Nonsteroidal Anti-inflammatory Drug Use and Serum Total Estradiol in Postmenopausal Women

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Abstract

Laboratory and epidemiologic evidence suggest that nonsteroidal anti-inflammatory drug (NSAID) use may be inversely related to the risk of breast cancer; however, the mechanism by which NSAIDs may protect against the development of this disease is uncertain. The objective of this observational study was to assess the relationship between current NSAID use and endogenous estradiol levels, an established breast cancer risk factor. To evaluate this aim, we conducted a cross-sectional investigation among 260 postmenopausal women who were not recently exposed to exogenous hormones. Information on current NSAID use (aspirin, cyclooxygenase-2 inhibitors, and other NSAIDs combined) was collected using a questionnaire at the time of blood draw. Estradiol was quantified in serum by radioimmunoassay. General linear models were used to evaluate the association between NSAID use and serum total estradiol. The age-adjusted and body mass index–adjusted geometric mean serum estradiol concentration among NSAID users (n = 124) was significantly lower than nonusers of NSAIDs (n = 136; 17.8 versus 21.3 pmol/L; P = 0.03). Further adjustment for additional potential confounding factors did not substantially alter estimates (17.7 versus 21.2 pmol/L; P = 0.03). To our knowledge, this report is the first to examine the relationship between NSAID use and serum estradiol in postmenopausal women. These cross-sectional findings suggest that NSAID use may be associated with lower circulating estradiol levels, potentially representing one mechanism through which NSAIDs exert protective effects on breast cancer. (Cancer Epidemiol Biomarkers Prev 2008;17(3):680–7)

Introduction

Although breast cancer is a major public health problem, little is known about preventing this disease. Experimental studies have reported a protective effect of nonsteroidal anti-inflammatory drugs (NSAIDs), such as aspirin and ibuprofen, against mammary carcinogenesis (1-3), and accumulating evidence from both case-control and cohort studies suggests that use of NSAIDs may be associated with a modest decreased risk of breast cancer in women (4-10). However, findings are mixed (11-17). Clarifying the association between NSAID use and the development of breast cancer is potentially of great importance clinically. NSAIDs are widely used, readily available, and inexpensive agents. If they were shown to be chemopreventive, they could have a substantial effect on public health.

Although the mechanisms by which NSAIDs may protect against breast cancer are not fully understood, data suggest that the protective effect may be attributed in part to the ability of NSAIDs to decrease the formation of prostaglandin E_2 (PGE_2) by blocking cyclooxygenase (COX)-1 and/or COX-2 activity. One possible mechanism by which the COX/PGE_2 cascade promotes breast cancer is via increasing estrogen production, as exposure to endogenous estrogens has been shown to play a causal role in the development of some breast cancers (18).

PGE_2 up-regulates aromatase activity (19), the enzyme that converts androgens to estrogens, leading to increased estrogen synthesis. In postmenopausal women, aromatatic conversion of androgens is the primary source of circulating estrogens, and suppression of this enzyme has been shown to have a profound effect on both circulating estrogen levels (20) and breast cancer recurrence (21). Recently, dose-dependent decreases in aromatase activity were observed in breast cancer cells following treatment with NSAIDs, a COX-1 selective inhibitor, and COX-2 selective inhibitors (22). Therefore, NSAIDs may offer protection against breast cancer by reducing a woman's exposure to estrogen via the inhibition of aromatase activity. Indeed, laboratory results have shown that estradiol production is decreased in breast cells that are exposed to the selective COX-2 inhibitor celecoxib (23).

Although the above-mentioned pathway through which NSAIDs may decrease the development of breast cancer has been highlighted previously (24, 25), the association between NSAID use and circulating estradiol

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in women is currently unknown. Therefore, in this cross-sectional investigation, we asked whether differences in serum estradiol levels could be observed between self-reported NSAID users and nonusers in a population of postmenopausal women not taking menopausal hormone therapy.

Materials and Methods

Study Population. We used data from controls drawn from the Mammograms and Masses Study (MAMS), a case-control study of estrogen metabolites, mammographic breast density, and breast cancer risk. Details of the study methodologies have been presented elsewhere (26). In brief, 869 cancer-free women and 264 recently diagnosed breast cancer cases were recruited into the MAMS through the Magee-Womens Hospital Mammographic Screening and Diagnostic Imaging Program in the greater Pittsburgh area between September 2001 and May 2005. Women who were ages ≥18 years, who reported no previous personal history of cancer, with the exception of nonmelanoma skin cancer, and who could provide written informed consent were eligible for study enrollment. Participants in the MAMS include (*a*) breast cancer cases who were recruited from the Magee-Womens Surgical Clinic for an initial evaluation after newly diagnosed primary breast cancer (n = 264), (*b*) controls who were undergoing outpatient needle breast biopsy through the Breast Biopsy Service at Magee-Womens Hospital but who were not subsequently diagnosed with breast cancer (n = 313), (*c*) ''healthy'' controls who received screening mammography through Magee-Womens Hospital or through Pittsburgh Magee Womancare Centers (n = 538), and (*d*) an additional 18 participants whose blood was dedicated solely to an ancillary study of intraindividual cytokine and hormone level reproducibility. To increase recruitment of the ''healthy'' control group, study flyers were attached to screened negative mammogram reports mailed to patients between November 2003 and April 2005. The MAMS is approved by the University of Pittsburgh's Institutional Review Board and all participants provided written informed consent at the time of study entry.

Subsample Selection. Participants were selected for the present study if they met the following eligibility criteria: (*a*) healthy controls recruited only via study flyers through Magee-Womens Hospital or through Pittsburgh Magee Womancare Centers (n = 453), because these participants completed a self-administered questionnaire on the day of blood draw; (*b*) postmenopausal, defined as having no menstrual bleeding during the year before enrollment, having undergone a bilateral oophorectomy, or having a hysterectomy without bilateral oophorectomy and ages ≥50 years. We measured follicle-stimulating hormone for women ages <55 years at blood draw who had a hysterectomy without bilateral oophorectomy (n = 5); all five participants had follicle-stimulating hormone levels above 40 mIU/mL (range, 49.1-185.2), consistent with follicle-stimulating hormone elevation in the postmenopausal range (27); (*c*) did not use hormone therapy within 3 months of enrollment; and (*d*) did not report using vaginal estrogen creams, oral contraceptives, selective estrogen receptor modulators, or corticosteroids on the day of blood draw. Ninety-eight premenopausal women, 55 postmenopausal women using hormone therapy, 24 women using selective estrogen receptor modulators, 5 participants on corticosteroids, and 1 participant later found to have a personal history of breast cancer were excluded from the study. Two hundred and seventy participants met the above-mentioned criteria.

Covariate Information. A standardized, self-administered questionnaire was used to gather exposure information. Participants in the subsample completed the questionnaire at study enrollment on the day of blood draw. Information collected included demographic data, current use of medication and supplements, reproductive history, family medical history, past exogenous hormone use, and lifestyle factors, such as smoking status and alcohol intake. Alcohol use (g/d) in the past year was calculated as reported previously (28). Age at onset of menopause was defined according to the methods formerly described by the Women's Health Initiative (29), where age at menopause corresponded to the age of a woman's last natural menstrual bleeding, bilateral oophorectomy, or age a woman began using hormone therapy. For a hysterectomized woman without a bilateral oophorectomy, age at menopause was the earliest age at which she began using hormone therapy or first had menopausal symptoms. If neither occurred and her age at hysterectomy was ≥50 years, then age at menopause was her age at hysterectomy. Age at menopause was undeterminable in seven participants. Years since menopause were calculated by subtracting the age at menopause from the age at enrollment.

Assessment of NSAID Use. The primary exposure variable ''current NSAID use'' was collected on the day of blood draw. On the self-administered questionnaire, participants were asked to report all prescribed and over-the-counter medications that were currently being used. The question asked, ''Are you CURRENTLY taking any medications (prescription or over-the-counter, including aspirin and ibuprofen)?'' If a participant responded affirmatively, she was prompted to ''please list them in this table.'' Dosage data were collected but not analyzed as many participants knew only the number of tablets taken rather than the actual dose. The questionnaire was reviewed for completeness by a trained research nurse (study coordinator), who queried participants if further clarification was needed. Each medication reported in the table was subsequently assigned a code using a therapeutic classification system as indexed in the Nurse Practitioners' Prescribing Reference, which is updated quarterly (30). Participants who listed aspirin, COX-2 inhibitor, or other non-aspirin NSAID use on the questionnaire were considered ''current NSAID users.'' Participants who did not list using a NSAID were considered ''current NSAID nonusers.'' Because acetaminophen is generally reported to be a poor inhibitor of the COX-1/COX-2 enzymes (31) and its mechanism of action has yet to be resolved, we classified acetaminophen users as nonusers of NSAIDs (n = 12) unless they also reported taking a NSAID (n = 6).

Two additional NSAID exposure variables were considered in relation to estradiol levels, a secondary exposure variable and a NSAID variable constructed from the primary and secondary variables. The secondary

NSAID exposure variable was from the participant's yes-or-no response to the study phlebotomist's question at blood draw, ''Have you taken any aspirin or anti-inflammatory agents in the last 48 h?'' No effort was undertaken to determine the specific agent the participant had used. Therefore, this variable is more subjective in that responses were based solely on each individual's perception of what constitutes an anti-inflammatory agent and aspirin. The secondary exposure variable was used in conjunction with the primary NSAID exposure variable to construct a third variable labeled ''consistent NSAID use.'' ''Consistent NSAID users'' listed on the questionnaire that they were currently taking a medication that was an aspirin, COX-2 inhibitor, or non-aspirin NSAID and also verbally reported that they took an aspirin or other anti-inflammatory agent in the past 48 h. ''Consistent NSAID nonusers'' did not list using any NSAID nor did they state having taken an aspirin or anti-inflammatory agent in the past 48 h. This latter variable was created as an attempt to reduce potential NSAID use/nonuse misclassification. None of the participants in this analysis were missing any of the NSAID exposure variable data. Exposure data were collected and coded without knowledge of estradiol levels.

Clinical Measures. The study coordinator obtained physical measurements (height and weight) and recorded information on a standardized form. After the participant removed her shoes and heavy clothing, weight was measured at a standing position to the nearest 0.1 kg using a standard balance beam; standing height was measured at full inspiration to the nearest 0.1 cm. All anthropometric measurements were taken twice and were repeated if the first two measurements differed by more than 0.5 cm or 0.5 kg. The mean of the measurements was used in the analysis. Body mass index (BMI) was calculated as weight (kg) divided by the square of height (m^2).

Forty milliliters of peripheral nonfasting blood were collected from the participants at study enrollment. All samples were processed on site at the Magee-Womens Hospital Satellite General Clinical Research Center according to standard protocols. After processing, the samples were aliquoted into 1 mL cryovials in which RBC, serum, plasma, and buffy coat were separated. Samples were stored at or below -70°C before laboratory analyses.

Laboratory Analyses. Serum samples were used for the quantification of total estradiol (sex hormone binding globulin and albumin-bound plus unbound estradiol) and were assayed at the Royal Marsden Hospital in England. Estradiol concentrations were measured by RIA after ether extraction, using a highly specific rabbit antiserum raised against an estradiol-6-carboxymethyloxime-bovine serum albumin conjugate (EIR) and Third-Generation Estradiol [I125] reagent DSL 39120 (Diagnostic Systems Laboratories; ref. 32). The assay detection limit was 3 pmol/L by calculation from the 95% confidence limits of the zero standard. A random subset of 27 replicate quality-control samples was included to assess reproducibility; the calculated coefficient of variation between duplicates for estradiol was 14.5%. Laboratory personnel were masked to both subject identification and quality-control status.

Statistical Analyses. Wilcoxon's rank-sum test was used to compare selected continuous characteristics between current users and nonusers of NSAIDs and the χ^2 test or the Fisher's exact test was used to assess differences in categorical variables. The Kruskal-Wallis test was used to test for significant differences in continuous characteristics across estradiol tertile categories. A log transformation was applied to serum estradiol concentrations to obtain homoscedacity and an approximately normal distribution for linear model residuals. One participant was excluded from analyses because total estradiol levels were deemed unreliable by the laboratory. An additional nine participants with outlying estradiol values, defined as >2 SD from the mean of estradiol concentration (>150 pmol/L; range, 150-847 pmol/L), were removed from analyses because such high levels likely indicated that the women were not postmenopausal or did not correctly report current hormone use. Thus, the final analytic subsample included 260 women.

Cohen's κ statistic was calculated as a measure of agreement between the primary and secondary NSAID exposure variables. Differences in mean log estradiol levels between users and nonusers of NSAIDs were tested by the Student's t test. The general linear model approach was performed to calculate multivariable-adjusted estradiol levels and to assess differences in levels between NSAID users and nonusers. Adjusted means and confidence intervals for each NSAID category were quantified using the least-squares mean option of PROC GLM. Two adjusted models are presented. The first model was adjusted for age and BMI, which were deemed necessary covariates given their previously reported associations with both NSAID use (33) and estradiol levels (34, 35). The second model was further adjusted for variables found to be associated with NSAID use or estradiol levels within the study population (univariate association $P < 0.15$). The final multivariable model was adjusted for age (continuous), BMI (continuous), years since menopause (continuous), race (White versus non-White), and regular alcohol intake in the past year (none, <12 g/d, ≥12 g/d, entered as an indicator variable). Additional adjustment for family history of breast cancer, past hormone therapy use, smoking status, sex hormone binding globulin, and various reproductive factors yielded similar results and are not presented. The geometric mean estradiol concentrations were calculated by taking the anti-log of the least-squares means after adjustment. For each model, a plot of the studentized residuals versus the predicted values was examined to check whether the equality of variance assumption was met. A normal probability plot of the residuals was examined to assess normality. Assumptions of normality and homogeneity of variance were met for all models presented. Tests of statistical significance were two tailed, and given the exploratory nature of this work, we reported our results at the $P < 0.05$ significance level rather than correct for multiple comparisons. All analyses were done using SAS software version 9.1 (SAS Institute).

Results

Characteristics of the study population are shown in Table 1. The majority of participants (66.9%) were

Table 1. Distribution of selected characteristics by NSAID use among 260 postmenopausal women in the MAMS

Characteristic	Current NSAID use		*P*
	User (n = 124)	Nonuser (n = 136)	
Age at blood draw (y), mean (SD)	62.6 (8.1)	62.9 (8.7)	0.91
BMI (kg/m^2), mean (SD)	28.6 (6.0)	28.3 (6.1)	0.63
Years menopausal, mean (SD)*	14.1 (9.8)	14.2 (10.3)	0.94
Race, %			
White	96.8	89.7	0.03
Non-White	3.2	10.3	
Regular alcohol intake in past year, %			
None	66.1	77.9	0.10
<12 g/d	21.8	14.0	
≥12 g/d	12.1	8.1	

*Missing n = 7 for years menopausal.

overweight or obese (BMI ≥ 25 kg/m^2) and White (93.1%). Overall, 124 (47.7%) participants reported current NSAID use at the time of blood draw (Table 2). In this study, 25.0%, 12.3%, and 2.3% participants reported using only aspirin, non-aspirin NSAIDs, and COX-2 selective inhibitors, respectively, whereas 8.1% reported using at least two different types of NSAIDs (data not shown). One hundred forty (53.8%) women reported that they took aspirin or another anti-inflammatory agent within 48 h of blood draw. One hundred (38.5%) participants listed current use of a NSAID on the baseline questionnaire and verbally reported aspirin or anti-inflammatory use within 48 h of blood draw, and 96 (36.9%) reported no use of NSAIDs in both settings. The agreement between the primary and secondary exposure variables was moderate with a κ value of 0.51.

With the exception of race, current NSAID users and nonusers were statistically similar with regard to all other demographic characteristics (Table 1). Current users of NSAIDs were more likely to be White than nonusers (96.8% versus 89.7%; P = 0.03). Demographic differences between users and nonusers for all NSAID exposure variables (primary, secondary, and constructed) were similar, with the exception of BMI. Participants who reported aspirin or anti-inflammatory drug use within the past 48 h and those who were consistent users not greater BMIs than participants who reported no use (data not shown).

The geometric mean serum estradiol concentration for the study population was 19.5 pmol/L, with levels ranging from 3.3 to 140.0 pmol/L. As illustrated in Table 3, higher serum estradiol levels were associated with increasing BMI (P < 0.0001) and negatively associated with alcohol intake (P = 0.003). Although not statistically significant, it was observed that women with higher circulating estradiol levels were on average fewer years from menopause (P = 0.11). With the exception of alcohol intake, all associations persisted after controlling for BMI (data not shown). The association between alcohol intake and estradiol diminished after controlling for BMI.

After adjustment for age and BMI, current NSAID use was significantly inversely associated with serum estradiol concentrations (17.8 versus 21.3 pmol/L; P = 0.03; Table 4), with ~16.4% lower levels in users than nonusers of NSAIDs. The age- and BMI-adjusted association between use of the secondary NSAID exposure variable (aspirin or anti-inflammatory agent in the past 48 h) and estradiol was suggestive of an inverse effect, but this finding was not statistically significant (18.5 versus 20.9 pmol/L; P = 0.14). A slightly stronger association between NSAID use and estradiol levels was observed when comparing consistent users with consistent nonusers (17.5 versus 21.5 pmol/L; P = 0.03). Further adjustment for race, alcohol intake, and years menopausal only slightly increased the strength of association observed in the age- and BMI-adjusted analyses.

Figure 1 presents the adjusted geometric mean serum estradiol concentration by subcategory of NSAID use as defined by the cross-tabulation of the primary and secondary NSAID exposure variables. Three categories were defined, the two concordant groups (that is, ''No NSAIDs on medication list/No NSAIDs verbally'' and ''Yes NSAIDs on medication list/Yes NSAIDs verbally'') remained as separate exposure categories, whereas the two discordant groups (that is, ''No NSAIDs on medication list/Yes NSAIDs verbally'' and ''Yes NSAIDs on medication list/No NSAIDs verbally'') were collapsed into a single category. The three groups had significantly

Table 2. Self-reported NSAID use in the MAMS

NSAID use	n (%)
Primary exposure variable	
Current use*	
Nonuser	136 (52.3)
User	124 (47.7)
Secondary exposure variable	
Past 48 h use†	
Nonuser	120 (46.2)
User	140 (53.8)
Constructed exposure variable	
Consistent use‡	
Nonuser	96 (36.9)
User	100 (38.5)

*Current use: Based on participants' self-reported current medication list.
† Past 48 h use: Based on participants' verbal response to the question: ''Have you taken an aspirin or other anti-inflammatory drug in the past 48 h?''
‡ Consistent use: The agreement between current use and past 48 h use. Nonuser = Participant's current medication list did not indicate use of a NSAID and the participant verbally responded that she did not consume an aspirin or anti-inflammatory agent within 48 h of blood draw. User = Participant's current medication list indicated use of a NSAID and the participant verbally responded that she consumed an aspirin or anti-inflammatory agent within 48 h of blood draw.

Table 3. Distribution of selected characteristics by tertile of serum estradiol concentration among 260 postmenopausal women in the MAMS

Characteristic	Estradiol concentrations			*P*
	Tertile 1 (n = 91)	Tertile 2 (n = 81)	Tertile 3 (n = 88)	
Age at blood draw (y), mean (SD)	63.8 (8.5)	62.6 (8.5)	61.9 (8.2)	0.38
BMI (kg/m^2), mean (SD)	25.4 (4.4)	27.3 (4.9)	32.6 (6.2)	<0.0001
Years menopausal, mean (SD)*	15.6 (9.7)	13.5 (10.2)	13.1 (10.1)	0.11
Race, %				
White	95.6	92.6	90.9	0.46
Non-White	4.4	7.4	9.1	
Regular alcohol intake in past year, %				
None	60.4	72.8	84.1	0.003
<12 g/d	28.6	13.6	10.2	
≥12 g/d	11.0	13.6	5.7	

*Missing n = 7 for years menopausal.

different adjusted mean estradiol levels (P_{trend} = 0.02). Mean estradiol was lowest for participants who reported NSAID use for both measures and highest for participants who did not report use for either measure.

To assess the possible effects of acetaminophen use on the findings, all analyses were repeated, excluding acetaminophen users from the NSAID nonuser groups. Results did not differ substantially (data not shown). We also assessed unadjusted and adjusted relationships between all NSAID exposure variables and log-transformed sex hormone binding globulin, but no statistically significant relationships were observed (data not shown).

Discussion

In this cross-sectional investigation, we observed lower circulating estradiol levels among postmenopausal women reporting NSAID use. Specifically, we observed ~16% lower estradiol levels among current users than nonusers. Decreased estradiol levels were consistent regardless of how NSAID use was assessed (that is, self-reported current NSAID use on questionnaire, verbal reporting of use in past 48 h, and the agreement between these two variables). Further, the strength of association was slightly stronger when comparing participants who reported NSAID use at both the time of blood draw and within 48 h of blood draw to those who reported no use of NSAIDs for both measures. Associations were independent of age, BMI, and other potential confounding variables. As elevated serum estradiol levels have been linked to breast cancer risk, these results provide support to the growing body of evidence linking NSAID use to decreased breast cancer incidence.

Although findings in the literature are not completely consistent, results of several epidemiologic studies

Table 4. Unadjusted and adjusted geometric mean (95% CI) estradiol concentrations according to categories of NSAID use

NSAID use	Serum estradiol concentrations (pmol/L)					
	Model 1*	*P*	Model 2†	*P*	Model 3‡	*P*
Primary exposure variable						
Current use		0.11		0.03		0.03
Nonuser (n = 136)	21.0 (18.4-24.0)		21.3 (19.0-23.7)		21.2 (18.9-23.7)	
User (n = 124)	18.0 (15.7-20.7)		17.8 (15.9-20.0)		17.7 (15.7-19.9)	
Secondary exposure variable						
Past 48 h use		0.94		0.14		0.07
Nonuser (n = 120)	19.5 (16.9-22.4)		20.9 (18.5-23.5)		21.1 (18.7-23.8)	
User (n = 140)	19.6 (17.2-22.3)		18.5 (16.5-20.6)		18.1 (16.2-20.3)	
Constructed exposure variable						
Consistent use		0.39		0.03		0.02
Nonuser (n = 96)	20.3 (17.3-23.8)		21.5 (18.9-24.4)		21.4 (18.8-24.4)	
User (n = 100)	18.4 (15.8-21.5)		17.5 (15.4-19.8)		17.2 (15.1-19.6)	

Current use: Based on participant's self-reported current medication list. Past 48 h use: Based on participant's verbal response to the question ''Have you taken an aspirin or other anti-inflammatory drug in the past 48 h?'' Consistent use: The agreement between current NSAID use and past 48 h use. Consistent Nonuser = Participant's current medication list did not indicate use of a NSAID and the participant verbally responded that she did not consume an aspirin or anti-inflammatory agent within 48 h of blood draw. Consistent User = Participant's current medication list indicated use of a NSAID and the participant verbally responded that she consumed an aspirin or anti-inflammatory agent within 48 h of blood draw.

*Unadjusted model.

† Adjusted for age at blood draw (continuous) and BMI (continuous).

‡ Missing n = 7; adjusted for age at blood draw (continuous), BMI (continuous), race (White, non-White), years menopausal (continuous), and current alcohol intake (none, <12 g, ≥12 g, indicator variable).

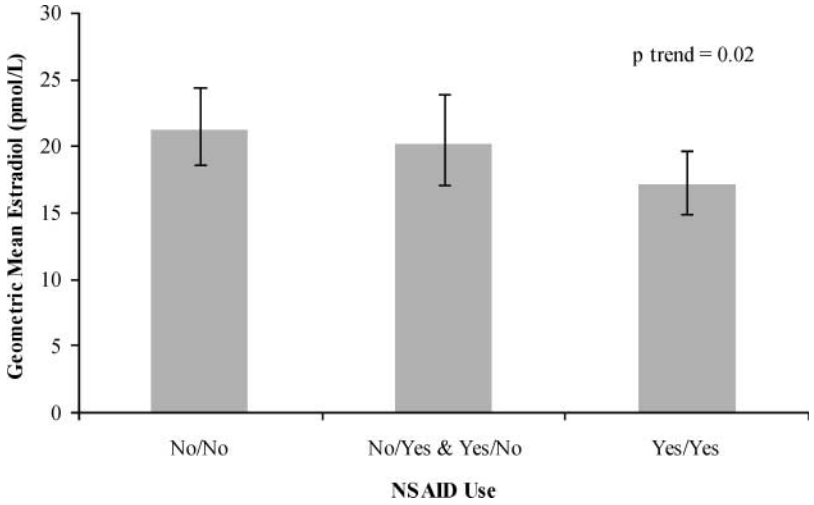

Figure 1. Adjusted geometric mean (95% CI) of estradiol according to self-reported NSAID use. Serum total estradiol was adjusted for age at blood draw, BMI, race, years menopausal, and current alcohol intake in a general linear model (n = 7 missing data). No/No = Participant's current medication list did not indicate use of a NSAID and the participant verbally responded that she did not consume an aspirin or anti-inflammatory agent within 48 h of blood draw. No/Yes = Participant's current medication list did not indicate use of a NSAID but participant verbally responded that she consumed an aspirin or anti-inflammatory agent within 48 h of blood draw. Yes/No = Participant's current medication list indicated use of a NSAID but the participant verbally responded that she did not consume an aspirin or anti-inflammatory agent within 48 h of blood draw. Yes/Yes = Participant's current medication list indicated use of a NSAID and the participant verbally responded that she consumed an aspirin or anti-inflammatory agent within 48 h of blood draw.

suggest that use of NSAIDs may reduce the risk of breast cancer (reviewed in ref. 36). The inconsistent findings across studies may be explained in part by differences in the definition of NSAID use, dosage and frequency data, and NSAID assessment periods. Notably, some studies suggest the decreased risk is stronger among estrogen receptor–positive breast cancers (37, 38) and, if true, would strengthen the hypothesis of an estrogen modulatory effect by NSAIDs. However, this relationship is not consistently observed (10, 39).

The reduced risk of breast cancer observed among NSAID users in epidemiologic studies may in part be mediated through the favorable effects of NSAIDs on PGE_2 production. Decreased PGE_2 synthesis may result in suppressed estradiol production in postmenopausal women and subsequently reduced breast cancer risk. In accordance with this biological paradigm, we observed that postmenopausal participants reporting NSAID use had lower estradiol levels. Therefore, this study adds credence to the epidemiologic data illustrating a protective effect between NSAID use and breast cancer incidence. As NSAID use is modifiable, a chemoprotective action attributed to its use could have a considerable public health effect. However, the risk-to-benefit ratio would need to be considered because NSAIDs have potentially serious side effects (40, 41).

The present study has limitations that deserve attention and that should be considered when evaluating the study findings. First, as this is a cross-sectional investigation, we cannot ascertain the temporal relationship between NSAID use and serum estradiol and causal conclusions cannot be made. Multiple measurements of NSAID use and serum estradiol may have resulted in more precise estimates. Additional limitations of this study include our inability to assess duration of NSAID use or dosage information, as duration of NSAID use was not collected and dosage data were deemed unreliable. Women exposed to a longer duration of NSAID use or larger doses may have more pronounced effects on circulating estradiol levels than occasional NSAID users (that is, as-needed) or those consuming smaller doses (that is, low-dose aspirin).

The sample size was not large enough to assess the effects of the different NSAIDs (e.g., aspirin and selective COX-2 inhibitors) on estradiol levels. Further, we cannot rule out exposure misclassification. The result of nondifferential misclassification of our exposure variable (NSAID use versus NSAID nonuse) would most likely bias the findings toward the null hypothesis and possibly underestimate the true association between NSAID use and serum estradiol. We attempted to reduce misclassification by repeating analyses limiting the sample to women who consistently reported NSAID use or nonuse. Further, although we attempted to control for potential confounders in the statistical analyses, we cannot rule out the possibility that women who are users of NSAIDs had a factor in common that we did not measure that is related to lower serum estradiol levels. Contrary to our findings, previous studies have observed positive associations between alcohol intake and postmenopausal endogenous estradiol levels; however, our results are consistent with others observing no association between alcohol and estradiol levels after adjustment for BMI (42). Finally, the lack of ethnic diversity and exclusion of premenopausal women in our sample limits the generalizability of the results.

Strengths of our study include the use of standardized instruments, reproducible measures of total estradiol, and the assessment of NSAID use on the same day as blood draw. The last strength is important, because the effect of NSAIDs on the inhibition of COX enzymes and PGE_2 synthesis occurs rapidly (43). Finally, the observed distribution of postmenopausal total estradiol levels and the self-reported prevalence of NSAID use in this population were similar to previous reports (35, 44).

In summary, we believe this to be one of the first reports on the association between NSAID use and postmenopausal estradiol levels. We found NSAID users to have significantly lower serum estradiol than nonusers which may account for the protective effect NSAID use has been observed to exhibit on breast cancer development. However, continued research efforts are needed to verify our findings.

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