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Analgesic Use in Relation to Sex Hormone and Prolactin Concentrations in Premenopausal Women

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Abstract

Purpose—Common analgesics (aspirin, non-aspirin NSAIDs, and acetaminophen) may be associated with hormone-related cancers, possibly via effects on sex hormone and prolactin concentrations. Methods: Between 1996–1999, 29,611 participants in the Nurses' Health Study II (NHSII) provided blood samples; 18,521 provided samples timed in the early follicular and mid-luteal phases of the menstrual cycle, the remainder provided untimed samples. We assessed the cross-sectional relationship between analgesic use and plasma sex hormone and prolactin concentrations among 2,034 premenopausal women, 32 to 54 years old, who served as controls in nested case-control studies, or participated in a within person hormone reproducibility study in the NHSII; this included 1700 timed and 334 untimed samples. Estrogens and progesterone were measured in timed samples; androgens and prolactin were measured in timed and untimed samples. Results: In multivariable models, non-aspirin NSAIDs were positively associated with follicular free estradiol (13.5% higher, use ≥4 days/week vs. non-users ($p=0.04$; $p_{trend}=0.11$)); results for follicular total estradiol were similar (13.2% higher, $p=0.06$; $p_{trend}=0.11$). Acetaminophen use was inversely associated with prolactin (11.8% lower, use 2 days/week vs. non-users, $p=0.01$, $p_{trend}=0.04$). Acetaminophen was also inversely associated with free testosterone (7.1% lower, use 2 days/week vs. non-users, $p=0.04$; $p_{trend}=0.04$). No other associations were observed with the other hormones, or with aspirin use.

Conclusions—There were no clear patterns between analgesic use and sex hormones in premenopausal women. Acetaminophen use may be modestly associated with prolactin and free testosterone. Our results do not support that analgesic use influences cancer risk through alterations in premenopausal circulating sex hormones or prolactin.

Keywords

analgesics; NSAID; aspirin; sex hormone; prolactin; premenopausal

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Introduction

Use of common analgesics, such as aspirin, non-aspirin NSAIDs, and acetaminophen, may be associated with the risk of breast [1–8], ovarian [9–18], endometrial [19], and colon [20–22] cancer; while evidence for colon cancer is consistent, the evidence from epidemiologic studies for reproductive cancers is somewhat inconsistent. The evidence for an association between analgesics and several hormone-related cancers is primarily for postmenopausal women, although in some studies premenopausal exposure or premenopausal cancer risk was assessed [8]. Prior research in premenopausal women in the Nurses' Health Study II did not show an association between analgesics and breast cancer risk [8]. Further, for some cancers, particularly colon cancer, long duration of use is most protective [23]. Some have hypothesized that such associations may be mediated, at least in part, by alterations in sex hormone concentrations or prolactin, which have been associated with risk of breast, ovarian, endometrial, and colorectal cancers [6, 24–30]. However, previous data examining the association between analgesics and circulating hormones has been in postmenopausal women [31–33], with no prior data in premenopausal women. Since cancer has a long latency period, it is important to understand the relationship between analgesic use and potential mediating factors, including sex hormone concentrations, in premenopausal women. Evaluating such relationships has the potential to improve the mechanistic understanding of these disease associations.

Therefore, we assessed the cross-sectional relationship of analgesic (aspirin, non-aspirin NSAID, and acetaminophen) use with plasma sex steroid hormone and prolactin concentrations in a sub-sample of 2,034 premenopausal women, ages 32 to 54 years old at blood draw, from the Nurses' Health Study II (NHSII).

Materials and Methods

Study Population

The NHSII was established in 1989, enrolling 116,430 female registered nurses, ages 25 to 42. The cohort continues to be followed biennially to update exposure variables and ascertain newly diagnosed disease. Between 1996 and 1999, 29,611 women (ages 32–54 years) provided a blood sample. Details of the blood collection are described elsewhere [34]. Briefly, premenopausal women who had not taken any exogenous hormones, been pregnant, or breastfed within 6 months (n = 18,521) completed a short questionnaire and provided timed blood samples on the 3rd to 5th day of their menstrual cycle (follicular sample), and 7 to 9 days before the anticipated start of their next cycle (luteal sample). Follicular plasma was aliquoted by the participant 8 to 24 hrs after collection and frozen. All other women (n = 11,090) provided a single untimed blood sample. Luteal and untimed samples were shipped via overnight courier on ice, processed by our laboratory, and separated into plasma, red blood cell, and white blood cell components. Samples have been stored in continuously monitored, liquid nitrogen freezers since collection.

Follow-up of the blood cohort as of June 2009 was 94.5%. Women included in this cross-sectional analysis were controls in one of several nested case-control studies with various endpoints, including breast cancer (n = 1268) [8], ovarian cancer (n = 46) [9], endometriosis (n = 592), and rheumatoid arthritis (n = 19) [35], or participants in hormone reproducibility studies (n = 109) [36]. This analysis was restricted to premenopausal women, who were defined as having timed samples, or among women who provided untimed samples, those whose periods had not ceased, or who reported having had a hysterectomy but with at least one ovary remaining, and were ≤ 47 (for non-smokers) or ≤ 45 (for smokers) years of age. The study was approved by the Committee on the Use of Human Subjects in Research at the Brigham and Women's Hospital (Boston, MA).

Exposure and Covariate Data

Information on exposures and covariates was obtained from biennial questionnaires and a questionnaire completed at blood collection. In 1993, 1995, 1997 and 1999, we requested information on the frequency of aspirin, non-aspirin NSAID, and acetaminophen use (never, 1, 2–3, 4–5, or ≥ 6 days/week); data on whether analgesic use was used ≥ 2 days per week was collected in 1989. We calculated frequency of use as the average of the frequencies reported in 1997 and 1999; analyses of duration incorporate data from 1989–1999. Age at menarche, height, and weight at age 18 were reported at baseline in 1989; oral contraceptive use and parity were updated with biennial questionnaires. Family history of breast cancer was assessed in 1989 and 1997. We adjusted for lactation history, smoking status, and physical activity as reported in 1997 and alcohol consumption as assessed in 1999. Current weight and details regarding blood collection date, time, and fasting status were reported on the blood questionnaire. Body mass index (BMI) at blood collection and at age 18 was calculated as weight in kilograms divided by height in meters squared (kg/m^2). A total of 80% of the study population provided blood samples within 10 months of responding to the 1997 questionnaire; 50% provided samples within 2.1 years of responding to the 1999 questionnaire.

Laboratory Assays

Hormone assay methods for estrogens, androgens, progesterone, and prolactin have been described previously [29, 37]. Briefly, plasma levels were assayed in up to nine batches. Estrone, estradiol, and estrone sulfate were assayed in luteal and follicular timed samples. Testosterone, androstenedione, and prolactin values were assayed in luteal and/or follicular timed samples as well as untimed samples. Progesterone was measured in luteal timed samples, and dehydroepiandrosterone (DHEA), DHEA-sulfate (DHEAS), and sex-hormone binding globulin (SHBG) were measured in luteal and untimed samples.

Estrogens (3 batches), testosterone (5 batches), androstenedione (2 batches), and progesterone (1 batch) were assayed at Quest Diagnostics (San Juan Capistrano, CA). Estrogens and testosterone were assayed by RIA following extraction and celite column chromatography. After extraction of estrone, enzyme hydrolysis, and column chromatography, estrone sulfate was assayed by RIA. Androstenedione was also assayed by RIA. Progesterone was assayed by RIA preceded by organic extraction. Four batches of estrogens and testosterone were assayed at Mayo Medical Laboratories using liquid chromatography-tandem mass spectrometry. Two batches of DHEA and androstenedione, and four batches of DHEAS, SHBG, and progesterone were assayed at the Royal Marsden Hospital. Androstenedione was assayed by RIA and DHEAS, sex-hormone binding globulin (SHBG), and progesterone were assayed by chemiluminescent immunoassay. The remaining batch of DHEAS was assayed at Mayo Medical Laboratories by chemiluminescent enzyme immunoassay. One batch of progesterone (RIA) and three batches of SHBG (chemiluminescent enzyme immunometric assay) were assayed at Massachusetts General Hospital (Boston MA) and one batch of SHBG and progesterone were assayed at the Children's Hospital Boston. Prolactin was measured using a microparticle enzyme immunoassay at the Massachusetts General Hospital, by the AxSYM Immunoassay system.

We included 10% blinded replicates in each batch to assess laboratory precision. Within-batch coefficients of variation were between 2% and 15% for all analytes, except a single batch progesterone (17%).

Free estradiol and free testosterone were calculated using the methods of Sodergard [38]. When follicular SHBG or testosterone concentrations were missing, concentrations from luteal or untimed samples were used. Follicular free estradiol calculated with luteal SHBG

and testosterone are highly correlated with calculations done using the timed follicular SHBG and testosterone (correlation coefficient from a subset of our data with both values (n=603) is 0.97).

Statistical Analyses

We excluded data with outlying values, as identified with the generalized extreme Studentized deviate many-outlier detection method [39], resulting in the exclusion of up to 13 values (range: 0 (estrone sulfate, DHEA, DHEAS) to 13 (prolactin)). We also excluded women with missing analgesic data. Following these exclusions, 2,034 women were included in our analyses with a total of 1700 timed and 334 untimed samples. Hormone concentrations in quality control samples differed by batch, indicating that there was some laboratory drift over time. Therefore, we adjusted all hormone levels for batch according to the methods described by Rosner et al [40].

For women with a follicular and luteal blood sample, we used the average of the two phases for testosterone, free testosterone, androstenedione, and prolactin because levels did not vary substantially by menstrual phase, and the average of follicular and luteal samples better represents long-term levels [36, 41]. We log-transformed hormone concentrations to improve normality and used generalized linear models to calculate adjusted geometric means for each hormone by category of analgesic use. We calculated the percent difference in the geometric means for the highest versus lowest category of use as $(e^{\beta} - 1) \times 100$. Lastly, we modeled a continuous variable weighted by the midpoint of each category of analgesic use, and calculated the *P*-trend using the Wald test [42]. P-trend for duration variables were calculated among users of the given analgesic.

Exposure variables for the frequency of analgesic use (days/week) were calculated using the average of weighted midpoints of the frequency categories in 1997 and 1999. Exposure variables were split into three or four categories, depending on the sample size. Duration of analgesic use was calculated from baseline in 1989 through 1999.

All models were adjusted for covariates known to be associated with analgesic use and/or hormone concentrations, including: age at blood draw (continuous), fasting status (<10, ≥10 h), time of day of blood draw (1–8 a.m., 9 a.m. to noon, 1–4 p.m., 5 p.m. to midnight), race/ethnicity (Caucasian, other), BMI at blood draw (continuous), duration of past oral contraceptive use (never, <4, ≥ 4 years), age at first birth/parity (nulliparous, age at first birth <25/1–2 children, 25–29/1–2 children, ≥ 30/1–2 children, <25/ ≥ 3 children, 25–29/ ≥ 3 children, ≥ 30/ ≥ 3 children), physical activity (<3, 3 to <9, 9 to <18, 18 to <27, ≥ 27 MET-h/wk), smoking history (never, past, current), alcohol intake (0, >0–10, >10–20, >20–30, >30 g/d), and use of other analgesics (yes, no). Models for luteal, random, and average of timed samples were also adjusted for date of blood draw (continuous) and difference between luteal blood draw date and date of next menstrual period (3–7, 8–21 days, unknown/untimed). Since we adjusted for batch using the previously described methods [40], we did not further include laboratory batch in the model. We also considered other potential confounders, including duration of breastfeeding, age at menarche, BMI at age 18, and family history of breast cancer; however, these variables did not change the results and were not included in our final model.

We assessed whether the association between each analgesic and hormone was modified by age (<45 versus ≥ 45 years) or BMI at blood draw (<25 versus ≥ 25 kg/m^2). We tested for effect modification by modeling an interaction term between each potential modifier and a continuous variable weighted by the midpoint of each category of analgesic use frequency, and calculating the Wald test. For all exposures, we conducted *a priori* sensitivity analyses restricted to ovulatory cycles for luteal estrogens (defined as mid-luteal progesterone ≥ 400

ng/dL) and women without a pre-existing condition that could influence analgesic use or hormone concentrations (uterine fibroids, rheumatoid arthritis (for women selected as controls for outcomes other than rheumatoid arthritis), osteoarthritis, or premenstrual syndrome). All analyses were conducted using SAS software, version 9.2 (SAS Institute Inc., Cary, NC); all p values were two sided and considered statistically significant if <0.05.

Results

The mean age at blood draw was 42.7 years. On average, participants were slightly overweight and moderately physically active (Table 1). Regular non-aspirin NSAID use (at least once per week in both 1997 and 1999) was more common (29.4%) than regular aspirin use (7.6%) or acetaminophen use (14.6%). Regular use of aspirin increased more from 1997 to 1999 (11.8% to 15.4%) compared to non-aspirin NSAIDs (40.8% to 41.9%) and acetaminophen (22.7% to 24.6%). Frequency and quantity of analgesic use in 1997 was moderately correlated with use in 1999 (Spearman $r = 0.47–0.52$ for aspirin, acetaminophen, or non-aspirin NSAIDs), whereas correlations between the use of different analgesics were weak (Spearman $r = 0.09–0.26$). Age-adjusted and multivariable models (MV) were similar, so only MV results are presented.

Aspirin

There was little evidence of an association between aspirin use by frequency or duration and any of the plasma hormones (Table 2). Percent differences comparing use ≥ 2 times per week to nonusers ranged from −10.6% for the follicular estradiol/testosterone ratio to 10.1% for DHEA (all p>0.05). Longer duration of aspirin use was suggestively associated with higher follicular estrone levels (14% higher levels associated with ≥ 5 years use as compared to no use (p=0.04; p_{trend}=0.06), but unassociated with any of the other hormones in the analysis (data not shown). Use ≥ 2 times per week as compared to no use was associated with lower progesterone (8.8% difference, p=0.04; p_{trend}=0.24) when analyses were restricted to women ovulatory in the cycle of collection. Frequency of aspirin use was positively associated with follicular estrone (p_{trend}=0.01) and follicular free estradiol (p_{trend}=0.02), and inversely associated with DHEAS (p_{trend}=0.03) in women without a pre-existing condition that may be associated both with hormone levels and analgesic use.

There was evidence that the associations between aspirin and luteal estradiol and estrone, the luteal estradiol/testosterone ratio, and prolactin all varied by level of BMI ($p_{interaction}$<0.05). Among women with BMI ≥ 25, more frequent use of aspirin was inversely associated with luteal estradiol (14.6% lower (p=0.01), use ≥ 2 days/week vs. nonusers), whereas there was no association among women with BMI <25 (comparable change: 0.02% difference (p=0.98)). The luteal estradiol/testosterone ratio was similarly impacted by BMI, with an inverse association among women with BMI ≥25 (comparable change: 14.3% lower (p=0.03)), with no association in women with BMI <25 (comparable change: 2.4% difference (p=0.69)). The effect modification for the remaining hormones was less clear with no significant associations in either BMI strata. There was no effect modification by age.

Non-Aspirin NSAIDs

More frequent use of non-aspirin NSAIDS was associated with higher follicular free estradiol (13.5% higher in women reporting use ≥4 days/ week vs. nonusers (p=0.04; p_{trend}=0.11)) and suggestively higher follicular total estradiol (comparable change of 13.2%, p=0.06; p_{trend}=0.11) (Table 3). Duration of non-aspirin NSAID use was not associated with duration of either hormone (follicular free estradiol, 5.9% difference ≥5 yrs vs. no use, p=0.27; p_{trend} among users=0.74; follicular total estradiol: 6.7% difference ≥5 yrs vs. no use,

p=0.24; p_{trend} among users=0.60. There was no evidence of an association between frequency or duration of non-aspirin NSAIDS and the remainder of the hormones.

In sensitivity analyses restricted to samples collected during an ovulatory cycle, frequency of NSAID use was inversely associated with luteal estrone (8.1% lower, use ≥4 days/ week vs. nonusers, p=0.04; p_{trend}=0.52) and the luteal estrone/androstenedione ratio (comparable change: 20.9% lower, p=0.01; p_{trend}=0.05). These associations were attenuated and not statistically significant after excluding women with preexisting conditions (data not shown).

The associations of non-aspirin NSAIDs and luteal estradiol and progesterone, free testosterone, and the luteal estradiol/testosterone ratio varied by BMI ($p_{interaction}$≤0.03). Use of non-aspirin NSAIDs ≥4 days/week vs. nonusers was associated with lower levels of progesterone (28.6% lower, p=0.01) among women with BMI ≥25, but not associated among women with BMI <25 (5.8% higher, p=0.67). Non-aspirin NSAIDs were inversely associated with the luteal estradiol/testosterone ratio only among women with BMI ≥25 (comparable change: 15.2% lower, p=0.04). There was no consistent effect modification by age (data not shown).

Acetaminophen

Frequency of acetaminophen use was significantly inversely associated with prolactin and free testosterone levels (Table 4). Compared to women reporting no acetaminophen use, prolactin levels were 11.8% lower (p=0.01, p_{trend} = 0.04) and free testosterone levels were 7.1% lower (p=0.04, p_{trend}= 0.04) among women who used acetaminophen ≥2 days per week. Duration of acetaminophen use was similarly inversely associated with free testosterone, with 10.5% lower free testosterone levels in women reporting use ≥5 years duration (p=0.02) as compared to nonusers (p_{trend} =0.80), as well as DHEAS, with 16.6% lower DHEAS levels associated with duration ≥ 5 years as compared to nonusers (p=0.02, p_{trend} = 0.04). Duration of acetaminophen use was not associated with prolactin (7.8% difference (p=0.16) comparing ≥ 5 years duration to nonusers; p_{trend} =0.46). Acetaminophen use was not associated with the other hormones, or ratios of hormones, in this analysis or in the sensitivity analyses. Results for prolactin were consistent after exclusion of anovulatory cycles. The associations were similar when stratifying by BMI or age (data not shown).

Although our analyses were based on *a priori* hypotheses, we evaluated the statistical significance of the primary results after adjustment for multiple comparisons. For each analgesic exposure, we conducted 19 Wald tests corresponding to the P_{trend} for each hormone across frequency categories of the exposure. Thus, using the conservative Bonferroni correction with 19 unique hormone tests, the adjusted level was 0.05/19=0.003. No associations in the primary analysis remained statistically significant at this adjusted level.

Discussion

In this large, cross-sectional analysis of premenopausal women, we observed higher follicular free and total estradiol associated with more frequent non-aspirin NSAID use, as well as lower concentrations of prolactin and free testosterone with higher acetaminophen use. No clear associations were observed between any type of analgesic use and luteal estradiol, estrone, estrone sulfate, testosterone, androstenedione, and estrogen/androgen ratios.

This is the first study to evaluate the association between NSAID use and sex hormone and prolactin concentrations in premenopausal women. Three previous studies evaluated these associations in postmenopausal women [31–33]. The largest study to date, by Gates et al,

observed a significant inverse association of both total NSAID and acetaminophen use with plasma concentrations of estradiol and free estradiol [31], in agreement with previous research [32]. McTiernan et al did not observe associations between analgesics and estradiol; however women who reported regular use of NSAIDs had lower prolactin concentrations and higher DHEA concentrations compared to non-users [33]. In contrast, we observed positive associations between non-aspirin NSAID use and follicular estradiol in this premenopausal population and an inverse association between acetaminophen use and free testosterone. We observed a similar relationship as McTiernan et al between analgesic use and prolactin, however our results were limited to acetaminophen use.

There are some important differences that may be especially pronounced when comparing associations in pre- and postmenopausal women. While androgens and prolactin only vary modestly across the menstrual cycle, compared to postmenopausal women, estrogen concentrations in premenopausal women vary widely, thus it may be more difficult to observe true relationships with hormone concentrations in one blood sample. However, our data are unique in that premenopausal women provided timed samples, allowing for more accurate assessment of relationships with sex hormones during luteal or follicular phases of the menstrual cycle.

This analysis was exploratory and there are no confirmed mechanisms between analgesic use and estrogen, prolactin, or DHEA/DHEAS concentrations among premenopausal women. However, analgesics have the potential to reduce the risk of hormone-related cancers by lowering prostaglandin synthesis through aromatase inhibition. The aromatase enzyme catalyzes the conversion of testosterone to estradiol and androstenedione to estrone [29]. The concentrations of both COX-2 and prostaglandins, particularly prostaglandin E_2 (PGE_2), are increased in the presence of inflammation and other stimuli as well as in tumor and metastatic tissue [43]. When human adipose stromal cells were exposed to PGE_2, aromatase activity was significantly increased compared to controls [44]. Since NSAIDs reduce COX-1 and COX-2, and thus prostaglandins, it is possible that such use could reduce aromatase activity. In postmenopausal women the expected result of aromatase inhibition would be lower estrogen levels; however, in premenopausal women reduced aromatase activity may result in higher estrogen levels as a result of compensatory feedback loops [45]. However, since we did not observe clear associations between NSAID use and premenopausal sex hormones, this mechanism may be more important in postmenopausal women in whom an important source of estrogens is aromatase activity in adipose tissue.

Recent evidence suggests that acetaminophen operates through a similar pathway to inhibit COX-2 [46]. Prolactin gene expression in human T cells is stimulated by PGE_2 [47]. Thus, acetaminophen use may lower PGE_2, possibly decreasing prolactin concentrations [48–51]. Acetaminophen may also have anti-gonadotropic effects through glutathione depletion and decreased concentrations of follicle-stimulating hormones, or hormone agonist/antagonist activity due to similarities in chemical stability compared to estradiol and progesterone [46, 52].

This study has some limitations. The cross-sectional study design allows the possibility of factors, such as pre-existing medical conditions that influence both analgesic use and sex hormone concentrations at the time of data collection. We observed some differences in associations when excluding women with pre-existing conditions related to increased analgesic use. However, among a subset of NHSII participants who were included in a separate analgesics sub-study, the most common indications for analgesic use were muscle/joint pain, cardiovascular prevention (for aspirin only), headaches, and backaches [53]. These conditions are unlikely to be strongly associated with the hormones of interest, with the exception of headaches [54]. The exact frequency and quantity of analgesic use at blood

draw was unknown, however, we were able to average the estimated frequency through questionnaires over two years near the time of blood draw. Since analgesic use in 1997 was moderately correlated with use in 1999, it is likely that analgesic use averaged over these two years is a relatively good representation of long-term use. Hormone concentrations were measured at a single blood draw, but the intra-class correlation coefficients for within-person repeated measures of these hormones over time are relatively high, except for the estrogens and prolactin [36]. Lastly, while our sample size was relatively large, we may have had inadequate power to detect small differences in hormones concentrations, especially at the extreme categories of analgesic use where samples sizes were smaller.

This study also has several strengths. This is the first study to evaluate the relationship between analgesic use and sex hormone concentrations in premenopausal women. We had a large study population with data on the concentration of multiple hormones of interest and detailed information on potential confounders collected near the blood draw. Notably, the blood draw samples are timed within the menstrual cycle, allowing accurate assessment of hormone concentrations during luteal and follicular phases. There was also minimal confounding by measured confounders making residual confounding unlikely.

Our study provides some evidence for an association of NSAID use with follicular estradiol levels and of acetaminophen use with free testosterone and prolactin concentrations in premenopausal women. Further research is needed to confirm the relationships observed in this population. Long-term assessment of analgesic exposure is needed to evaluate the temporal component of this relationship and additional large-scale, prospective observational studies of hormone-related cancers among premenopausal women would improve further evaluation of these associations. Understanding the determinants of premenopausal hormone concentrations is important for many hormone-related diseases that may initiate during premenopausal years.

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Table 1

Characteristics at blood draw of 2034 premenopausal women in the Nurses' Health Study II

	Mean (SD) or %
Age in years	42.7 (4.0)
Body mass index (kg/m^2)	25.7 (6.1)
Physical activity (METs/week)	18.0 (17.7)
Parous, %	81.0
Parity (among parous women)	2.3 (0.9)
Age at first birth in years (among parous women)	26.6 (4.4)
Past oral contraceptive use, %	85.1
Duration of oral contraceptive use in months (among past users)	54.2 (45.5)
Alcohol intake (grams/day)	4.1 (7.0)
Current smoker, %	7.9
Regular aspirin use*, %	7.6
Regular acetaminophen use*, %	14.6
Regular use of other analgesics*, %	29.4
	Median (10th–90th percentile)
Estradiol, pg/mL	
Follicular	46.6 (22.1 – 100.9)
Luteal	134 (72 – 238)
Free estradiol, pg/mL	
Follicular	0.6 (0.3 – 1.2)
Luteal	1.7 (0.9 – 2.9)
Estrone, pg/mL	
Follicular	40.4 (25.1 – 67.7)
Luteal	84.2 (51.0 – 143.6)
Estrone sulfate, pg/mL	
Follicular	661 (297 – 1518)
Luteal	1459 (572 – 3320)
DHEA, ng/dL (luteal/random)	612 (346 – 1127)
DHEAS, ug/dL (luteal/random)	86.9 (39.5 – 163.0)
Progesterone, ng/dL (luteal)	1398 (249 – 2695)
Testosterone, ng/dL§	23.6 (14.3 – 36.9)
Free testosterone, ng/dL§	0.2 (0.1 – 0.4)
Androstenedione, ng/dL§	100 (61 – 164)
Prolactin, ng/mL§	14.6 (8.3 – 28.8)
Ratio of follicular estrone/androstenedione	0.4 (0.2 – 0.8)
Ratio of luteal estrone/androstenedione	0.8 (0.4 – 1.4)
Ratio of follicular estradiol/testosterone	2.2 (1.1 – 5.4)

	Mean (SD) or %
Ratio of luteal estradiol/testosterone	5.9 (2.8 – 10.4)

* Use more than once per week in both 1997 and 1999

§ Average of follicular and luteal measures, or untimed

Table 2

Adjusted geometric mean levels of sex steroid hormones by average frequency of aspirin use in 1997 and 1999 among 2034 premenopausal women in the Nurses' Health Study II

		Frequency of use (days/week)*			Percent difference†	P-value for trend‡
	N	0	1	2+		
Maximum N		1634	183	217		
Estradiol, pg/mL						
Follicular	1422	47	47	49	5.6	0.18
Luteal	1553	133	127	126	−5.9	0.25
Luteal – ovulatory cycles	1342	138	133	130	−5.4	0.26
Free estradiol, pg/mL						
Follicular	1386	0.58	0.60	0.62	5.5	0.20
Luteal	1537	1.7	1.6	1.6	−4.3	0.58
Luteal – ovulatory cycles	1325	1.7	1.7	1.7	−4.0	0.53
Estrone, pg/mL						
Follicular	1442	40	44	43	7.1	0.07
Luteal	1601	86	82	80	−6.3	0.12
Luteal – ovulatory cycles	1377	86	84	81	−5.0	0.26
Estrone sulfate, pg/mL						
Follicular	447	679	732	615	−9.4	0.44
Luteal	452	1413	1559	1296	−8.3	0.64
Luteal – ovulatory cycles	404	1455	1534	1276	−12.3	0.37
DHEA, ng/dL (luteal/random)	479	611	612	672	10.1	0.16
DHEAS, ug/dL (luteal/random)	1254	84	87	80	−4.1	0.15
Progesterone, ng/dL (luteal)	1619	1075	1135	980	−8.9	0.23
Progesterone, ng/dL (luteal-ovulatory cycles)	1386	1529	1468	1394	−8.8**	0.24
Testosterone, ng/dL§	2000	23	23	24	1.7	0.99
Free testosterone, ng/dL§	1939	0.19	0.20	0.20	3.5	0.53
Androstenedione, ng/dL§	633	100	98	98	−2.4	0.24
Prolactin, ng/mL§	1314	15	14	16	2.6	0.77

		Frequency of use (days/week)*			Percent difference†	P-value for trend‡
	N	0	1	2+		
Ratio of follicular estrone/androstenedione	468	0.43	0.43	0.43	−0.5	0.77
Ratio of luteal estrone/androstenedione	487	0.76	0.76	0.80	6.2	0.09
Ratio of luteal estrone/ androstenedione – ovulatory cycles	435	0.77	0.75	0.78	1.6	0.35
Ratio of follicular estradiol/testosterone	437	2.4	1.9	2.1	−10.6	0.42
Ratio of luteal estradiol/testosterone	1553	5.7	5.3	5.4	5.3	0.73
Ratio of luteal estradiol/testosterone – ovulatory cycles	1334	6.0	5.6	5.7	−4.2	0.87

NOTE: Adjusted for age at blood draw (continuous), fasting status at blood draw (follicular and luteal phase), date and time of blood draw (follicular and luteal phase), race/ethnicity, parity, age at first birth, BMI (continuous), physical activity, smoking history, duration of oral contraceptive use among past users, alcohol intake, and frequency of use of other analgesics

*Average of frequency reported in 1997 and 1999

†Percent difference for highest vs. lowest category of aspirin use; calculated using $e^{\beta}-1$

‡Weighted by the midpoint of each frequency category (0, 1, 2–3, 4–5, or 6+ days of use per week) and calculated using the Wald test

§Average of follicular and luteal measures, or untimed

**P<0.05

Table 3

Adjusted geometric mean levels of sex steroid hormones by average frequency of non-aspirin NSAID use in 1997 and 1999 among 2034 premenopausal women in the Nurses' Health Study II

		Frequency of use (days/week)*				Percent difference†	P-value for trend‡
	N	0	1	2 to 3	4+		
Maximum N		949	567	342	176		
Estradiol, pg/mL							
Follicular	1422	47	47	46	53	13.2	0.11
Luteal	1553	132	133	133	125	−5.5	0.98
Luteal – ovulatory cycles	1342	136	136	142	130	−4.8	0.77
Free estradiol, pg/mL							
Follicular	1386	0.59	0.58	0.58	0.66	13.5**	0.11
Luteal	1537	1.7	1.7	1.7	1.5	−8.2	0.54
Luteal – ovulatory cycles	1325	1.7	1.7	1.8	1.5	−9.6**	0.40
Estrone, pg/mL							
Follicular	1442	41	41	41	41	1.1	0.96
Luteal	1601	85	85	87	79	−7.2	0.64
Luteal – ovulatory cycles	1377	85	85	88	78	−8.1**	0.52
Estrone sulfate, pg/mL							
Follicular	447	655	663	734	715	9.2	0.24
Luteal	452	1363	1419	1483	1474	8.2	0.28
Luteal – ovulatory cycles	404	1373	1484	1584	1352	−1.6	0.75
DHEA, ng/dL (luteal/random)	479	606	612	685	572	−5.7	0.67
DHEAS, ug/dL (luteal/random)	1254	83	88	84	77	−6.2	0.25
Progesterone, ng/dL (luteal)	1619	1090	1069	1061	960	−12.1	0.20
Progesterone, ng/dL (luteal-ovulatory cycles)	1386	1512	1476	1559	1526	0.9	0.44
Testosterone, ng/dL§	2000	23	23	23	23	−0.5	0.79
Free testosterone, ng/dL§	1939	0.20	0.20	0.19	0.19	−4.3	0.33
Androstenedione, ng/dL§	633	100	98	102	98	−1.9	0.90
Prolactin, ng/mL§	1314	15	14	16	16	2.4	0.24

	N	Frequency of use (days/week)* 0	1	2 to 3	4+	Percent difference†	P-value for trend‡
Ratio of follicular estrone/androstenedione	468	0.44	0.43	0.44	0.39	−12.5	0.36
Ratio of luteal estrone/androstenedione	487	0.76	0.79	0.76	0.65	−15.1	0.29
Ratio of luteal estrone/ androstenedione – ovulatory cycles	435	0.78	0.80	0.77	0.61	−20.9 **	0.05
Ratio of follicular estradiol/testosterone	437	2.3	2.2	2.4	2.4	1.7	0.65
Ratio of luteal estradiol/testosterone	1553	5.7	5.6	5.7	5.3	−7.1	0.40
Ratio of luteal estradiol/ testosterone – ovulatory cycles	1334	5.9	5.8	6.2	5.5	−7.5	0.54

NOTE: Adjusted for age at blood draw (continuous), fasting status at blood draw (follicular and luteal phase), date and time of blood draw (follicular and luteal phase), race/ethnicity, parity, age at first birth, BMI (continuous), physical activity, smoking history, duration of oral contraceptive use among past users, alcohol intake, and frequency of use of other analgesics

*Average of frequency reported in 1997 and 1999

†Percent difference for highest vs. lowest category of acetaminophen use; calculated using $e^{\beta}-1$

‡Weighted by the midpoint of each frequency category (0, 1, 2–3, 4–5, or 6+ days of use per week) and calculated using the Wald test

§Average of follicular and luteal measures, or untimed

**P<0.05

Table 4

Adjusted geometric mean levels of sex steroid hormones by average frequency of acetaminophen use in 1997 and 1999 among 2034 premenopausal women in the Nurses' Health Study II

		Frequency of use (days/week)*			Percent difference†	P-value for trend‡
	N	0	1	2+		
Maximum N		1367	457	210		
Estradiol, pg/mL						
Follicular	1422	47	47	49	5.1	0.21
Luteal	1553	133	131	126	−5.4	0.09
Luteal – ovulatory cycles	1342	138	135	130	−5.9	0.11
Free estradiol, pg/mL						
Follicular	1386	0.58	0.60	0.60	2.8	0.41
Luteal	1537	1.7	1.7	1.6	−3.4	0.32
Luteal – ovulatory cycles	1325	1.7	1.7	1.6	−5.8	.24
Estrone, pg/mL						
Follicular	1442	41	40	39	−6.5	0.20
Luteal	1601	86	84	82	−4.7	0.18
Luteal – ovulatory cycles	1377	86	84	81	−5.7	0.19
Estrone sulfate, pg/mL						
Follicular	447	684	676	610	−10.8	0.49
Luteal	452	1463	1261	1346	−8.0	0.42
Luteal – ovulatory cycles	404	1468	1328	1471	0.2	0.88
DHEA, ng/dL (luteal/random)	479	637	582	578	−9.3	0.20
DHEAS, ug/dL (luteal/random)	1254	85	82	79	−6.7	0.47
Progesterone, ng/dL (luteal)	1619	1070	1071	1064	−0.5	0.50
Progesterone, ng/dL (luteal-ovulatory cycles)	1386	1518	1524	1416	−6.7	0.32
Testosterone, ng/dL§	2000	23	23	22	−4.8	0.10
Free testosterone, ng/dL§	1939	0.20	0.20	0.18	−7.1**	0.04
Androstenedione, ng/dL§	633	100	98	99	−1.3	0.72
Prolactin, ng/mL§	1314	15	15	14	−11.8**	0.04
Ratio of follicular estrone/androstenedione	468	0.42	0.47	0.43	1.8	0.29

	N	Frequency of use (days/week)* 0	1	2+	Percent difference†	P-value for trend‡
Ratio of luteal estrone/androstenedione	487	0.76	0.78	0.73	−4.7	0.64
Ratio of luteal estrone/ androstenedione – ovulatory cycles	435	0.77	0.80	0.75	−2.5	0.87
Ratio of follicular estradiol/testosterone	437	2.3	2.5	2.4	7.9	0.33
Ratio of luteal estradiol/testosterone	1553	5.6	5.6	5.8	4.1	0.75
Ratio of luteal estradiol/testosterone – ovulatory cycles	1334	5.9	6.0	5.8	−2.1	0.68

NOTE: Adjusted for age at blood draw (continuous), fasting status at blood draw (follicular and luteal phase), date and time of blood draw (follicular and luteal phase), race/ethnicity, parity, age at first birth, BMI (continuous), physical activity, smoking history, duration of oral contraceptive use among past users, alcohol intake, and frequency of use of other analgesics

*Average of frequency reported in 1997 and 1999

†Percent difference for highest vs. lowest category of acetaminophen use; calculated using $e^{\beta}-1$

‡Weighted by the midpoint of each frequency category (0, 1, 2–3, 4–5, or 6+ days of use per week) and calculated using the Wald test

§Average of follicular and luteal measures, or untimed

**P<0.05