Research Article

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Analgesic Use and Sex Steroid Hormone Concentrations in Postmenopausal Women

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Abstract

Prior epidemiologic studies suggest that regular use of analgesics may decrease risk of breast and ovarian cancer. We explored possible hormone-mediated mechanisms for these associations by examining the relationship between use of aspirin, nonaspirin nonsteroidal anti-inflammatory drugs (NSAID), and acetaminophen and sex steroid hormone concentrations among 740 postmenopausal women in the Nurses' Health Study. All women reported their analgesic use in 1988 or 1990 and provided a blood sample in 1989 to 1990. We calculated adjusted geometric mean estrogen and androgen levels for each category of analgesic use and calculated the *P* value for trend with increasing frequency of use. There was no association between days of use per month of aspirin, nonaspirin NSAIDs, or acetaminophen in 1990 and hormone levels (all $P_{trend} \geq 0.09$). However, we observed significant inverse trends between the estimated number of aspirin tablets per month in 1988 and concentrations of estrone ($P_{trend} = 0.04$) and estrone sulfate ($P_{trend} = 0.03$). In analyses of total (aspirin and nonaspirin) NSAID use in 1990, women who used NSAIDs at least 15 days per month had significantly lower levels of estradiol compared with women with no NSAID use ($P_{trend} = 0.03$). Frequency of use of all analgesics (aspirin, nonaspirin NSAIDs, and acetaminophen) in 1990 was inversely associated with concentrations of estradiol ($P_{trend} = 0.001$), free estradiol ($P_{trend} = 0.01$), estrone sulfate ($P_{trend} = 0.03$), and the ratio of estradiol to testosterone ($P_{trend} = 0.04$). Among postmenopausal women, regular users of aspirin and other analgesics may have lower estrogen levels than nonusers, which could contribute to a decreased risk of breast or ovarian cancer among analgesic users. *Cancer Epidemiol Biomarkers Prev; 19(4); 1033–41. ©2010 AACR.*

Introduction

Regular use of aspirin, nonaspirin nonsteroidal anti-inflammatory drugs (NSAID), and acetaminophen has been inversely associated with risk of breast and ovarian cancer in some epidemiologic studies (1-11); however, overall, the data are inconclusive and the potential mechanisms are unclear. Meta-analyses generally suggest that use of aspirin or nonaspirin NSAIDs decreases breast cancer risk by 12% to 25% (1-6). NSAID use also has been inversely associated with ovarian cancer risk in a few studies (7-10), but meta-analyses generally have not supported an association (2, 12, 13). Acetaminophen does not seem to influence breast cancer risk (5, 14) but has been inversely associated with risk of ovarian cancer in some (11), but not all (15, 16), studies.

Analgesics may inhibit breast or ovarian carcinogenesis in part by influencing hormone levels. Aspirin and nonaspirin NSAIDs inhibit the activity of aromatase, an enzyme involved in the conversion of androgens to estrogens, by decreasing cyclooxygenase (COX) expression and prostaglandin synthesis (17). The expression of the aromatase gene (*CYP19*) has been positively correlated with *COX-1* and *COX-2* expression in human breast tissue (18). Acetaminophen has lower anti-inflammatory activity and is not considered an NSAID, although it seems to inhibit the COX-1 and COX-2 enzymes under certain conditions (19-21). Other properties of acetaminophen that could contribute to an inverse association with breast and/or ovarian cancer risk include an antigonadotropic effect through glutathione depletion and decreased follicle-stimulating hormone concentrations, or hormone-agonist/-antagonist activity due to chemical similarity to estradiol and progesterone (22).

Given the possible hormone-mediated mechanisms for an inverse association between analgesic use and risk of breast or ovarian cancer, we examined the cross-sectional relationship between use of aspirin, nonaspirin NSAIDs, and acetaminophen and sex steroid hormone concentrations among 740 postmenopausal women in the Nurses' Health Study (NHS).

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Materials and Methods

Study population

The Nurses' Health Study was established in 1976 when 121,700 female registered nurses ages 30 to 55 y responded to a mailed questionnaire about their life-style factors and medical histories. Study participants completed follow-up questionnaires every 2 y, providing information on new diagnoses of disease and updated information on risk factors.

In 1989 to 1990, when participants were 43 to 70 y of age, 32,826 women provided a blood sample. Details of the collection are described elsewhere (23). Briefly, participants arranged to have their blood drawn and shipped with an icepack through overnight mail. Upon receipt at our laboratory, each blood specimen was processed and stored in continuously monitored liquid nitrogen freezers at a temperature of −130°C or colder. At the time of blood collection, participants completed a short questionnaire that included questions on current weight, postmenopausal hormone (PMH) use, and menopausal status.

Women included in this analysis were controls in a nested case-control study of breast cancer (24, 25). All participants were postmenopausal, had not used PMH within 3 mo before blood collection, had no history of cancer, and reported their analgesic use on the 1988 or 1990 questionnaire. Women were considered postmenopausal if they reported having a natural menopause (no menstrual cycles during the previous 12 mo) or a bilateral oophorectomy, or if they had a hysterectomy without bilateral oophorectomy and were ages ≥56 y (for nonsmokers) or ≥54 y (for smokers; ref. 26). The Committee on the Use of Human Subjects in Research at Brigham and Women's Hospital, Boston, MA approved this analysis.

Exposure and covariate data

In 1988, we used a questionnaire that requested information on the frequency of aspirin use (never, 1-4, 5-14, 15-21, or ≥22 d per month) and the usual number of tablets per day of use (0, 1, 2, 3-4, 5-6, or ≥7 tablets). In 1990 and 1992, we requested the average number of days of use per month of acetaminophen, aspirin, and other anti-inflammatory drugs, using the same categories as the 1988 questionnaire. In our analyses, we combined the top two frequency categories (15-21 and ≥22 d/mo), due to the small number of women who reported 15 to 21 d of use per month.

We obtained information on covariates of interest from the biennial questionnaires or the questionnaire completed at blood draw. We calculated body mass index (BMI) using weight reported at blood draw and height reported on the baseline (1976) questionnaire. If weight at blood draw was missing, we used the value from the 1990 questionnaire to calculate BMI. Physical activity was reported in 1988 and alcohol intake in 1990. Parity and age at first birth (AFB) were collected through 1984. Age at menopause was collected on every biennial questionnaire, and in our analysis, we used each participant's first reported age at menopause. Smoking history was assessed in 1990, and data on PMH use were collected both at blood draw and in 1990.

Laboratory assays

Details of the laboratory methods are described elsewhere (24, 27). Briefly, plasma levels of each hormone were assayed in up to six batches. Estradiol, estrone, androstenedione, and testosterone were measured at the Quest Diagnostics' Nichols Institute (San Juan Capistrano, CA) using RIA, after extraction and column chromatography. For estrone sulfate, the first batch was assayed at the University of Massachusetts Medical Center's Longcope Steroid Radioimmunoassay Laboratory (Worcester, MA), and subsequent batches were assayed at the Nichols Institute. Estrone sulfate concentrations were determined by RIA of estrone, after enzyme hydrolysis, extraction, and column chromatography. For sex hormone binding globulin, which was used to calculate free estradiol and free testosterone, the first two batches were assayed at the Longcope Laboratory, and subsequent batches were assayed at the Reproductive Endocrinology Unit Laboratory at Massachusetts General Hospital (Boston, MA) using the AxSYM Immunoassay system (Abbott Diagnostics). Free estradiol and free testosterone were calculated using the methods of Sodergard et al. (28).

Estradiol, estrone sulfate, testosterone, and sex hormone binding globulin were assayed in controls matched to breast cancer cases diagnosed through June 2000, and estrone and androstenedione were assayed in controls matched to cases diagnosed through June 1998. We included 10% blinded replicates in each batch to assess laboratory precision. Within-batch coefficients of variation were between 8% and 10% for all analytes except androstenedione (12%). The limits of detection were 2 pg/mL for estradiol, 10 pg/mL for estrone, 40 pg/mL for estrone sulfate, 5 ng/dL for androstenedione, and 2 ng/dL for testosterone. When hormone values were reported as less than the detection limit, which occurred for estrone (n = 13), estradiol (n = 2), and estrone sulfate (n = 5), we set the value to half the limit of detection.

Statistical analysis

For each hormone, we excluded from the analysis women with missing data due to assay difficulties or low sample volume. In addition, we excluded six participants with outlying values for estradiol (n = 1), androstenedione (n = 2), or testosterone (n = 3), based on the generalized extreme studentized deviate many-outlier detection approach (29). For each analysis, we also excluded women who were missing data on the exposure of interest. After these exclusions, 740 women were eligible for our analyses.

We log transformed the values for each hormone to improve normality and used generalized linear models to calculate adjusted geometric means for each hormone by category of analgesic use in 1988 or 1990.

Table 1. Characteristics at blood draw of 740 postmenopausal women in the Nurses' Health Study

	Mean (SD) or %
Age (y)	61.5 (4.7)
BMI (kg/m^2)	26.2 (4.8)
Physical activity (METS/wk)	15.7 (19.3)
Parous, %	95.0
Parity (among parous women)	3.6 (1.7)
AFB in years (among parous women)	25.8 (3.5)
Age at natural menopause (y)	50.5 (3.2)
Past postmenopausal hormone (PMH) use, %	31.2
Duration of PMH use among past users (mo)	35.5 (44.4)
Alcohol intake (g/d)	5.4 (10.3)
Current smoker, %	13.1
Regular aspirin use,*%	31.2
Regular use of nonaspirin NSAIDs*, %	19.1
Regular acetaminophen use*, %	17.3
	Median (10th-90th percentile)
Estradiol, pg/mL	7 (4-14)
Free estradiol, pg/mL	0.10 (0.04-0.25)
Estrone, pg/mL	25 (14-43)
Estrone sulfate, pg/mL	197 (86-522)
Testosterone, ng/dL	22 (12-39)
Free testosterone, ng/dL	0.21 (0.09-0.44)
Androstenedione, ng/dL	57 (29-109)
Ratio of estrone/androstenedione	0.43 (0.22-0.78)
Ratio of estradiol/testosterone	0.31 (0.16-0.61)

*Use more than once per week in 1990.

We calculated the percent difference in the geometric means for the highest versus lowest category of use of each analgesic as $(e^{\beta} - 1) \times 100$. In addition, we modeled a continuous variable weighted by the midpoint of each frequency category, and calculated the P value for trend using the Wald test (30).

We created exposure variables for the estimated number of aspirin tablets per month in 1988 and the frequency (days/month) of total NSAID or total analgesic use in 1990. We calculated the number of aspirin tablets per month by multiplying the number of days of use per month by the usual number of tablets per day of use, using the midpoint of each category. We calculated the frequency of use of aspirin plus nonaspirin NSAIDs or all analgesics combined (aspirin, nonaspirin NSAIDs, and acetaminophen) by summing the midpoints of the frequency categories for each analgesic of interest.

We adjusted all models for laboratory batch, age at blood draw (continuous), fasting status (<10 versus ≥10 h), date (≤6/89, 7/89-12/89, 1/90-6/90, ≥7/90) and time of day (1-8 a.m., 9 a.m. to noon, 1-4 p.m., 5 p.m. to midnight) of blood draw, AFB/parity (nulliparous, AFB <25/1-4 children, AFB 25-29/1-4 children, AFB ≥30/1-4 children, AFB <25/≥5 children, AFB ≥25/≥5 children), BMI (continuous), physical activity (<3, 3 to <9, 9 to <18, 18 to <27, ≥27 MET-h/wk), smoking history (never, past, current), duration of PMH use among past users (continuous), age at menopause (<45, 45-49, 50-54, ≥55 y), and alcohol intake (0, >0-10, >10-20, >20-30, >30 g/d). Additionally, in the analyses of aspirin, nonaspirin NSAID, acetaminophen, and total NSAID use in 1990, we adjusted for frequency of use of other analgesics using the categories noted above. We also considered other potential confounders, including duration of breastfeeding, duration of past oral contraceptive use, age at menarche, and hysterectomy without bilateral oophorectomy; however, these variables did not change the results and therefore were not included in our final model.

We assessed whether the association between each analgesic and hormone levels differed by category of physical activity (<9 versus ≥9 MET-h/wk), PMH use (never versus ever), time since menopause (<10 versus ≥10 y), and BMI (<25 versus ≥25 kg/m^2), which is a major determinant of hormone levels in postmenopausal women (23, 31). We tested for effect modification by modeling an interaction term between each potential modifier of interest and a continuous variable weighted by the midpoint of each category of analgesic use frequency, and calculating the Wald test. Finally, we examined the associations among women with consistent analgesic use by limiting the analysis to women who reported regular use (≥5 d/mo) of the same analgesic in both 1988 and 1990 (aspirin only) or 1990 and 1992, or infrequent use (0-4 d/mo) at both time points. All P values were two sided andconsidered statistically significant if ≤0.05.

Results

The mean age at blood draw was 61.5 years (Table 1). On average, participants were slightly overweight and moderately physically active. Regular aspirin use (more than once per week; 31.2%) was more common than regular use of acetaminophen (17.3%) or nonaspirin NSAIDs (19.1%) in 1990. Regular aspirin use was slightly lower in 1988 than in 1990; in 1988, 29.3% of participants reported regular aspirin use, whereas 20.8% reported regular use in both 1988 and 1990 and 59.1% reported no use or use of less than or equal to once per week at both time points. Frequency of aspirin use in 1988 was moderately correlated with use in 1990 (Spearman r = 0.65), whereas correlations between the use of the different types of analgesics in 1990 were weak (Spearman r = 0.01-0.22).

There were no associations between the number of days of use per month of aspirin, nonaspirin NSAIDs, or acetaminophen in 1990 and levels of estrogens, androgens, or the ratio of estrogens to androgens

Table 2. Adjusted geometric mean hormone levels by frequency of analgesic use in 1990 among postmenopausal women in the Nurses' Health Study

	N	Frequency of use (d/mo)*				Percent difference†	P_{trend}‡
		0	1-4	5-14	15+		
Aspirin							
Maximum *n*	638	300	139	69	130		
Estradiol, pg/mL	617	6.9	7.2	7.0	6.6	−3.6	0.37
Free estradiol, pg/mL	588	0.10	0.10	0.10	0.09	−2.7	0.45
Estrone, pg/mL	485	24.1	24.7	24.2	26.5	10.1	0.11
Estrone sulfate, pg/mL	616	203	211	234	194	−4.5	0.51
Testosterone, ng/dL	621	21.4	22.0	20.5	22.0	2.8	0.77
Free testosterone, ng/dL	606	0.20	0.22	0.20	0.21	5.9	0.76
Androstenedione, ng/dL	484	54.4	56.0	52.3	61.3	12.8	0.11
Ratio of estrone/androstenedione	462	0.47	0.46	0.48	0.47	0.30	0.85
Ratio of estradiol/testosterone	588	0.36	0.35	0.37	0.33	−2.7	0.24
Nonaspirin NSAIDs							
Maximum *n*	596	387	95	35	79		
Estradiol, pg/mL	581	7.0	7.2	5.7	6.4	−8.9	0.14
Free estradiol, pg/mL	553	0.10	0.10	0.08	0.09	−11.4	0.12
Estrone, pg/mL	464	24.8	23.8	21.3	24.0	−3.1	0.66
Estrone sulfate, pg/mL	573	207	230	176	183	−11.3	0.12
Testosterone, ng/dL	579	21.5	22.5	19.9	19.8	−7.8	0.18
Free testosterone, ng/dL	564	0.21	0.22	0.19	0.18	−12.1	0.09
Androstenedione, ng/dL	462	55.1	57.2	53.0	51.4	−6.8	0.34
Ratio of estrone/androstenedione	443	0.49	0.45	0.44	0.47	−1.1	0.84
Ratio of estradiol/testosterone	551	0.36	0.35	0.31	0.34	−1.7	0.44
Acetaminophen							
Maximum *n*	606	339	162	47	58		
Estradiol, pg/mL	588	7.2	6.7	7.0	6.4	-9.9	0.23
Free estradiol, pg/mL	558	0.10	0.10	0.10	0.09	−7.9	0.47
Estrone, pg/mL	472	24.9	25.4	24.2	22.7	−8.9	0.19
Estrone sulfate, pg/mL	583	213	213	176	190	−10.8	0.14
Testosterone, ng/dL	589	21.0	21.3	22.3	21.7	3.7	0.55
Free testosterone, ng/dL	572	0.20	0.21	0.23	0.21	5.2	0.44
Androstenedione, ng/dL	471	54.4	57.6	58.6	53.0	−2.7	0.79
Ratio of estrone/androstenedione	451	0.49	0.46	0.47	0.45	−4.3	0.26
Ratio of estradiol/testosterone	559	0.37	0.35	0.34	0.34	−3.2	0.18

NOTE: Adjusted for age at blood draw, laboratory batch, fasting status at blood draw, date and time of blood draw, parity, AFB, BMI at blood draw, physical activity, smoking history, duration of PMH use among past users, age at menopause, alcohol intake, and frequency of use of other analgesics.

*Top two frequency categories (15-21 and ≥22 d/mo) are combined due to the small number of women who reported 15 to 21 d of use per month.

†Percent difference for highest vs lowest category of use (15+ vs 0 d/mo), calculated using $(e^{\beta} - 1) \times 100$; no statistically significant differences.

‡Weighted by the midpoint of each frequency category (0, 2.5, 9.5, or 23 d/mo) and calculated using the Wald test.

(Table 2). However, using the more detailed data on aspirin use from 1988, we observed significant inverse trends between the estimated number of aspirin tablets per month and concentrations of estrone (P_{trend} = 0.04) and estrone sulfate (P_{trend} = 0.03; Table 3). There were no statistically significant differences in hormone levels among women in the highest versus lowest category of aspirin use for any of the hormones examined (data not shown).

In the analyses of frequency of total (aspirin plus nonaspirin) NSAID use in 1990, we observed significant inverse associations with estradiol and free estradiol and a borderline significant inverse association with estrone sulfate (Table 4). Mean estradiol levels were 10.5% lower

among women who used NSAIDs at least 15 days per month, compared with women with no NSAID use (6.4 versus 7.2 pg/mL, respectively; P_{trend} = 0.03). Similarly, mean concentrations of free estradiol were 10.6% lower (0.09 versus 0.10 pg/mL, respectively; P_{trend} = 0.04) and mean estrone sulfate levels were 11.1% lower (188 versus 211 pg/mL, respectively; P_{trend} = 0.07) among women in the highest versus lowest category of NSAID use. These associations were strengthened when we added acetaminophen and considered the use of all analgesics combined in 1990 (Table 4). Women in the highest versus lowest category of total analgesic use had significantly lower levels of estradiol (15.2% lower; P_{trend} = 0.001) and free estradiol (12.9% lower; P_{trend} = 0.01), and a lower ratio of estradiol to testosterone (4.1% lower; P_{trend} = 0.04). In addition, we observed a significant inverse trend between frequency of total analgesic use and estrone sulfate (P_{trend} = 0.03), but the difference in estrone sulfate levels for women in the highest versus lowest category of total analgesic use (12.6% lower) was not statistically significant.

The primary confounder in our analysis was BMI. When we adjusted our models for age, laboratory batch, and date, time, and fasting status of blood draw only (age-adjusted model), none of the analgesic/hormone associations were statistically significant. For example, women in the highest versus lowest category of total analgesic use had 4.4% lower levels of estradiol in the age-adjusted model, compared with 15.2% lower in the final multivariable-adjusted model, and the *P* values for trend were 0.31 and 0.001, respectively. However, after adding BMI to the age-adjusted model, the results were similar to those for the final model; for the association with total analgesic use, estradiol concentrations were 14.9% lower among women in the highest versus lowest category of use, and the P_{trend} was 0.001. All of the significant associations from the final multivariable-adjusted model remained statistically significant in the model adjusted for age, laboratory batch, date/time/fasting status of blood draw, and BMI (data not shown). Supplementary Table S1 shows the percent difference in hormone levels for women in the highest versus lowest category of analgesic use in 1990 and the *P* value for trend, before and after adjustment for BMI.

In addition to the covariates in our final model, we also examined potential confounding by conditions that may influence analgesic use, including fracture, hypertension, rheumatoid arthritis, and uterine fibroids. However, adjusting for these preexisting conditions did not materially change our results (data not shown).

We observed significant interactions with BMI for several associations (Supplementary Table S2). In stratified analyses, the association between aspirin use in 1990 and free estradiol was inverse only among women with BMI of <25 kg/m^2 ($P_{interaction}$ = 0.005); comparing women in the highest versus lowest category of aspirin use, the percent difference in free estradiol levels was −19.4% (P_{trend} = 0.01) among lean women and +4.9%

Table 3. Adjusted geometric mean hormone levels by estimated number of aspirin tablets per month in 1988 among postmenopausal women in the Nurses' Health Study

	N	Estimated aspirin tablets/mo*					Percent difference†	P_{trend}‡
		0	1-10	11-30	31-50	50+		
Maximum *n*	716	276	247	100	25	68		
Estradiol, pg/mL	694	7.0	6.9	7.2	6.7	6.6	−4.5	0.45
Free estradiol, pg/mL	663	0.10	0.10	0.10	0.10	0.09	−5.8	0.39
Estrone, pg/mL	545	24.7	25.2	24.2	23.4	22.7	−7.9	0.04
Estrone sulfate, pg/mL	687	204	216	207	213	179	−12.1	0.03
Testosterone, ng/dL	697	21.3	21.9	21.1	19.3	20.5	−3.7	0.08
Free testosterone, ng/dL	681	0.20	0.22	0.20	0.19	0.19	−6.0	0.12
Androstenedione, ng/dL	545	55.4	56.8	55.5	53.3	53.0	−4.4	0.07
Ratio of estrone/androstenedione	522	0.47	0.48	0.45	0.45	0.47	−0.02	0.68
Ratio of estradiol/testosterone	659	0.35	0.35	0.36	0.40	0.34	−1.0	0.72

NOTE: Adjusted for age at blood draw, laboratory batch, fasting status at blood draw, date and time of blood draw, parity, AFB, BMI at blood draw, physical activity, smoking history, duration of PMH use among past users, age at menopause, and alcohol intake.

*Calculated as the midpoint of each frequency category (0, 2.5, 9.5, 18, or 26 d/mo) multiplied by the midpoint of each category of usual number of tablets taken on each day of use (0, 1, 2, 3.5, 5.5, or 7.5 tablets).

†Percent difference for highest vs lowest category of aspirin use (50+ vs 0 tablets/mo), calculated using (e^{β} − 1) × 100; no statistically significant differences.

‡Weighted by the midpoint of each frequency category (0, 2.5, 9.5, 18, or 26 d/mo) multiplied by the midpoint of each category of usual number of tablets taken on each day of use (0, 1, 2, 3.5, 5.5, or 7.5 tablets); calculated using the Wald test.

Table 4. Adjusted geometric mean hormone levels by frequency of total NSAID or total analgesic use in 1990 among postmenopausal women in the Nurses' Health Study

	N	Frequency of use (d/mo)*				Percent difference†	P_{trend}‡
		0	1-4	5-14	15+		
Aspirin and nonaspirin NSAID use‡							
Maximum *n*	691	230	154	107	200		
Estradiol, pg/mL	670	7.2	7.1	7.1	6.4	−10.5§	0.03
Free estradiol, pg/mL	638	0.10	0.10	0.10	0.09	−10.6	0.04
Estrone, pg/mL	528	24.7	23.5	24.9	24.6	−0.11	0.74
Estrone sulfate, pg/mL	665	211	211	221	188	−11.1	0.07
Testosterone, ng/dL	671	21.9	21.8	20.6	21.0	−4.3	0.35
Free testosterone, ng/dL	655	0.21	0.22	0.20	0.20	−4.3	0.26
Androstenedione, ng/dL	526	55.3	54.8	54.1	56.2	1.6	0.75
Ratio of estrone/androstenedione	504	0.48	0.45	0.49	0.47	−1.3	0.91
Ratio of estradiol/testosterone	635	0.37	0.35	0.37	0.34	−3.0	0.15
Total analgesic use‡,‖							
Maximum *n*	719	148	168	156	247		
Estradiol, pg/mL	698	7.5	7.1	7.1	6.3	−15.2§	0.001
Free estradiol, pg/mL	665	0.10	0.10	0.10	0.09	−12.9§	0.01
Estrone, pg/mL	554	25.0	24.5	24.6	24.3	−2.6	0.69
Estrone sulfate, pg/mL	692	216	210	222	189	−12.6	0.03
Testosterone, ng/dL	699	22.2	21.4	21.7	20.9	−5.8	0.32
Free testosterone, ng/dL	682	0.21	0.21	0.22	0.20	−2.1	0.59
Androstenedione, ng/dL	552	56.4	53.2	58.0	55.0	−2.5	0.99
Ratio of estrone/androstenedione	530	0.49	0.49	0.45	0.47	−1.6	0.44
Ratio of estradiol/testosterone	663	0.38	0.35	0.36	0.33	−4.1§	0.04

NOTE: Adjusted for age at blood draw, laboratory batch, fasting status at blood draw, date and time of blood draw, parity, AFB, BMI at blood draw, physical activity, smoking history, duration of PMH use among past users, age at menopause, and alcohol intake, plus frequency of use of acetaminophen for analyses of aspirin and nonaspirin NSAID use.

*Top two frequency categories (15-21 and ≥22 d/mo) are combined due to the small number of women who reported 15 to 21 d of use per month.

†Percent difference for highest vs lowest category of use (15+ vs 0 d/mo), calculated using $(e^{\beta} - 1) \times 100$.

‡Calculated using the sum of variables for frequency of use of each relevant type of analgesic, in which each variable was weighted by the midpoint of each frequency category (0, 2.5, 9.5, or 23 d/m); P_{trend} weighted by the midpoint of each frequency category and calculated using the Wald test.

§$P \leq 0.05$.

‖Frequency of use of aspirin, nonaspirin NSAIDs, and acetaminophen.

(P_{trend} = 0.61) among overweight women. However, other associations were similar by BMI category or were more strongly inverse among overweight women, and there was no clear pattern of interactions with BMI across the different analgesics and hormones. Although there were a few significant interactions with physical activity, PMH use, and time since menopause, there was no consistent pattern of effect modification by these variables (data not shown).

Among women (*n* = 329-545) with consistent analgesic use across two follow-up cycles, the results generally were similar to those for all women (data not shown). However, some associations were no longer statistically significant, likely due to the smaller sample size. In secondary analyses, results after excluding women within 4 years of menopause (*n* = 60) were similar to those for all women (data not shown).

Although our analyses were based on *a priori* hypotheses, we secondarily evaluated the statistical significance of the results after adjustment for multiple comparisons. Using the conservative Bonferroni correction with 28 unique exposure/hormone combinations, the adjusted α level was 0.05/28 = 0.002. At this level of significance, only the association between total analgesic use and estradiol remained statistically significant (P_{trend} = 0.001).

Discussion

Our results suggest that postmenopausal women who regularly use aspirin, nonaspirin NSAIDs, or

acetaminophen may have lower estrogen levels than women who infrequently use analgesics. We observed significant inverse associations between total NSAID use and concentrations of estradiol and free estradiol, and between total analgesic use and levels of estradiol, free estradiol, estrone sulfate, and the ratio of estradiol to testosterone. There were no associations with the frequency of use (days/month) of each individual type of analgesic in 1990, when data on the number of tablets per day were unavailable; however, we observed significant inverse associations between the estimated number of aspirin tablets per month in 1988 and concentrations of estrone and estrone sulfate. Although some associations varied by category of BMI, physical activity, PMH use, or time since menopause, there was no clear pattern of effect modification by any of these variables.

Two previous studies have examined the association between NSAID use and hormone levels in postmenopausal women. In an analysis of 274 participants in the Women's Health Initiative Dietary Modification Trial, regular use of NSAIDs (defined as use at least twice per week) was not significantly associated with levels of estrogens, testosterone, or androstenedione (32). However, this study did not examine associations with dose or frequency of NSAID use, and participants were asked to refrain from using NSAIDs during the 48 hours before blood draw, which may have limited the ability to detect associations. In another study of NSAID use and estradiol levels among 260 women, both current use reported at blood draw and consistent use (defined as self-reported current use plus use within 48 hours before blood draw) were inversely associated with serum levels of total estradiol (33). Associations with dose and frequency of NSAID use were not assessed. Neither study evaluated interactions with BMI or other potential effect modifiers.

Aromatase inhibition due to decreased prostaglandin synthesis is a plausible mechanism by which analgesics may decrease risk of hormone-related cancers in women. In the presence of inflammation and other stimuli, COX-2 expression is upregulated and levels of prostaglandins, particularly prostaglandin E_2, increase (17). Human adipose stromal cells exposed to prostaglandin E_2 have higher levels of cyclic AMP (cAMP) and a 50-fold increase in aromatase activity, compared with control cells, due to cyclic AMP–induced stimulation of the *CYP19* promoter II (34). The aromatase enzyme catalyzes the conversion of testosterone to estradiol and androstenedione to estrone; in postmenopausal women, adipose aromatase is the primary source of circulating estrogens (35, 36). Multiple studies have observed strong positive associations between levels of endogenous estrogens and breast cancer risk in postmenopausal women (37). In contrast, data on endogenous estrogen concentrations and ovarian cancer risk are sparse (37); however, studies of genetic variation in the *CYP19* gene (38) and studies of estrogen-related exposures, such as BMI and PMH (39), suggest a possible role of estrogens in ovarian carcinogenesis, particularly for the endometrioid histologic subtype (40, 41). Aromatase inhibitors, which are used in the treatment of estrogen receptor–positive postmenopausal breast cancer, significantly decrease estrogen concentrations without altering androgen concentrations (42). This suggests that analgesics that decrease aromatase activity through the suppression of COX expression and prostaglandin synthesis also may decrease estrogen concentrations and potentially risk of breast or ovarian cancer. Several studies of NSAID use and breast cancer risk have reported an inverse association only for hormone receptor–positive breast tumors (43-45), supporting the aromatase inhibition hypothesis, but other studies have reported no difference by hormone receptor status (5, 46-50).

Although we observed significant inverse associations between several analgesic variables and estrogen concentrations, not all of our results were consistent with those expected if analgesics influence hormone levels through aromatase inhibition. In particular, there was no association between frequency of use of aspirin, nonaspirin NSAIDs, or acetaminophen individually in 1990 and levels of any estrogen. In addition, the estimated number of aspirin tablets per month in 1988 was significantly inversely associated with estrone and estrone sulfate concentrations but not levels of estradiol or free estradiol, although the observed associations were in the hypothesized direction. Limitations of our data that may have contributed to these inconsistencies include the unavailability of information on the exact frequency of use or dose of each analgesic in 1990 and the availability of a single blood sample for each woman. However, the intraclass correlation coefficients for within-person repeated measures of these hormones over time are high (51), suggesting that a single blood sample reflects long-term values. Although analgesic use reported at the time of the 1988 or 1990 questionnaire may not have accurately reflected use at the time of blood draw, the results were essentially unchanged in analyses restricted to women who were consistent analgesic users across two follow-up cycles and who were therefore more likely to have had the same level of use at blood draw. Although our study population is large, we may have had inadequate power to detect small differences in hormone levels, particularly for analyses of the extreme categories of analgesic use in which the number of women in the highest frequency category is relatively small. The cross-sectional design also is a limitation of our analysis, as it is impossible to determine whether differences in hormone levels were related to analgesic use or some other factor, such as a preexisting condition influencing both hormone levels and analgesic use. Although there was no evidence of confounding by several conditions examined, including fracture, hypertension, rheumatoid arthritis, and uterine fibroids, it is possible that other conditions may have confounded the associations. According to the Supplementary Data collected for a subset of participants (52), the most common indications for frequent analgesic use in 1999 were muscle/joint pain, cardiovascular prevention (aspirin only), headache, and backache. Although

the indications for use at the time of blood draw may have differed slightly from those in 1999, these conditions, with the possible exception of headaches (53), are unlikely to be strongly related to estrogen concentrations.

This analysis also has several strengths, including the large study population with data on multiple hormones of interest, and the detailed information on analgesic use and potential confounders of interest collected near the time of blood draw. In addition, with the exception of BMI, which we controlled for continuously, there was minimal confounding by the covariates included in our analysis, making it unlikely that the results were due to residual confounding.

Our results provide modest support for an inverse association between analgesic use and estrogen concentrations in postmenopausal women. Additional research is needed to confirm this association and to determine whether the decrease in estrogen concentrations due to analgesic use translates to a lower risk of breast or ovarian cancer. Randomized trials of aspirin, nonaspirin NSAID, and acetaminophen use and changes in hormone levels would be a logical next step in elucidating these associations, including the magnitude of the effect, the most beneficial analgesics, and the relevant dose. This research may have important public health implications if an inverse association between analgesic use and risk of breast or ovarian cancer is confirmed, as analgesics could be easily implemented as a chemopreventive and may decrease risk of several cancers.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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