneity between studies. In the absence of heterogeneity ($p>0.20$ for all estimates), we pooled data from all three studies and used overall quartile medians in tests for trend in pooled analyses. Models were adjusted for matching factors (except antibiotic use) via conditional logistic regression of matched pairs, and multivariable models were further adjusted for parity (nulliparous, 1, 2, 3, >3), OC use (never, <1 year, 1–5 years, ≥5 years), IUD use (ever, never), tubal ligation (yes, no), hysterectomy (yes, no), family history of ovarian cancer (yes, no), smoking (current, past, never), and BMI (continuous). Pooled analyses included an interaction term between cohort and IUD use, as prior work suggests different associations for this exposure between the cohorts (41, 42).

We considered different associations for Type II (high-grade serous, poorly differentiated) vs. Type I (low-grade serous, endometrioid, clear cell, mucinous) ovarian cancers. Serous ovarian carcinomas with unknown grade were classified as Type II ovarian cancers, while cases without information on tumor histology were excluded from histotype analyses. To calculate the associations, we used polytomous logistic regression, adjusted for matching factors, and to test for heterogeneity we used a likelihood ratio test comparing the polytomous model to a model with constant associations for Type II and Type I ovarian cancers. We evaluated multiplicative effect modification by BMI, aspirin use and smoking using unconditional logistic regression adjusted for matching factors and covariates by including cross-product terms between each level of these categorical variables and PGE-M in our models and conducting a likelihood ratio test. Per the World Health Organization, the definition of overweight differs by race/ethnicity, so SWHS women (Chinese) were classified as overweight if BMI ≥24 kg/m^2, and NHS/NHSII (primarily non-Hispanic white)

were classified as overweight if BMI ≥25 kg/m^2 (43). Aspirin use was defined as aspirin, non-aspirin NSAID or acetaminophen use in the last 72 hours (NHS), aspirin use in the last 72 hours (NHSII), aspirin or non-aspirin NSAID use in the last 7 days (SWHS), or regular use of aspirin (NHS/NHSII: ≥2 times per week; SWHS: ≥3 times per week) over the past two years. Smoking status was dichotomized as ever versus never, given the low number of current smokers.

We used SAS 9.4 by SAS Institute Inc., Cary, NC, USA for all analyses. Statistical tests assumed 2-sided p-values with α=0.05.

RESULTS

We included 304 (123 NHS, 71 NHSII, 110 SWHS) cases of epithelial ovarian cancer and 600 matched controls with urinary PGE-M measures. The control populations differed across cohorts with respect to PGE-M levels, age, menopausal status, hysterectomy, OC use, IUD use, tubal ligation, and regular use of aspirin-based medications (Table 1). Smoking prevalence was low in all three studies. Overall, parity and OC use were less common among ovarian cancer cases compared to controls, while family history of ovarian cancer was more common. Among controls, regular aspirin use was associated with lower urinary PGE-M, while smoking was associated with higher urinary PGE-M levels (Supplemental Table 1).

There was no evidence of an association across PGE-M quartiles and risk of ovarian cancer in study-specific multivariable models using study-specific quartile cutpoints (top versus bottom quartile, NHS OR=0.59, 95%CI=0.30–1.15, p-trend=0.10; NHSII OR=0.93, 95%CI=0.31–2.83, p-trend=0.93; SWHS OR=1.18, 95% CI=0.51–2.73, p-trend=0.48; Supplemental Table 2), or when considering common quartile cutpoints (top versus bottom quartile, NHS OR=0.62, 95%CI=0.32–1.20, p-trend=0.12; NHSII OR=0.76, 95%CI=0.21–2.72, p-trend=0.97; SWHS OR=1.10, 95% CI=0.42–2.88, p-trend=0.71; Table 2). No significant heterogeneity was observed (p>0.40 for all estimates) so the cohorts were pooled (top versus bottom quartile, OR=0.80, 95% CI=0.51–1.27; p-trend=0.37, Table 2). In the combined study population, there was no heterogeneity by tumor histology (p=0.53; Table 3).

The maximum time from urine collection to ovarian cancer diagnosis was 17.8 years with a median time of 5.7 years. As a sensitivity analysis, we removed cases diagnosed within one year of urine collection from our analyses (Supplemental Table 3). Results were similar to the main analysis (top versus bottom quartile OR=0.86, 95%CI=0.54–1.39, p-trend=0.65). We also stratified by median time from collection to case diagnosis (Supplemental Table 3) and observed no statistically significant evidence of an association between urinary PGE-M and ovarian cancer for those with a case diagnosis in the 5.7 years immediately following biospecimen collection (top versus bottom quartile OR=1.10, 95%CI=0.56–2.15, p-trend=0.92), or after the first 5.7 years (top versus bottom quartile OR=0.64, 95%CI=0.32–1.28, p-trend=0.25).

We hypothesized a priori that the association between pre-diagnosis urinary PGE-M and ovarian cancer risk would differ by levels of inflammatory exposures, including aspirin use and BMI. Results did not differ by aspirin use (p-interaction=0.59; Table 4). For example, among women who reported recent or regular use of aspirin, the OR comparing the top versus bottom quartile was 0.80 (95%CI=0.36–1.77; p-trend=0.46) and, among women who did not report aspirin use, the OR was 0.89 (95%CI=0.50–1.61; p-trend=0.77). Results also did not differ when stratifying by BMI (p-interaction=0.82). Among normal weight women, the OR comparing the top versus bottom quartile was 0.65 (95%CI=0.34–1.24; p-trend=0.29) and, among overweight or obese women, the OR was 1.02 (95%CI=0.52–1.99; p-trend=0.85). Results stratified by smoking status were not statistically different (p=0.14) but trended in opposite directions. Among never smokers, the OR comparing the top versus bottom quartile was 1.13 (95%CI=0.65–1.96; p-trend=0.55) and, among ever smokers, the OR was 0.46 (95%CI=0.20–1.08; p-trend=0.06).

A prior SWHS study of urinary PGE-M and breast cancer that was conducted among women with a very low smoking prevalence observed a dose-response association among post-menopausal women with BMI <25kg/m^2 (33), so we also conducted a post-hoc analysis restricted to normal weight women who had never smoked (Supplemental Table 4). There was evidence of a positive association between PGE-M and ovarian cancer risk when comparing the top and bottom PGE-M quartiles in an unadjusted model (OR=2.40; 95%CI=0.92–6.29; p-trend=0.04), but this finding was attenuated by adjustment for ovarian cancer risk factors (OR=1.60; 95%CI=0.50–5.14; p-trend=0.29).

Given that aspirin and non-aspirin NSAIDs can affect PGE-M levels we decided to further consider the influence of aspirin use on the association between PGE-M levels and risk of ovarian cancer, and evaluate the potential for effect modification by PGE-M levels in the association between aspirin and risk of ovarian cancer. First, we conducted an additional post-hoc sensitivity analysis restricting our study population to those who had not reported aspirin (SWHS/NHS/NHSII), non-aspirin NSAID (SWHS/NHS) or acetaminophen (NHS) use in the past 72 hours (NHS/NHSII) or past week (SWHS). Results from this analysis were very similar to the main analysis. For example, the OR comparing the top versus bottom quartile was 0.84 (95%CI=0.48–1.46, p-trend=0.96, Supplemental Table 5), while the comparable OR from the main analysis was 0.80 (95%CI=0.51–1.27, p-trend=0.37, Table 2). Second, we conducted an exploratory analysis among women in NHS/NHSII with both PGE-M and aspirin data to consider if PGE-M levels may alter the association between aspirin use and ovarian cancer risk (Supplemental Table 6). Considering standard dose (325mg) aspirin, among those with low PGE-M levels, we observed a 1.44-fold (95%CI=0.62–3.38) increased odds of ovarian cancer among current regular users of standard dose aspirin compared to never regular users, and among those with high PGE-M levels the comparable odds ratio was 0.86 (95%CI=0.25–2.91). For low dose (≤100mg) aspirin, among those with low PGE-M levels, we observed lower odds of ovarian cancer among current regular users of low dose aspirin compared to never regular users (OR=0.73; 95%CI=0.36–1.47), and among those with high PGE-M levels the comparable odds ratio was 1.14 (95%CI=0.51–2.58). There was no statistically significant evidence of heterogeneity by PGE-M level for standard dose (p-heterogeneity=0.36) or low-dose (p-heterogeneity=0.89) aspirin, but these findings merit further exploration in a larger study.

DISCUSSION

In this large, prospective nested case-control study including primarily non-Hispanic white and Chinese women, we observed that pre-diagnosis urinary PGE-M levels were not significantly associated with risk of ovarian cancer. Further, we did not observe any significant interactions by recent aspirin use, BMI, or smoking, which are key inflammatory factors that may interact with the prostaglandin synthesis pathway (23, 30, 44–48). Our post-hoc analysis of aspirin use and risk of ovarian cancer by PGE-M level reported no statistically significant evidence of heterogeneity by PGE-M level; however, our observation of a possible lower risk of ovarian cancer among current low-dose aspirin users with low PGE-M levels but not high PGE-M levels merits further evaluation in a larger study.

Research on ovarian cancer biology supports a role for prostaglandins in ovarian carcinogenesis. One *in vitro* study reported that the COX2 inhibitor NS-398 reduced PGE2 in ovarian cancer cells (19), and research by the same group and others observed PGE2 in the ascites of ovarian cancer patients (19), COX2 expression in ovarian tumors (12–15, 19, 49, 50), and poorer survival among patients with COX2+ ovarian cancer (15–19). In our recent work, we observed COX1 expression, COX2 expression, or both in many ovarian cancer cases (15, 51); however, when we evaluated the associations between aspirin or non-aspirin NSAID use and ovarian cancer risk by tumor expression of these markers, there was no evidence of heterogeneity (51). Our results are consistent with a recent evaluation of fatty acid metabolites in the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial, which reported no significant associations between COX-derived prostanoids and risk of ovarian cancer (52). It is possible that prostanoid-dependent inflammatory mechanisms are important to ovarian cancer growth and metastasis, but prostanoid-independent inflammatory mechanisms are more relevant to ovarian cancer development.

Epidemiologic research has reported positive associations between PGE-M levels and risk of colorectal cancer (25, 26), colorectal adenoma (27), gastric cancer (28, 29), small cell lung cancer (30), and pancreatic cancer (31). These associations varied substantially in magnitude, from a 5.6-fold (95%CI=2.4–13.5) higher risk of colorectal cancer for women comparing PGE-M levels in the highest versus lowest quartile to a 1.63-fold (95%CI=1.01–2.63) increased risk of pancreatic cancer comparing the highest versus lowest tertile. Our results were more similar to studies of postmenopausal breast cancer, which did not report a positive association overall, but did report associations among specific, albeit small (n<150), subgroups (e.g., low BMI (33), non-regular users of NSAIDs (34)). Consistent with existing literature, we observed a positive association between urinary PGE-M and inflammatory factors, including smoking (38, 53) and, to a lesser extent, increasing BMI (34). We also observed a possible positive association between urinary PGE-M and ovarian cancer among normal-weight women who had never smoked. This analysis was motivated by the NHS study of PGE-M, aspirin and adenoma risk, in which anti-inflammatory drug use was associated with lower adenoma risk among women with mid-high levels of PGE-M but not low levels of PGE-M (27). Multiple obesity pathways are related to prostaglandin synthesis or signaling (44–46) and any modest effects of prostaglandins on ovarian cancer risk may be eclipsed by more extensive dysregulation of inflammation among those with high BMI, so this finding warrants further exploration in future studies.

Urinary PGE-M may reflect both inflammatory and anti-inflammatory factors, including the use of aspirin and non-aspirin NSAIDs. Use of NSAIDs, including aspirin (23, 47, 48), ibuprofen (30), and indomethacin (48), have been associated with lower PGE-M levels. Our three study populations reported very different patterns of aspirin use. Prevalence of aspirin use was nearly 50% among NHS participants, approximately 15% among NHSII participants, and fewer than 5% among SWHS participants. Despite these differences in usage patterns, we observed similar associations between PGE-M levels and ovarian cancer in NHS/NHSII and SWHS overall and when stratified by aspirin use.

This study had several strengths, including pre-diagnosis urine collection and the ability to account for differences between laboratories via recalibration of all participant PGE-M measures to a common standard. For example, we minimized the potential for reverse causation by collecting the urine biospecimen prior to ovarian cancer diagnosis, and we were able to conduct a sensitivity analysis excluding cases (and matched controls) with diagnosis dates within one year of specimen collection. Another strength was detailed covariate information that allowed us to control for important confounders and evaluate effect modification by inflammatory exposures. Further, the inclusion of study populations with different racial/ethnic backgrounds and different aspirin usage patterns increases generalizability of the observed associations.

We also recognize several important limitations of our study. Biomarker validity is one potential concern. While urinary PGE-M reflects systemic PGE2 levels (22, 24), it is unclear if urinary PGE-M levels are reflective of PGE2 exposure in the peritoneal cavity. However, other systemic markers (e.g., C-reactive protein, androgens) have been associated with ovarian cancer risk (35, 54, 55). Another important limitation is that we only obtained one PGE-M measure, although our prior study demonstrated a reasonable intraclass correlation over 2 years and we did not observe an association in cases diagnosed within 5.7 years of urine collection.

The decision to pool NHS/NHSII/SWHS led to additional strengths and limitations for this analysis. Importantly, pooling improved power, though our sample size remained limited with respect to detecting modest associations, examining associations with specific histotypes, and detecting effect modification. Additionally, SWHS, NHS and NHSII had slightly different urine collection protocols (e.g., all SWHS samples were spot urine and nearly all NHS/NHSII were first morning), thus we were unable to account for differences in these protocols in the analysis. While the NHS/NHSII were able to match on more factors that could explain variability in PGE-M than SWHS (e.g., timing of collection within the menstrual cycle), it is important to note that the SWHS previously detected associations between urinary PGE-M and other cancer types (25, 28, 29, 32, 33).

In summary, we observed no evidence of an association between pre-diagnosis urinary PGE-M levels and risk of ovarian cancer, despite the modest inverse association of aspirin and non-aspirin NSAIDs with risk of ovarian cancer in these and other populations (1–7). Overall, and particularly when considered in conjunction with our finding that associations between NSAID use and ovarian cancer risk do not differ by tumor expression of COX1 or COX2 (51), the results of this study suggest that regulation of the prostaglandin synthesis

pathway may not be the most important link between NSAID use and ovarian carcinogenesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations list:

PGE2	prostaglandin E2
NHS	Nurses' Health Study
SWHS	Shanghai Women's Health Study
LC/MS	liquid chromatography-mass spectrometry
OR	odds ratio
CI	confidence interval
NSAIDs	non-steroidal anti-inflammatory drugs
COX	cyclooxygenase
HT	hormone therapy
IUD	intrauterine device
OC	oral contraceptive
CVs	coefficients of variation

REFERENCES

1. Baandrup L, Faber MT, Christensen J, Jensen A, Andersen KK, Friis S, et al. Nonsteroidal anti-inflammatory drugs and risk of ovarian cancer: systematic review and meta-analysis of observational studies. Acta Obstet Gynecol Scand. 2013 3;92(3):245–55. [PubMed: 23240575]
2. Baandrup L, Kjaer SK, Olsen JH, Dehlendorff C, Friis S. Low-dose aspirin use and the risk of ovarian cancer in Denmark. Ann Oncol. 2015 4;26(4):787–92. [PubMed: 25538177]

3. Peres LC, Camacho F, Abbott SE, Alberg AJ, Bandera EV, Barnholtz-Sloan J, et al. Analgesic medication use and risk of epithelial ovarian cancer in African American women. Br J Cancer. 2016 3 29;114(7):819–25. [PubMed: 26908324]
4. Trabert B, Ness RB, Lo-Ciganic WH, Murphy MA, Goode EL, Poole EM, et al. Aspirin, nonaspirin nonsteroidal anti-inflammatory drug, and acetaminophen use and risk of invasive epithelial ovarian cancer: a pooled analysis in the Ovarian Cancer Association Consortium. J Natl Cancer Inst. 2014 2;106(2):djt431. [PubMed: 24503200]
5. Zhang D, Bai B, Xi Y, Wang T, Zhao Y. Is aspirin use associated with a decreased risk of ovarian cancer? A systematic review and meta-analysis of observational studies with dose-response analysis. Gynecol Oncol. 2016 8;142(2):368–77. [PubMed: 27151430]
6. Trabert B, Poole EM, White E, Visvanathan K, Adami H, Anderson GL, et al. Aspirin, Nonsteroidal Anti-inflammatory Drug, and Acetaminophen Use and Risk of Ovarian Cancer: An Analysis in the Ovarian Cancer Cohort Consortium (OC3). Submitted. 2018.
7. Barnard ME, Poole EM, Curhan GC, Eliassen AH, Rosner BA, Terry KL, et al. Association of Analgesic Use With Risk of Ovarian Cancer in the Nurses' Health Studies. JAMA Oncol. 2018 12 1;4(12):1675–82. [PubMed: 30286239]
8. Ulrich CM, Bigler J, Potter JD. Non-steroidal anti-inflammatory drugs for cancer prevention: promise, perils and pharmacogenetics. Nat Rev Cancer. 2006 2;6(2):130–40. [PubMed: 16491072]
9. Umar A, Steele VE, Menter DG, Hawk ET. Mechanisms of nonsteroidal anti-inflammatory drugs in cancer prevention. Semin Oncol. 2016 2;43(1):65–77. [PubMed: 26970125]
10. Vane JR, Botting RM. Mechanism of action of nonsteroidal anti-inflammatory drugs. Am J Med. 1998 3 30;104(3A):2S–8S; discussion 21S-2S.
11. Vane JR, Botting RM. The mechanism of action of aspirin. Thromb Res. 2003 6 15;110(5–6):255–8. [PubMed: 14592543]
12. Freedman RS, Wang E, Voiculescu S, Patenia R, Bassett RL Jr., Deavers M, et al. Comparative analysis of peritoneum and tumor eicosanoids and pathways in advanced ovarian cancer. Clin Cancer Res. 2007 10 1;13(19):5736–44. [PubMed: 17908963]
13. Kino Y, Kojima F, Kiguchi K, Igarashi R, Ishizuka B, Kawai S. Prostaglandin E2 production in ovarian cancer cell lines is regulated by cyclooxygenase-1, not cyclooxygenase-2. Prostaglandins Leukot Essent Fatty Acids. 2005 8;73(2):103–11. [PubMed: 15963707]
14. Rask K, Zhu Y, Wang W, Hedin L, Sundfeldt K. Ovarian epithelial cancer: a role for PGE2-synthesis and signalling in malignant transformation and progression. Mol Cancer. 2006;5:62. [PubMed: 17107625]
15. Beeghly-Fadiel A, Wilson AJ, Keene S, El Ramahi M, Xu S, Marnett LJ, et al. Differential cyclooxygenase expression levels and survival associations in type I and type II ovarian tumors. J Ovarian Res. 2018 2 27;11(1):17. [PubMed: 29482584]
16. Ali-Fehmi R, Che M, Khalifeh I, Malone JM, Morris R, Lawrence WD, et al. The effect of cyclooxygenase-2 expression on tumor vascularity in advanced stage ovarian serous carcinoma. Cancer. 2003 10 1;98(7):1423–9. [PubMed: 14508829]
17. Ali-Fehmi R, Morris RT, Bandyopadhyay S, Che M, Schimp V, Malone JM Jr., et al. Expression of cyclooxygenase-2 in advanced stage ovarian serous carcinoma: correlation with tumor cell proliferation, apoptosis, angiogenesis, and survival. Am J Obstet Gynecol. 2005 3;192(3):819–25. [PubMed: 15746677]
18. Ferrandina G, Lauriola L, Zannoni GF, Fagotti A, Fanfani F, Legge F, et al. Increased cyclooxygenase-2 (COX-2) expression is associated with chemotherapy resistance and outcome in ovarian cancer patients. Ann Oncol. 2002 8;13(8):1205–11. [PubMed: 12181243]
19. Denkert C, Kobel M, Pest S, Koch I, Berger S, Schwabe M, et al. Expression of cyclooxygenase 2 is an independent prognostic factor in human ovarian carcinoma. Am J Pathol. 2002 3;160(3):893–903. [PubMed: 11891188]
20. Kortz L, Dorow J, Ceglarek U. Liquid chromatography-tandem mass spectrometry for the analysis of eicosanoids and related lipids in human biological matrices: a review. J Chromatogr B Analyt Technol Biomed Life Sci. 2014 8 01;964:1–11.
21. Ricciotti E, FitzGerald GA. Prostaglandins and inflammation. Arterioscler Thromb Vasc Biol. 2011 5;31(5):986–1000. [PubMed: 21508345]

22. Catella F, Nowak J, Fitzgerald GA. Measurement of renal and non-renal eicosanoid synthesis. Am J Med. 1986;81(Suppl 2B):23–9.
23. Neale JR, Dean BJ. Liquid chromatography-tandem mass spectrometric quantification of the dehydration product of tetranor PGE-M, the major urinary metabolite of prostaglandin E(2) in human urine. J Chromatogr B Analyt Technol Biomed Life Sci. 2008 8 1;871(1):72–7.
24. Frolich JC, Wilson TW, Sweetman BJ, Smigel M, Nies AS, Carr K, et al. Urinary prostaglandins. Identification and origin. J Clin Invest. 1975 4;55(4):763–70. [PubMed: 1120781]
25. Cai Q, Gao YT, Chow WH, Shu XO, Yang G, Ji BT, et al. Prospective study of urinary prostaglandin E2 metabolite and colorectal cancer risk. J Clin Oncol. 2006 11 01;24(31):5010–6. [PubMed: 17075120]
26. Johnson JC, Schmidt CR, Shrubsole MJ, Billheimer DD, Joshi PR, Morrow JD, et al. Urine PGE-M: A metabolite of prostaglandin E2 as a potential biomarker of advanced colorectal neoplasia. Clin Gastroenterol Hepatol. 2006 11;4(11):1358–65. [PubMed: 16996805]
27. Bezawada N, Song M, Wu K, Mehta RS, Milne GL, Ogino S, et al. Urinary PGE-M levels are associated with risk of colorectal adenomas and chemopreventive response to anti-inflammatory drugs. Cancer Prev Res (Phila). 2014 7;7(7):758–65. [PubMed: 24824037]
28. Dong LM, Shu XO, Gao YT, Milne G, Ji BT, Yang G, et al. Urinary prostaglandin E2 metabolite and gastric cancer risk in the Shanghai women's health study. Cancer Epidemiol Biomarkers Prev. 2009 11;18(11):3075–8. [PubMed: 19861525]
29. Wang T, Cai H, Zheng W, Michel A, Pawlita M, Milne G, et al. A Prospective Study of Urinary Prostaglandin E2 Metabolite, Helicobacter pylori Antibodies, and Gastric Cancer Risk. Clin Infect Dis. 2017 5 15;64(10):1380–6. [PubMed: 28402440]
30. Murphey LJ, Williams MK, Sanchez SC, Byrne LM, Csiki I, Oates JA, et al. Quantification of the major urinary metabolite of PGE2 by a liquid chromatographic/mass spectrometric assay: determination of cyclooxygenase-specific PGE2 synthesis in healthy humans and those with lung cancer. Anal Biochem. 2004 11 15;334(2):266–75. [PubMed: 15494133]
31. Zhao J, Wang J, Du J, Xu H, Zhang W, Ni QX, et al. Urinary prostaglandin E2 metabolite and pancreatic cancer risk: case-control study in urban Shanghai. PLoS One. 2015;10(2):e0118004. [PubMed: 25679523]
32. Cui Y, Shu XO, Li HL, Yang G, Wen W, Gao YT, et al. Prospective study of urinary prostaglandin E2 metabolite and pancreatic cancer risk. Int J Cancer. 2017 12 15;141(12):2423–9. [PubMed: 28815606]
33. Cui Y, Shu XO, Gao YT, Cai Q, Ji BT, Li HL, et al. Urinary prostaglandin E2 metabolite and breast cancer risk. Cancer Epidemiol Biomarkers Prev. 2014 12;23(12):2866–73. [PubMed: 25214156]
34. Kim S, Taylor JA, Milne GL, Sandler DP. Association between urinary prostaglandin E2 metabolite and breast cancer risk: a prospective, case-cohort study of postmenopausal women. Cancer Prev Res (Phila). 2013 6;6(6):511–8. [PubMed: 23636050]
35. Poole EM, Lee IM, Ridker PM, Buring JE, Hankinson SE, Tworoger SS. A prospective study of circulating C-reactive protein, interleukin-6, and tumor necrosis factor alpha receptor 2 levels and risk of ovarian cancer. Am J Epidemiol. 2013 10 15;178(8):1256–64. [PubMed: 23966559]
36. Schernhammer ES, Hankinson SE. Urinary melatonin levels and breast cancer risk. J Natl Cancer Inst. 2005 7 20;97(14):1084–7. [PubMed: 16030307]
37. Zheng W, Chow WH, Yang G, Jin F, Rothman N, Blair A, et al. The Shanghai Women's Health Study: rationale, study design, and baseline characteristics. Am J Epidemiol. 2005 12 1;162(11): 1123–31. [PubMed: 16236996]
38. Duffield-Lillico AJ, Boyle JO, Zhou XK, Ghosh A, Butala GS, Subbaramaiah K, et al. Levels of prostaglandin E metabolite and leukotriene E(4) are increased in the urine of smokers: evidence that celecoxib shunts arachidonic acid into the 5-lipoxygenase pathway. Cancer Prev Res (Phila). 2009 4;2(4):322–9. [PubMed: 19336727]
39. Eliassen AH, Hendrickson SJ, Brinton LA, Buring JE, Campos H, Dai Q, et al. Circulating carotenoids and risk of breast cancer: pooled analysis of eight prospective studies. J Natl Cancer Inst. 2012 12 19;104(24):1905–16. [PubMed: 23221879]
40. Rosner B Percentage points for a generalized ESD many-outlier procedure. Technometrics. 1983;25(2):165–72.

41. Huang Z, Gao Y, Wen W, Li H, Zheng W, Shu XO, et al. Contraceptive methods and ovarian cancer risk among Chinese women: A report from the Shanghai Women's Health Study. Int J Cancer. 2015 8 1;137(3):607–14. [PubMed: 25556333]
42. Tworoger SS, Fairfield KM, Colditz GA, Rosner BA, Hankinson SE. Association of oral contraceptive use, other contraceptive methods, and infertility with ovarian cancer risk. Am J Epidemiol. 2007 10 15;166(8):894–901. [PubMed: 17656616]
43. Expert Consultation WHO. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. Lancet. 2004 1 10;363(9403):157–63. [PubMed: 14726171]
44. Amor S, Iglesias-de la Cruz MC, Ferrero E, Garcia-Villar O, Barrios V, Fernandez N, et al. Peritumoral adipose tissue as a source of inflammatory and angiogenic factors in colorectal cancer. Int J Colorectal Dis. 2016 2;31(2):365–75. [PubMed: 26493186]
45. Jin M, Long ZW, Yang J, Lin X. Correlations of IGF-1R and COX-2 Expressions with Ras and BRAF Genetic Mutations, Clinicopathological Features and Prognosis of Colorectal Cancer Patients. Pathol Oncol Res. 2018 1;24(1):45–57. [PubMed: 28188432]
46. Subbaramaiah K, Morris PG, Zhou XK, Morrow M, Du B, Giri D, et al. Increased levels of COX-2 and prostaglandin E2 contribute to elevated aromatase expression in inflamed breast tissue of obese women. Cancer Discov. 2012 4;2(4):356–65. [PubMed: 22576212]
47. Navarro SL, Kantor ED, Song X, Milne GL, Lampe JW, Kratz M, et al. Factors Associated with Multiple Biomarkers of Systemic Inflammation. Cancer Epidemiol Biomarkers Prev. 2016 3;25(3): 521–31. [PubMed: 26908433]
48. Seyberth HW, Sweetman BJ, Frolich JC, Oates JA. Quantifications of the major urinary metabolite of the E prostaglandins by mass spectrometry: evaluation of the method's application to clinical studies. Prostaglandins. 1976 2;11(2):381–97. [PubMed: 772759]
49. Klimp AH, Hollema H, Kempinga C, van der Zee AG, de Vries EG, Daemen T. Expression of cyclooxygenase-2 and inducible nitric oxide synthase in human ovarian tumors and tumor-associated macrophages. Cancer Res. 2001 10 1;61(19):7305–9. [PubMed: 11585770]
50. Matsumoto Y, Ishiko O, Deguchi M, Nakagawa E, Ogita S. Cyclooxygenase-2 expression in normal ovaries and epithelial ovarian neoplasms. Int J Mol Med. 2001 7;8(1):31–6. [PubMed: 11408945]
51. Barnard ME, Hecht JL, Rice MS, Gupta M, Harris HR, Eliassen AH, et al. Anti-Inflammatory Drug Use and Ovarian Cancer Risk by COX1/COX2 Expression and Infiltration of Tumor-Associated Macrophages. Cancer Epidemiol Biomarkers Prev. 2018 12;27(12):1509–17. [PubMed: 30377203]
52. Hada M, Edin ML, Hartge P, Lih FB, Wentzensen N, Zeldin DC, et al. Pre-diagnostic serum levels of fatty acid metabolites and risk of ovarian cancer in the Prostate, Lung, Colorectal, and Ovarian Cancer (PLCO) Screening Trial. Cancer Epidemiol Biomarkers Prev. 2018 9 27.
53. Gross ND, Boyle JO, Morrow JD, Williams MK, Moskowitz CS, Subbaramaiah K, et al. Levels of prostaglandin E metabolite, the major urinary metabolite of prostaglandin E2, are increased in smokers. Clin Cancer Res. 2005 8 15;11(16):6087–93. [PubMed: 16115954]
54. Zeng F, Wei H, Yeoh E, Zhang Z, Ren ZF, Colditz GA, et al. Inflammatory Markers of CRP, IL6, TNFalpha, and Soluble TNFR2 and the Risk of Ovarian Cancer: A Meta-analysis of Prospective Studies. Cancer Epidemiol Biomarkers Prev. 2016 8;25(8):1231–9. [PubMed: 27277846]
55. Ose J, Poole EM, Schock H, Lehtinen M, Arslan AA, Zeleniuch-Jacquotte A, et al. Androgens Are Differentially Associated with Ovarian Cancer Subtypes in the Ovarian Cancer Cohort Consortium. Cancer Res. 2017 7 15;77(14):3951–60. [PubMed: 28381542]

Table 1.

Age-standardized characteristics of ovarian cancer cases and controls at the time of urine collection (NHS 2000–2002, NHSII 1996–1999, SWHS 1997–2000)

	NHS		NHSII		SWHS	
	Control (n=247)	Case (n=123)	Control (n=141)	Case (n=71)	Control (n=212)	Case (n=110)
PGEM ng/ creatinine mg, mean (SD)	6.1(3.5)	5.9(3.8)	4.8(3.3)	5.0(3.8)	7.4(4.3)	7.6(3.9)
Age at specimen collection, mean (SD) *†	68.0(6.5)	67.9(6.4)	44.8(4.5)	44.6(4.5)	53.1(8.1)	53.0(8.0)
Years from collection to diagnosis, mean (SD) *	--	5.9(3.9)	--	7.6(5.0)	--	5.5(2.9)
BMI, mean (SD)	25.7(4.0)	25.6(4.5)	26.2(6.2)	27.7(7.2)	24.0(3.6)	24.6(3.7)
Menopausal status and hormone therapy (HT)‡, %						
- Premenopausal, %	1.2	0.8	80.5	82.4	47.0	44.3
- Postmenopausal/no HT, %	37.7	31.8	5.8	5.4	45.6	45.7
- Postmenopausal/HT use, %	59.9	67.1	5.8	5.5	6.5	9.1
- Unknown, %	1.2	0.3	8.0	6.7	0.9	1.0
Parity, %						
- Nulliparous, %	3.2	4.1	20.7	23.7	2.7	4.6
- 1 child, %	6.1	5.7	8.5	16.9	12.3	15.1
- 2 children, %	27.9	32.9	44.0	45.5	29.4	22.5
- 3 children, %	25.1	27.3	19.0	8.3	26.0	27.0
- 4+ children, %	37.7	30.1	7.8	5.6	29.5	30.8
Ever IUD use, %	5.7	4.2	3.5	4.2	57.7	53.5
Oral contraceptive (OC) use, %						
- Never, %	53.5	47.3	13.4	18.4	76.6	78.6
- <1 year, %	12.1	15.6	9.2	12.5	6.1	10.3
- 1–5 years, %	19.4	21.5	40.4	43.5	7.0	6.7
- 5+ years, %	15.0	15.6	37.0	25.6	10.3	4.4
Tubal ligation, %	22.2	18.4	29.2	11.3	11.9	17.0
Hysterectomy, %	28.4	35.9	12.3	15.1	0.5	0.0
Family history of ovarian cancer§, %	2.8	5.7	1.4	4.3	0.0	0.0
Smoking status, %						
- Never, %	47.8	50.7	70.2	63.6	97.6	94.8
- Past, %	47.4	45.1	22.7	26.6	0.5	0.9
- Current, %	4.8	4.1	7.1	9.8	1.9	4.2
Regular aspirin use, %	44.6	52.2	13.6	18.2	1.8	4.4

Values are means (SD) or percentages and are standardized to the age distribution of the study population.

Values of polytomous variables may not sum to 100% due to rounding.

*Value is not age adjusted.

†Matching factor in NHS, NHSII, SWHS.

[‡] Menopausal status was a matching factor for all studies; HT use was a matching factor in NHS (2005–2015) and NHSII only.

[§] No SWHS participants in this nested case-control study reported a family history of ovarian cancer.

Table 2.

Urinary PGE-M levels and risk of invasive epithelial ovarian cancer in NHS, NHSII and SWHS

	PGE-M (ng/mg creatinine) quartiles				
	Q1* (low)	Q2	Q3	Q4 (high)	p-trend**
NHS					
Cases/controls	35/61	33/60	34/70	21/56	
†Model 1 OR (95% CI)	(ref)	0.97 (0.52–1.81)	0.84 (0.46–1.54)	0.66 (0.34–1.28)	0.19
‡Model 2 OR (95% CI)	(ref)	0.98 (0.51–1.89)	0.79 (0.41–1.50)	0.62 (0.32–1.20)	0.12
NHSII					
Cases/controls	28/62	22/37	15/25	6/17	
†Model 1 OR (95% CI)	(ref)	1.28 (0.66–2.47)	1.29 (0.61–2.74)	0.81 (0.29–2.25)	0.98
‡Model 2 OR (95% CI)	(ref)	1.08 (0.42–2.74)	1.43 (0.54–3.80)	0.76 (0.21–2.72)	0.97
SWHS					
Cases/controls	12/26	23/52	33/52	42/74	
†Model 1 OR (95% CI)	(ref)	0.96 (0.43–2.18)	1.40 (0.60–3.22)	1.27 (0.53–3.03)	0.45
‡Model 2 OR (95% CI)	(ref)	0.90 (0.37–2.17)	1.46 (0.59–3.63)	1.10 (0.42–2.88)	0.71
Pooled					
Cases/controls	75/149	78/149	82/147	69/147	
†Model 1 OR (95% CI)	(ref)	1.01 (0.69–1.49)	1.06 (0.71–1.58)	0.89 (0.58–1.37)	0.63
‡Model 2 OR (95% CI)	(ref)	0.95 (0.63–1.43)	0.99 (0.65–1.50)	0.80 (0.51–1.27)	0.37

*Quartile (Q) cutpoints are 3.78, 5.34 and 7.53 ng PGE-M/mg creatinine.

**Tests for trend use quartile medians.

†Conditional logistic regression.

‡Adjusted for parity, OC use, IUD use, tubal ligation, hysterectomy, family history of ovarian cancer, smoking, and BMI. Hysterectomy and family history of ovarian cancer were not adjusted for in the analysis of SWHS only, since prevalence of these factors was very low.

Table 3.

Urinary PGE-M levels and risk of ovarian cancer in NHS, NHSII and SWHS, by tumor histotype [*]

	PGE-M (ng/mg creatinine) quartiles					
	Q1[**] (low)	Q2	Q3	Q4 (high)	p-trend[†]	p-het
Controls	150	150	150	150		
Type II[‡]						
Cases	51	45	49	34		
[§]Model 1 OR (95% CI)	(ref)	0.93 (0.58–1.52)	0.98 (0.61–1.58)	0.73 (0.43–1.26)	0.31	
[¶]Model 2 OR (95% CI)	(ref)	0.86 (0.52–1.41)	0.86 (0.52–1.40)	0.65 (0.37–1.13)	0.15	
Type I[‡]						
Cases	19	27	23	19		
[§]Model 1 OR (95% CI)	(ref)	1.66 (0.85–3.24)	1.51 (0.76–3.02)	1.24 (0.59–2.59)	0.74	
[¶]Model 2 OR (95% CI)	(ref)	1.56 (0.79–3.08)	1.38 (0.68–2.82)	1.13 (0.53–2.42)	0.93	
						0.53

[*] Includes cases with histology data only.

[**] Quartile (Q) cutpoints are 3.78, 5.34 and 7.53 ng PGE-M/mg creatinine.

[†] Tests for trend use quartile medians.

[‡] Type II = high grade serous and serous of unknown grade; Type I = low grade serous, endometrioid, clear cell and mucinous.

[§] Unconditional polytomous logistic regression adjusting for matching factors.

[¶] Model 1, further adjusted for parity, OC use, IUD use, tubal ligation, hysterectomy, smoking, and BMI.

Table 4.

Association of urinary PGE-M levels and risk of invasive epithelial ovarian cancer in NHS, NHSII and SWHS stratified by inflammatory exposures

	PGE-M (ng/mg creatinine) quartile					
	Q1* (low)	Q2	Q3	Q4 (high)	p-trend**	p-int
No recent or regular aspirin use						
Cases/controls	38/88	48/102	55/101	50/113		
†Multivariate-adjusted OR (95% CI)	(ref)	0.98 (0.56–1.70)	1.14 (0.66–1.98)	0.89 (0.50–1.61)	0.77	
Recent or regular aspirin use						
Cases/controls	37/62	30/48	27/49	19/37		
†Multivariate-adjusted OR (95% CI)	(ref)	0.94 (0.48–1.86)	0.77 (0.38–1.54)	0.80 (0.36–1.77)	0.46	
						0.59
Normal weight‡						
Cases/controls	41/85	38/86	45/66	30/77		
†Multivariate-adjusted OR (95% CI)	(ref)	0.95 (0.53–1.70)	1.28 (0.70–2.34)	0.65 (0.34–1.24)	0.29	
Overweight‡						
Cases/controls	34/65	40/64	37/84	39/73		
†Multivariate-adjusted OR (95% CI)	(ref)	1.06 (0.56–1.99)	0.83 (0.45–1.53)	1.02 (0.52–1.99)	0.85	
						0.82
Never smoker						
Cases/controls	46/102	52/110	60/106	53/106		
†Multivariate-adjusted OR (95% CI)	(ref)	1.02 (0.61–1.71)	1.22 (0.73–2.04)	1.13 (0.65–1.96)	0.55	
Ever smoker						
Cases/controls	29/48	26/40	22/44	16/44		
†Multivariate-adjusted OR (95% CI)	(ref)	1.02 (0.48–2.13)	0.73 (0.34–1.58)	0.46 (0.20–1.08)	0.06	
						0.14

*Quartile (Q) cutpoints are 3.78, 5.34 and 7.53 ng PGE-M/mg creatinine

**Tests for trend use quartile medians.

†Unconditional logistic regression model adjusted for matching factors, parity, OC use, IUD use, tubal ligation, hysterectomy, family history of ovarian cancer, smoking (when stratifying by BMI and aspirin use), and BMI (when stratifying by aspirin use and smoking).

‡Per the World Health Organization, definition of overweight differs by race/ethnicity, so SWHS women (Chinese) are classified as overweight for BMI ≥24 kg/m^2, and NHS/NHSII (primarily non-Hispanic white) are classified as overweight if BMI ≥25 kg/m^2.