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Analgesic Use and Patterns of Estrogen Metabolism in Premenopausal Women

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Abstract

Analgesic use has been hypothesized to reduce the risk of some cancers, with inverse associations between analgesics and colon cancer, and suggestive inverse associations for breast cancer. Estrogen metabolites (EM) have genotoxic and estrogenic potential; genotoxicity may differ by hydroxylation pathway. Analgesic use may impact patterns of estrogen metabolism; effects of analgesics on disease risk could be mediated through these patterns. We conducted a cross-sectional study among 603 premenopausal women in the Nurses' Health Study II. Frequency of aspirin, non-aspirin non-steroidal anti-inflammatory drugs (NSAIDs), and acetaminophen use was reported via questionnaire; average frequency in 1997 and 1999 was calculated. Women provided urine samples between 1996-1999, collected during the mid-luteal phase of the menstrual cycle. Urinary EM were quantified using high-performance liquid chromatography-tandem mass spectrometry. We observed no association between analgesic use and estradiol, estrone, or specific pathways of estrogen metabolism. Women reporting more frequent aspirin use had lower methylated 2-catechols (e.g., 2-hydroxyestrone-3-methyl-ether, 2+ days/week vs. non-use: 0.95 vs. 1.21 pmol/mg creatinine, $p_{\text{difference}}=0.01$; $p_{\text{trend}}=0.07$). Non-aspirin NSAID use was positively associated with 17-epiestriol (4+ days/week vs. non-use: 2.48 vs. 1.52 pmol/mg creatinine; $p_{\text{difference}}=0.01$, $p_{\text{trend}}=0.11$). Acetaminophen use was positively associated with total EM (2+ days/week vs. non-use: 236 vs. 198 pmol/mg creatinine; $p_{\text{difference}}=0.02$, $p_{\text{trend}}=0.11$), 2-hydroxyestrone-3-methyl ether (1.6 vs. 1.1 pmol/mg creatinine; $p_{\text{difference}}<0.01$, $p_{\text{trend}}=0.02$), and 16 α -hydroxyestrone (17 vs. 12 pmol/mg creatinine $p_{\text{difference}}=0.01$, $p_{\text{trend}}=0.05$). This study provides the first assessment of analgesic use and patterns of estrogen metabolism in women. While we observed some associations between analgesics and individual EM, no clear patterns emerged.

Keywords

estrogen metabolism; urinary estrogens; premenopausal; analgesics; NSAIDs

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Conflict of Interest

The authors declare that they have no conflict of interest.

Introduction

Prior studies suggest inverse associations between analgesic use, specifically nonsteroidal anti-inflammatory drugs (NSAIDs), and cancer risk, though results are not consistent across different types of cancer [1-3]. The inverse association between NSAID use and colon cancer is well documented [4, 5]; results for breast cancer are less consistent, but suggest a possible protective effect [1, 2, 6-9]. Sex steroids, including estrogens, have previously been shown to be associated with colon and breast cancers [10-13], and analgesics may impact these cancers through patterns of estrogen metabolism.

We previously described the associations between analgesics and premenopausal plasma sex steroids [14], in the only study of this association to date. We observed few associations between analgesics and plasma estradiol and estrone (parent estrogens), with the exception of non-aspirin NSAIDs. Plasma free estradiol, assessed in the follicular phase, was significantly higher among women reporting use 4 days/week vs. non-users (13.5% higher with use 4 days/week vs. non-users; $p_{\text{difference}}=0.04$, $p_{\text{trend}}=0.11$). In the luteal phase, after excluding women providing a blood sample during an anovulatory cycle, non-aspirin NSAID use 4 days/week vs. no use was associated with 9.6% lower free estradiol and 8.1% lower estrone ($p_{\text{difference}}<0.05$). To our knowledge, there are no prior data on the association between analgesic use and patterns of estrogen metabolism in premenopausal women.

Estrogen is metabolized by hydroxylation at the 2-, 4-, and 16- carbon positions, denoting three different pathways, and estrogen metabolites (EM) have been shown to have differential biologic effects [15, 16]. Little is known about patterns of estrogen metabolism in premenopausal women beyond 2-hydroxyestrone and 16 α -hydroxyestrone, the two metabolites that previously have been best and most frequently measured. Recent advances allow quantification of 15 estrogens and estrogen metabolites using liquid chromatography-tandem mass spectrometry [17, 18]. Given our limited understanding of correlates of estrogen metabolism, we assessed the relationship between analgesic use and 15 urinary estrogens and EM, as well as EM grouped in metabolic pathways, in a cross-sectional analysis in the Nurses' Health Study II (NHSII).

Methods

Study Population

The NHSII was established in 1989 when 116,430 female registered nurses, ages 25 to 42, completed and returned a mailed questionnaire. The cohort continues to be followed through biennial questionnaires. Between 1996 and 1999, 29,611 participants provided blood and urine samples. Details of the blood and urine collection have been published previously [19, 20]. Briefly, premenopausal participants who had not used oral contraceptives, been pregnant or breastfeed within the past 6 months provided blood samples timed within the early follicular (3-5 days of menstrual cycle) and mid-luteal (anticipated 7-9 days prior to next menstrual cycle) phase of the menstrual cycle ($n=18,521$); the remainder provided untimed samples. A single urine sample was collected during the mid-luteal phase. Participants completed a questionnaire at the time of blood and urine collection and returned samples, packed with an ice pack, to our laboratory where they were processed; 93% of samples were received within 26 hours of collection. Participants returned postcards with the date of their next menstrual period to allow precise calculation of luteal day of collection. Samples have been stored since collection at -130°C in continuously monitored liquid nitrogen freezers.

This cross-sectional analysis includes 603 premenopausal women who provided a luteal urine sample and were selected as controls in a nested case-control study of breast cancer (n=493) [19] or were participants in a reproducibility study in the NHSII (n=110) [21].

This investigation was approved by the Institutional Review Board of the Brigham and Women's Hospital.

Exposure and Covariate Data

Analgesic use was reported on the biennial questionnaires. Participants reported use of acetaminophen, aspirin or aspirin-containing products, and NSAIDs separately. Regular analgesic use (2+ days/week) was reported in 1989; frequency of analgesic use (never, 1, 2-3, 4-5, 6+ days/week) was reported on the 1993-1999 biennial questionnaires. We calculated frequency of analgesic use as the average of the frequencies reported in 1997 and 1999 and duration of analgesic use using data collected from 1993-1999.

Covariate data were collected on the biennial questionnaires, as well as the questionnaire returned with the blood and urine samples. Specially, age, whether the sample was the first morning urine sample, and weight (used to calculate BMI), were reported on the questionnaire completed at sample collection. Luteal day of collection was calculated using the date of next menstrual period provided on a returned postcard. The remaining covariates were reported on the biennial questionnaires (year of collection): alcohol consumption (1997 and 2001, averaged), physical activity (1997 and 2001, averaged), usual menstrual cycle length and pattern (1993), age at first birth and parity (1997), height (used to calculate BMI, 1989), current smoking (1997), and frequency of other analgesic use (1997 and 1999, averaged).

Laboratory Assays

Urine samples were assayed for estrogen metabolites at the Laboratory of Proteomics and Analytical Technology, SAIC-Frederick, Inc., Frederick, MD using liquid chromatography-tandem mass spectrometry (LC-MS/MS). In brief, LC-MS/MS was performed with a TSQ Quantum-AM triple quadrupole mass spectrometer coupled with a Surveyor high performance liquid chromatography system (Thermo, San Jose, CA). Both the liquid chromatography system and mass spectrometer were controlled by Xcalibur software (Thermo). Assay details have been published previously [17, 19, 22].

Estrogen metabolite values were adjusted for creatinine to account for urine volume. Creatinine was measured in three batches at the Endocrine Core Laboratory at Emory University (Atlanta, GA), Boston Children's Hospital (Boston, MA), and Brigham and Women's Hospital (Boston, MA). Plasma progesterone was used to determine whether the menstrual cycle of urine collection was ovulatory (progesterone >400 ng/dL). Progesterone was assayed at Quest Diagnostics-Nichols Institute (San Juan Capistrano, CA) and the Royal Marsden Hospital (London, U.K.).

Masked quality control samples (10%) were included in all projects. Overall coefficients of variation (CVs) based on these samples were <7% for all of the EMs with the exception of 4-methoxyestrone (17%) and 4-methoxyestradiol (15%). CVs for creatinine were 9.2% and CVs for progesterone were 17%.

Statistical Analyses

Urinary EM (pmol/mL) were adjusted for creatinine, resulting in picomoles per milligram of creatinine, and log transformed to achieve a more normal distribution. Outliers were assessed using the extreme Studentized deviate, many-outlier procedure [23], resulting in the

exclusion of up to 16 outlying values (2-methoxyestradiol). EM were evaluated individually, as well as by grouped by pathway (e.g. 2-hydroxylation pathway, catechols) and pathway ratios.

Frequency of each type of analgesic use (days/week) was analyzed as the average of weighted midpoints of the reported 1997 and 1999 frequencies. Exposure variables were split into three (aspirin, acetaminophen) or four (non-aspirin NSAIDs) categories, depending on the sample size. Duration of each type of analgesic use was calculated from 1989 to 1999, among participants reporting analgesic use 2 days per week (i.e., regular users).

We used generalized linear models to calculate geometric means. Statistical models adjusted for: age (years; continuous), laboratory batch (1, 2), days between collection and next menstrual period (<5, 6-7, 8-9, 10 days), first morning urine (yes, no), body mass index (kg/m^2 ; continuous), height (inches; continuous), physical activity (MET-hrs/week; continuous), menstrual cycle pattern (extremely regular/no more than 1-2 days deviation from expected, very regular/within 3-4 days, regular/within 5-7 days, usually/always irregular), usual menstrual cycle length (<26, 26-31, 32 days), age at first birth/parity (nulliparous, age at first birth <25 years/1-2 children, age at first birth 25 to 29 years/1-2 children, age at first birth 30 years/1-2 children, age at first birth <25 years/ 3 children, age at first birth 25 years/ 3 children), ovulatory status of collection cycle (yes, no), alcohol consumption (non-drinker, up to 3 drinks/month, 3 drinks/month to 2 drinks/week, 2 drinks/week to 5 drinks/week, more than 5 drinks/weeks), smoking status (never, past, current), and frequency of other analgesic use (e.g., aspirin analyses were adjusted for acetaminophen and non-aspirin NSAID use).

Analyses were conducted in SAS version 9.3 (Cary, NC). P-values were considered statistically significant if $p < 0.05$.

Results

Participant characteristics were similar across frequency of use of all analgesics combined (Table 1). Women were on average 43 years of age at urine collection, and predominantly parous, past users of oral contraceptives, and non-smokers. The majority of participants provided a first morning urine sample and provided a sample during an ovulatory menstrual cycle. A higher proportion of women reporting analgesic use 4 days/week provided a sample during an anovulatory menstrual cycle, as compared to women reporting no analgesic use (14.9% vs. 9.4% anovulatory).

Overall, similar patterns of baseline characteristics were observed across frequencies of each individual analgesic (data not shown). However, there were differences in parity, smoking, luteal day of collection and proportion of samples provided in anovulatory cycles. Parity was lower among aspirin and non-aspirin NSAIDs users compared to non-users (e.g., non-aspirin NSAIDs, 4 days/week vs. 0 days/week 77.2% vs. 86.0% parous), but higher in acetaminophen users (2 days/week vs. 0 days/week 91.8% vs. 82.0% parous). Prevalence of current smoking was elevated in the highest category of non-aspirin NSAID use (4 days/week: 17.2%). Finally, a lower proportion of acetaminophen users (2 days/week) provided a sample outside 3-10 days before next menstrual period (7.1%) or in an anovulatory menstrual cycle (4.7%), while a higher proportion of aspirin users (2 days/week) provided a sample outside of this window (18.5%) or in an anovulatory menstrual cycle (18.1%).

Aspirin use was not associated with total EM or the parent estrogens, estradiol and estrone (Table 2). More frequent aspirin use was inversely associated with methylated catechols, with significant differences between non-users and use 2 days per week (non-users vs. use 2 days per week: 10.4 vs. 8.6 pmol/mg creatinine, $p_{\text{difference}}=0.04$, $p_{\text{trend}}=0.13$). Results

were similar for methylated 2-catechols, 2-methoxyestradiol, and 2-hydroxyestrone-3-methyl ether. Following these associations, aspirin was positively associated with the EM ratios that included methylated catechols in the denominator (e.g. 2-catechols/methylated 2-catechols, non-users vs. use 2 days per week: 5.3 vs. 6.2 pmol/mg creatinine, $p_{\text{difference}}=0.04$, $p_{\text{trend}}=0.05$). We observed no association between aspirin and estrogen metabolites on the 4- or 16-hydroxylation pathways, and no association between duration of regular aspirin use and any of the EM (data not shown).

We observed no associations between non-aspirin NSAID use and EM, with the exception of 17-epiestriol (Table 3). Non-aspirin NSAIDs were positively associated with 17-epiestriol (non-users vs. use 4 days/week: 1.52 vs. 2.48 pmol/mg creatinine, $p_{\text{difference}}=0.01$, $p_{\text{trend}}=0.11$). In analyses considering duration of regular non-aspirin NSAID use, longer duration of use was associated with lower 2-methoxyestrone (never users vs. 6 years of regular use: 8.46 vs. 4.93 pmol/mg creatinine, $p_{\text{difference}}=0.01$, $p_{\text{trend}}=0.01$) and 16 α -hydroxyestrone (never users vs. 6 years of regular use: 15.50 vs. 11.79, $p_{\text{difference}}=0.16$, $p_{\text{trend}}=0.04$) (data not shown).

Acetaminophen use was positively associated with total EM, as well as individual metabolites on the 2- and 16-hydroxylation pathways (Table 4). As compared to non-users, participants using acetaminophen 2 days per week had significantly higher total EM (non-users vs. 2 days per week: 198 vs. 236 pmol/mg creatinine, $p_{\text{difference}}=0.02$, $p_{\text{trend}}=0.11$), 2-hydroxyestrone-3-methyl ether (non-users vs. 2 days per week: 1.1 vs. 1.6 pmol/mg creatinine, $p_{\text{difference}}<0.01$, $p_{\text{trend}}=0.02$) and 16 α -hydroxyestrone (non-users vs. 2 days per week: 12 vs. 17 pmol/mg creatinine, $p_{\text{difference}}=0.01$, $p_{\text{trend}}=0.05$). Acetaminophen use was inversely associated with several of the metabolic ratios including the 4-/2-catechol ratio, 4-/2-pathway ratio, 4-/16-pathway ratio, and the 2-pathway/parent estrogens ratio. Longer duration of acetaminophen use was positively associated with methylated catechols (non-users vs. 6 years use: $p_{\text{difference}}=0.09$, $p_{\text{trend}}=0.04$), methylated 2-catechols (non-users vs. 6 years use: $p_{\text{difference}}=0.08$, $p_{\text{trend}}=0.03$), 2-hydroxyestrone-3-methyl ether (non-users vs. 6 years use: $p_{\text{difference}}<0.01$, $p_{\text{trend}}<0.01$), and 16 α -hydroxyestrone (non-users vs. 6 years use: $p_{\text{difference}}=0.03$, $p_{\text{trend}}=0.06$) (data not shown).

We conducted sensitivity analyses restricted to women with ovulatory cycles (plasma luteal progesterone >400 ng/dL; n=542), who provided a first morning urine sample (n=472), who were not perimenopausal at time of urine collection (i.e., reported onset of menopause within 4 years of urine collection; n=529), whose urine sample was collected within 4-10 days of their next menstrual period (n=516), and who did not report a medical condition that may be associated with both estrogen metabolism and analgesic use (uterine fibroids, premenstrual syndrome, rheumatoid arthritis, and osteoarthritis; (n=465)). Overall, results were similar in these subgroups (data not shown).

We examined whether the association between analgesics and EM differed by BMI and found no significant differences between women with BMI <25 vs. those with BMI $\geq$ 25 for aspirin and acetaminophen. The association between non-aspirin NSAIDs and estriol was significantly different by BMI ($p_{\text{heterogeneity}}=0.02$), with an inverse association in those with BMI <25 (non-users vs. 4 days per week: 31 vs. 22 pmol/mg creatinine, $p_{\text{difference}}=0.09$, $p_{\text{trend}}=0.06$) and a positive association in those with BMI $\geq$ 25 (non-users vs. 4 days per week: 34 vs. 44 pmol/mg creatinine, $p_{\text{difference}}=0.17$, $p_{\text{trend}}=0.06$). In addition, many of the pathway ratios with the 16-hydroxylation pathway in the denominator were significantly different between women with BMI <25 and $\geq$ 25 (e.g., 2-pathway/16-pathway: BMI <25, non-users vs. 4 days per week: 0.94 vs. 1.06 pmol/mg creatinine, $p_{\text{difference}}=0.61$, $p_{\text{trend}}=0.04$; BMI $\geq$ 25, non-users vs. 4 days per week: 0.85 vs. 0.56 pmol/mg creatinine, $p_{\text{difference}}=0.09$, $p_{\text{trend}}=0.03$; $p_{\text{interaction}}=0.01$).

Finally, we restricted the sample for each analgesic to exclusive users of that analgesic (i.e., for non-aspirin NSAIDs excluded participants reporting aspirin or acetaminophen use). We were unable to evaluate associations between analgesics and EM in the exclusive aspirin and acetaminophen user subgroups because there were very few exclusive users. Results for exclusive users of non-aspirin NSAIDs (N=352 in the analysis; 158 users) were not materially different than the overall results.

Discussion

In this detailed evaluation of the association between analgesic use and patterns of estrogen metabolism in premenopausal women we observed few consistent associations between analgesics and urinary luteal EM. We observed inverse associations between frequent aspirin use and EM on the 2-hydroxylation pathway, including the methylated 2-catechols, 2-methoxyestradiol and 2-hydroxyestrone-3-methyl ether. However, use of non-aspirin NSAIDs was not associated with any EM, with the exception of 17-epiestriol, for which we observed a positive association. Acetaminophen use was positively associated with total estrogens and EM, 2-hydroxyestrone-3-methyl ether, and 16 α -hydroxyestrone. Results were consistent in normal weight and overweight/obese women for aspirin and acetaminophen while there was some evidence that BMI modified the effect of non-aspirin NSAIDs on patterns of estrogen metabolism.

To our knowledge, this study is the first to evaluate analgesic use and patterns of estrogen metabolism, and there is only a single prior study evaluating the association between analgesic use and premenopausal estrogens [14]. In our previous study of analgesic use and premenopausal plasma hormones, including estradiol and estrone, we observed significantly higher follicular free estradiol and lower luteal free estradiol and estrone with increased frequency of non-aspirin NSAID use (luteal differences statistically significant after restricting to ovulatory cycles). There were no differences in plasma estrogen levels associated with aspirin or acetaminophen use [14]. We observed no associations between analgesic use and urinary luteal estrone or estradiol in the current analysis. Our prior analysis investigated plasma parent estrogens whereas the present study investigated urinary estrogen metabolites; this may limit comparability between the two studies. Luteal estrone and estradiol from plasma and urinary samples are modestly correlated (Spearman correlations: estrone, $r=0.56$; estradiol, $r=0.36$) [21].

A possible mechanism linking analgesics and estrogens may be via their effect on cyclooxygenases (e.g., COX-1, COX-2) and prostaglandins [24, 25]. While evidence is more established for NSAID inhibition of COX-1 and COX-2, data suggest acetaminophen may also act through reductions in COX-2 [26]. Inhibition of COX-1 and COX-2 results in lower prostaglandin E2 and reduced aromatase (CYP19) activity, the key enzyme in the conversion of testosterone to estradiol and androstenedione to estrone [27]. The effect of aromatase inhibition may be differential by menopausal status, with lower aromatase resulting in lower estrogen levels in postmenopausal women but higher estrogen levels in premenopausal women, due to compensatory feedback loops [28]. In the current study, we observed higher total estrogens and estrogen metabolites with more frequent acetaminophen use, but no associations between aspirin and non-aspirin NSAIDs and total estrogens and estrogen metabolites. To our knowledge, the influence of COX and prostaglandins on estrogen metabolism has not been previously studied.

One limitation to our study is that we have only a single urine sample per participant, however a reproducibility study suggests stability over three years for the three hydroxylation pathways (interclass correlation coefficient (ICC) range: 0.52 for the 16-hydroxylation pathway to 0.72 for the 2-hydroxylation pathway) [21]. Analgesic use was

collected in close temporal proximity to blood collection in this cross-sectional analysis, and therefore we cannot rule out reverse causation. Urine samples were collected during the luteal phase of the menstrual cycle, therefore we cannot make comparisons to plasma follicular results. Additionally, we have limited data on duration of analgesic use in this cohort given the limited number of regular analgesic users.

The strengths include the fact that this analysis is the first to examine the association between analgesics and patterns of estrogen metabolism in all 15 urinary estrogens and estrogen metabolites. The study population is well characterized, with detailed information on analgesic use and data on potential confounders collected at or near urine collection, allowing a thorough evaluation of the association between analgesics and EM.

In summary, in this cross-sectional analysis we found few consistent associations between analgesic use and patterns of estrogen metabolism in premenopausal women. Further research is warranted to confirm the associations we observed with individual estrogen metabolites for use of aspirin, non-aspirin NSAIDs, and acetaminophen.

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Table 1

Characteristics of study population by the frequency of any analgesic use at urine collection in the Nurses' Health Study II (n=603)

	Frequency of any analgesic use (days/week)			
	0	1	2 to 3	4+
	194	150	134	125
Age in years	42.6 (4.0)	42.6 (3.7)	43.1 (3.7)	43.4 (3.8)
Body mass index (kg/m ²)	23.9 (3.7)	24.7 (4.0)	25.3 (4.1)	26.9 (4.9)
Height (inches)	64.9 (2.4)	65.4 (2.0)	64.8 (2.3)	65.2 (2.0)
Physical activity (MET-hr/week) ^a	23.1 (19.2)	20.7 (15.7)	22.4 (15.4)	22.4 (17.7)
Parous, %	85.8	77.8	85.4	76.0
Parity ^b	2.1(1.0)	1.7(1.0)	2.0(1.0)	1.8(1.0)
Age at first birth ^b (years)	26.5(3.6)	26.3(4.1)	26.7(4.2)	26.2(3.7)
Past oral contraceptive use, %	77.9	87.0	89.2	87.4
Alcohol intake (g/day) ^c	3.7(5.2)	4.3(5.0)	4.7(6.6)	3.6(4.4)
Current smoker, %	5.9	6.4	7.4	8.9
Age at menarche <12 yrs, %	19.2	19.9	21.2	25.7
Menstrual cycle length 26-31 days, %	72.5	60.0	67.4	74.1
Regular menstrual cycles ^d	97.1	92.7	96.5	93.8
First morning urine, %	88.3	75.3	80.2	78.5
Luteal day outside 3-10 days before next period, %	12.7	11.8	15.9	14.1
Anovulatory cycle, %	9.4	5.7	7.7	14.9
Creatinine, mg/L	1150 (542)	1132 (439)	1170 (503)	1034 (446)

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

^a Average of 1997 and 2001 activities.

^b Among parous women.

^c Average of 1995 and 1999 intakes.

^d Menstrual cycles begin within 5 to 7 days of expected

Table 2

Adjusted geometric means (pmol/mg creatinine) of urinary estrogen and estrogen metabolite levels by average frequency of aspirin use in 1997 and 1999: Nurses' Health Study II (n = 603)

	<u>Aspirin Frequency of use (tablet/wk)</u>			p for difference *	p for trend
	0	1	2+		
N	486	44	73		
Total estrogens and estrogen metabolites	199	219	193	0.60	0.91
Parent estrogens	41	47	39	0.57	0.74
Estrone	28	30	26	0.56	0.80
Estradiol	14	15	13	0.51	0.70
Catechols	61	66	58	0.61	0.82
2-Catechols	54	57	51	0.61	0.83
2-Hydroxyestrone	48	50	45	0.63	0.86
2-Hydroxyestradiol	5.3	6.2	5.1	0.73	0.95
4-Catechols					
4-Hydroxyestrone	5.6	6.1	5.1	0.45	0.70
Methylated catechols	10.4	10.2	8.6	0.04	0.13
Methylated 2-catechols	10.0	9.7	8.3	0.05	0.14
2-Methoxyestrone	7.8	7.6	6.6	0.08	0.18
2-Methoxyestradiol	0.72	0.69	0.60	0.06	0.10
2-Hydroxyestrone-3-methyl ether	1.21	1.26	0.95	0.01	0.07
Methylated 4-catechols	0.21	0.25	0.21	0.84	0.66
4-Methoxyestrone	0.13	0.16	0.14	0.93	0.20
4-Methoxyestradiol	0.05	0.05	0.04	0.21	0.27
2-Hydroxylation pathway	65	68	60	0.37	0.57
4-Hydroxylation pathway	5.9	6.7	5.6	0.61	0.89
16-Hydroxylation pathway	70	72	72	0.74	0.51
16 α -Hydroxyestrone	12	13	12	0.76	0.75
Estriol	30	31	32	0.55	0.47
17-Epiestriol	1.62	1.44	1.67	0.81	0.77
16-Ketoestradiol	15	15	13	0.20	0.11
16-Epiestriol	6.3	7.3	6.6	0.54	0.41
<i>Ratios of metabolic pathways</i>					
4-Catechols/2-catechols	0.10	0.10	0.10	0.88	0.87
2-Catechols/16-pathway	0.76	0.77	0.73	0.74	0.76
Catechols/16-pathway	0.86	0.82	0.84	0.89	0.93
4-Pathway/2-pathway	0.09	0.11	0.09	0.95	0.92
2-Pathway/16-pathway	0.92	0.85	0.87	0.63	0.69
4-pathway/16-pathway	0.09	0.09	0.08	0.70	0.78
2,4-pathway/16-pathway	1.0	0.97	0.98	0.66	0.70
2-pathway/4,16-pathway	0.82	0.80	0.76	0.50	0.53
2-Catechols/methylated 2-catechols	5.3	5.7	6.2	0.04	0.05

	<u>Aspirin Frequency of use (tablet/wk)</u>			p for difference *	p for trend
	0	1	2+		
4-Catechols/methylated 4-catechols	25	25	24	0.82	0.66
Catechols/methylated catechols	5.8	6.2	6.7	0.04	0.05
Parent estrogens/estrogen metabolites	1.5	1.3	1.5	0.82	0.94
2-Pathway/parent estrogens	0.14	0.14	0.14	0.92	0.88
4-Pathway/parent estrogens	1.7	1.5	1.7	0.61	0.51
16-Pathway/parent estrogens	0.27	0.29	0.27	0.90	0.89
2-Hydroxyestrone/16 α -hydroxyestrone	3.9	3.7	4.0	0.83	0.66

Adjusted for age (continuous), laboratory batch, luteal day, first morning urine, BMI (continuous), alcohol consumption, physical activity (continuous), menstrual cycle length, cycle pattern, age at first birth/parity, height, anovulatory cycle, current smoking, average frequency of other analgesic use

*
P for difference between 2+ tablets per week and 0 tablets per week

Table 3

Adjusted geometric means (pmol/mg creatinine) of urinary estrogen and estrogen metabolite levels by average frequency of non-aspirin NSAID use in 1997 and 1999: Nurses' Health Study II (n = 603)

	<u>Non-Aspirin NSAID Frequency of use (tablet/wk)</u>				p for difference *	p for trend
	0	1	2-3	4+		
N	277	181	105	40		
Total estrogens and estrogen metabolites	202	199	196	199	0.87	0.78
Parent estrogens	41	42	41	41	0.87	0.42
Estrone	28	28	26	27	0.68	0.99
Estradiol	13	14	14	13	0.87	0.47
Catechols	60	60	67	59	0.88	0.27
2-Catechols	52	53	59	52	0.95	0.24
2-Hydroxyestrone	46	47	53	46	0.98	0.25
2-Hydroxyestradiol	5.1	5.3	6.0	5.0	0.79	0.18
4-Catechols						
4-Hydroxyestrone	5.7	5.4	5.6	5.6	0.93	0.50
Methylated catechols	10	10	10	9	0.40	0.99
Methylated 2-catechols	10.0	9.5	9.8	9.0	0.40	0.98
2-Methoxyestrone	8.0	7.4	7.5	6.9	0.26	0.60
2-Methoxyestradiol	0.72	0.68	0.70	0.67	0.60	0.92
2-Hydroxyestrone-3-methyl ether	1.2	1.1	1.3	1.2	0.84	0.28
Methylated 4-catechols	0.21	0.21	0.23	0.22	0.77	0.31
4-Methoxyestrone	0.13	0.13	0.15	0.15	0.48	0.14
4-Methoxyestradiol	0.05	0.05	0.05	0.05	0.66	0.34
2-Hydroxylation pathway	63	64	72	61	0.83	0.24
4-Hydroxylation pathway	6.0	5.8	5.9	6.2	0.85	0.50
16-Hydroxylation pathway	71	70	64	78	0.37	0.43
16 α -Hydroxyestrone	13	12	11	13	0.99	0.89
Estriol	31	31	27	32	0.96	0.95
17-Epiestriol	1.52	1.66	1.55	2.48	0.01	0.11
16-Ketoestradiol	15	14	13	17	0.17	0.36
16-Epiestriol	6.3	6.4	6.3	7.2	0.18	0.08
<i>Ratios of metabolic pathways</i>						
4-Catechols/2-catechols	0.10	0.10	0.10	0.11	0.80	0.82
2-Catechols/16-pathway	0.74	0.75	0.88	0.66	0.48	0.79
Catechols/16-pathway	0.85	0.84	0.92	0.76	0.46	0.94
4-Pathway/2-pathway	0.10	0.09	0.09	0.10	0.86	0.81
2-Pathway/16-pathway	0.90	0.90	1.01	0.79	0.42	0.98
4-pathway/16-pathway	0.09	0.08	0.09	0.08	0.55	0.88
2,4-pathway/16-pathway	1.0	1.0	1.1	0.9	0.38	0.96
2-pathway/4,16-pathway	0.79	0.81	0.93	0.70	0.38	0.92
2-Catechols/methylated 2-catechols	5.2	5.5	5.7	5.7	0.35	0.14

	Non-Aspirin NSAID Frequency of use (tablet/wk)				p for difference *	p for trend
	0	1	2-3	4+		
4-Catechols/methylated 4-catechols	26	24	23	25	0.84	0.82
Catechols/methylated catechols	5.8	6.0	6.2	6.2	0.43	0.19
Parent estrogens/estrogen metabolites	1.53	1.49	1.60	1.51	0.88	0.60
2-Pathway/parent estrogens	0.15	0.13	0.14	0.15	0.96	0.88
4-Pathway/parent estrogens	1.7	1.7	1.6	1.9	0.29	0.64
16-Pathway/parent estrogens	0.27	0.28	0.27	0.25	0.29	0.24
2-Hydroxyestrone/16 α -hydroxyestrone	3.6	4.0	4.4	3.6	0.93	0.40

Adjusted for age (continuous), laboratory batch, luteal day, first morning urine, BMI (continuous), alcohol consumption, physical activity (continuous), menstrual cycle length, cycle pattern, age at first birth/parity, height, anovulatory cycle, current smoking, average frequency of other analgesic use

*
P for difference between 4+ tablets per week and 0 tablets per week

Table 4

Adjusted geometric means (pmol/mg creatinine) of urinary estrogen and estrogen metabolite levels by average frequency of acetaminophen use in 1997 and 1999: Nurses' Health Study II (n = 603)

	<u>Acetaminophen Frequency of use (tablet/wk)</u>			p for difference *	p for trend
	0	1	2+		
N	429	122	52		
Total estrogens and estrogen metabolites	198	191	236	0.02	0.11
Parent estrogens	41	39	45	0.37	0.49
Estrone	28	26	30	0.37	0.58
Estradiol	14	13	14	0.54	0.45
Catechols	61	57	69	0.33	0.85
2-Catechols	54	50	62	0.25	0.69
2-Hydroxyestrone	48	44	56	0.21	0.64
2-Hydroxyestradiol	5.3	5.0	5.9	0.40	0.79
4-Catechols					
4-Hydroxyestrone	5.8	4.9	5.2	0.44	0.24
Methylated catechols	9.9	10.4	11.4	0.19	0.44
Methylated 2-catechols	9.5	9.9	11.1	0.17	0.43
2-Methoxyestrone	7.4	8.1	8.2	0.43	0.72
2-Methoxyestradiol	0.70	0.68	0.71	0.95	0.43
2-Hydroxyestrone-3-methyl ether	1.1	1.2	1.6	<0.01	0.02
Methylated 4-catechols	0.21	0.26	0.22	0.60	0.32
4-Methoxyestrone	0.13	0.16	0.14	0.54	0.31
4-Methoxyestradiol	0.05	0.06	0.06	0.36	0.27
2-Hydroxylation pathway	65	61	75	0.20	0.61
4-Hydroxylation pathway	6.2	5.3	5.5	0.37	0.17
16-Hydroxylation pathway	70	66	81	0.12	0.19
16 α -Hydroxyestrone	12	11	17	0.01	0.05
Estriol	30	31	31	0.77	0.61
17-Epiestriol	1.6	1.6	1.6	0.99	0.65
16-Ketoestradiol	14	14	16	0.29	0.29
16-Epiestriol	6.4	6.1	6.6	0.83	0.81
<i>Ratios of metabolic pathways</i>					
4-Catechols/2-catechols	0.11	0.09	0.08	0.07	0.03
2-Catechols/16-pathway	0.76	0.75	0.75	0.94	0.61
Catechols/16-pathway	0.86	0.86	0.80	0.59	0.38
4-Pathway/2-pathway	0.10	0.09	0.08	0.05	0.05
2-Pathway/16-pathway	0.91	0.92	0.87	0.74	0.52
4-pathway/16-pathway	0.09	0.08	0.07	0.09	0.05
2,4-pathway/16-pathway	1.0	1.0	1.0	0.64	0.43
2-pathway/4,16-pathway	0.81	0.81	0.82	0.96	0.72
2-Catechols/methylated 2-catechols	5.5	5.0	5.9	0.43	0.62

	<u>Acetaminophen Frequency of use (tablet/wk)</u>			p for difference *	p for trend
	0	1	2+		
4-Catechols/methylated 4-catechols	28	18	24	0.54	0.15
Catechols/methylated catechols	6.0	5.5	6.3	0.55	0.82
Parent estrogens/estrogen metabolites	1.5	1.6	1.6	0.73	0.84
2-Pathway/parent estrogens	0.15	0.14	0.12	0.13	0.05
4-Pathway/parent estrogens	1.66	1.69	1.79	0.41	0.45
16-Pathway/parent estrogens	0.27	0.26	0.26	0.35	0.56
2-Hydroxyestrone/16 α -hydroxyestrone	3.9	4.0	3.2	0.24	0.24

Adjusted for age (continuous), laboratory batch, luteal day, first morning urine, BMI (continuous), alcohol consumption, physical activity (continuous), menstrual cycle length, cycle pattern, age at first birth/parity, height, anovulatory cycle, current smoking, average frequency of other analgesic use

*
P for difference between 2+ tablets per week and 0 tablets per week