Analgesic Use and Circulating Estrogens, Androgens, and Their Metabolites in the Women's Health Initiative Observational Study

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ABSTRACT

Though studies have observed inverse associations between use of analgesics (aspirin, NSAIDs, and acetaminophen) and the risk of several cancers, the potential biological mechanisms underlying these associations are unclear. We investigated the relationship between analgesic use and serum concentrations of estrogens, androgens, and their metabolites among postmenopausal women to provide insights on whether analgesic use might influence endogenous hormone levels, which could in turn influence hormone-related cancer risk. The study included 1,860 postmenopausal women from two case–control studies nested within the Women's Health Initiative Observational Study. Analgesic use was reported at study baseline. Fifteen estrogens and estrogen metabolites and 12 androgens and androgen metabolites were quantified in baseline serum by LC/MS-MS. Linear regression with inverse probability weighting, stratified by menopausal hormone therapy (MHT) use, was used to estimate adjusted geometric mean concentrations of each hormone by analgesic use. Among women not currently using MHT ($n = 951$), low-dose aspirin (<100 mg) use was associated with a higher serum concentration of estrone, estradiol, and 2, 4, and 16 hydroxylated metabolites. Use of regular-dose aspirin (≥100 mg), non-aspirin NSAIDs, and acetaminophen was not associated with serum concentrations of estrogens, androgens, or their metabolites. This study highlights the importance of examining aspirin use by dose and suggests that low-dose aspirin may influence endogenous estrogen concentrations.

Prevention relevance: This study explores a potential pathway by which analgesic medications such as aspirin may prevent hormone-related cancers. The findings support a positive association between low-dose aspirin use and endogenous estrogens, indicating that further elucidation of the interplay between low-dose aspirin, estrogen concentrations, and cancer risk is needed.

Introduction

Regular use of aspirin and other NSAIDs has been associated with a lower risk of several hormone-related cancers in postmenopausal women, including breast (1–3), ovarian (4, 5), and endometrial cancers (6). The potential biological mechanisms linking NSAID use to these cancers remain uncertain.

Aspirin and other NSAIDs inhibit the cyclooxygenase (COX) enzymes, thereby blocking the synthesis of prostaglandins and thromboxanes (7). Acetaminophen, another analgesic medication, also inhibits the COX enzymes, albeit to a lesser extent (8). Prostaglandins are often pro-inflammatory and can promote tumorigenesis via pathways involved in cell proliferation, migration, apoptosis, and angiogenesis (9), while thromboxanes promote platelet aggregation and may facilitate the generation of a favorable metastatic niche (10). Analgesics may thus lower cancer risk by downregulating these pathways.

For hormone-related cancers, analgesics could also potentially protect against cancer by modulating estrogen production (11). Prostaglandin E_2 (PGE_2) has been shown to increase activity of aromatase (12, 13), the enzyme that converts androstenedione to estrone and testosterone to estradiol, and so analgesics may reduce aromatase activity via inhibition of PGE_2. In line with this hypothesis, COX inhibitors were found to decrease aromatase activity in breast cancer cell lines (14). Given that aromatization of androgens is the principal source of estrogens for postmenopausal women (15) and that

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circulating estrogens are positively associated with risk of postmenopausal breast, endometrial, and non-serous ovarian cancers (16–20), it is plausible that analgesics could lower cancer risk among postmenopausal women by decreasing endogenous estrogen production.

To date, few population-based studies have examined associations between analgesic use and circulating estrogens and androgens among postmenopausal women. Two studies reported inverse associations between NSAID use and serum concentrations of estradiol (21, 22), while a third study observed NSAID users to have higher concentrations of dehydroepiandrosterone sulfate (DHEAS), an adrenal androgen that serves as a precursor for more potent androgens and estrogens (23). However, no study has compared associations by dose of analgesic, which is important given the distinct pharmacodynamics and metabolic effects of low-dose versus regular-dose aspirin (24). Furthermore, very few studies have examined associations for estrogen or androgen metabolites, which is necessary to elucidate the precise pathways through which analgesics may influence cancer risk. A study of premenopausal women observed analgesics to be associated with urinary levels of specific estrogen metabolites (25), but this has not yet been examined in postmenopausal women.

We thus examined associations between use of analgesics, including low-dose aspirin, regular-dose aspirin, non-aspirin NSAIDs, and acetaminophen, and circulating levels of estrogens, androgens, and their metabolites among postmenopausal women in the Women's Health Initiative (WHI)-Observational Study (OS). We hypothesized that analgesic users would have lower levels of circulating estrogens and higher levels of circulating androgens compared with non-users of these medications and that the patterns of association would differ by metabolic pathway and aspirin dose.

Materials and Methods

Study population

The current study included 1,864 postmenopausal women, selected from the WHI-OS (n = 93,676, age 50–79 years; refs. 26, 27), for nested case–control studies of ovarian and endometrial cancers (19, 20, 28, 29). These women were included in the current study because they had detailed hormone measurements available. Cases were women diagnosed with ovarian or endometrial cancer between WHI-OS enrollment in 1993–1998 and 2012; controls were selected from the women who were cancer free at the date of case diagnosis and frequency matched to cases based on age at baseline, year of blood draw, race/ethnicity, hysterectomy status prior to the index date, and current use of menopausal hormone therapy (MHT). At baseline, all cases and controls had no history of cancer other than nonmelanoma skin cancer as well as ≥1.1 mL of serum available. We combined the cases and controls for this cross-sectional analysis because serum samples were collected from a single draw at baseline, prior to cancer diagnosis (mean 6.7 years from baseline to cancer diagnosis for cases). We excluded 4 women with missing data on body mass index (BMI) from our analytic sample, yielding a sample size of 1,860. Analyses of aspirin also excluded 61 aspirin users missing information on the dose (13% of aspirin users), yielding a sample size of 1,799. The WHI-OS was approved by Institutional Review Boards at the Fred Hutchinson Cancer Research Center (Seattle, WA; WHI Clinical Coordinating Center) and all participating clinical centers and was conducted in accordance with the ethical principles of the Belmont Report and the U.S. Common Rule. All participants provided written informed consent.

Data availability

The data that support the findings of this study are available from the WHI-OS. Restrictions apply to the availability of these data, which were used under license for this study. Please see https://www.whi.org/page/working-with-whi-data for more information.

Ascertainment of medication use and key covariates

At the baseline visit, WHI-OS participants completed a self-administered questionnaire that collected information on demographics, medical and reproductive factors, and other cancer risk factors. Height and weight were measured by clinic staff and were used to calculate BMI in kg/m^2. A computer-assisted interview collected information about current medication use (30). To facilitate completion of this interview, participants were asked to bring all prescription and over-the-counter medications used regularly (≥2 times/week) over the previous 2 weeks. Use of low-dose aspirin (<100 mg), regular-dose aspirin (≥100 mg), non-aspirin NSAIDs, and acetaminophen were ascertained from this interview and used to classify each study participant as either a current, regular user (≥2 times/week) or non-user (<2 times/week) of each analgesic. Women who reported both low-dose and regular-dose aspirin use ($n = 4$) were classified as low-dose users for this analysis.

Laboratory assays

Stable isotype dilution LC/MS-MS was used to quantify combined conjugated and unconjugated concentrations of 15 estrogens and estrogen metabolites (estrone, estradiol, 2-hydroxyestrone, 2-methoxyestrone, 2-hydroxyestradiol, 2-methoxyestradiol, 2-hydroxyestrone-3-methyl ether, 4-hydroxyestrone, 4-methoxyestrone, 4-methoxyestradiol, 16α-hydroxyestrone, estriol, 16-ketoestradiol, 16-epiestriol, and 17-epiestriol). Five of the estrogens (estrone, estradiol, estriol, 2-methoxyestrone, and 2-methoxyestradiol) were also measured in unconjugated forms; their conjugated concentrations were then calculated by subtracting the unconjugated concentration from the combined concentration.

We also quantified concentrations of 12 androgens and androgen metabolites [DHEA, DHEAS, androstenedione (A4), testosterone (T), 5α-androstane-3,17-dione (5α-A), dihydrotestosterone (DHT), androsterone (ADT), dihydrotestosterone

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sulfate (DHTS), 3α-diol-3G, 3α-diol-17G, androsterone glucuronide (ADT-G), and etiocholanolone-glucuronide (EtioG)] using LC/MS-MS.

Full details of the methods have been described previously (31, 32). Laboratory coefficients of variation of masked technical replicates across batches were <6% for all estrogens and <11% for all androgens. Intraclass correlation coefficients (ICC) ranged from 0.77 to 0.997 (median 0.98 for the estrogens and 0.99 for the androgens).

Statistical analysis

Hormone values were log transformed to improve normality. Geometric mean (GM) concentrations in picomoles per liter (estrogens and estrogen metabolites) or nanomoles per liter (androgens and androgen metabolites) for each hormone were calculated by category of analgesic use using inverse probability weighted linear regression. For all analyses, inverse probability weights were used to account for the case–control sampling and represent the eligible WHI-OS cohort ($n = 56{,}109$) and were calculated as the inverse of the sampling fractions: 1 for all cases, and varying weights for control subjects, depending on their strata as defined by matching factors (33). The GMs were adjusted for baseline covariates including age at blood draw (in 5-year categories), year of blood draw (1993–96, 1997–98), race (White, non-White), BMI (<25, 25–29.9, ≥30 kg/m^2), time since menopause (<10, 10–19, ≥20 years), ever use of oral contraceptives (yes, no), and diabetes (yes, no). Given that current MHT use greatly alters circulating levels of estrogens and some androgens (34), we restricted the primary analyses to women who were never/former users of MHT at baseline ($n = 951$), and examined current MHT users ($n = 848$) in secondary analyses. For non-current MHT users, models were additionally adjusted for history of MHT use (never, former), and for current MHT users, models were additionally adjusted for formulation (use of estrogen + progesterone, use of unopposed estrogen, use of both estrogen + progesterone and unopposed estrogen). GMs were also calculated additionally adjusted for total estrone concentration to determine whether associations with total estrone were driving associations with downstream metabolites. Finally, we calculated GMs stratified by BMI, and adjusted for continuous BMI within strata, to explore whether BMI modified associations between analgesic use and circulating hormone levels.

To explore the association of analgesic use with specific estrogen metabolite pathways, we calculated the proportional concentrations of parent estrogens and metabolites from the 2-, 4-, and 16-hydroxylation pathways relative to the total estrogen concentration. We also examined ratios of unconjugated estradiol to testosterone and unconjugated estrone to androstenedione to assess whether analgesic use may have influenced the aromatization of these androgens into estrogens.

Focusing on never/former MHT users, we conducted several sensitivity analyses to test the robustness of our findings for aspirin use. To ensure that subclinical cancer did not impact the observed associations, we conducted an analysis limited to controls only ($n = 471$). We also conducted analyses among never users of MHT only ($n = 644$) and after excluding women with extreme values [defined as unconjugated estrone concentrations above 184 pmol/L (∼50 pg/mL), concentrations typically indicative of current MHT use (19), $n = 76$ women excluded]. Because diabetes is strongly associated with endogenous hormone levels (35) as well as with aspirin use, we conducted an analysis restricted to women without diabetes ($n = 920$). Finally, we conducted analyses stratified by history of cardiovascular disease (CVD, $n = 771$ women without a history of CVD, $n = 188$ women with a history of CVD), because history of CVD is an indication for a specific regimen of aspirin use (i.e., daily, low-dose use), and because the effects of low-dose aspirin on circulating hormones could potentially vary by indication for use. To account for the multiple comparisons (39 tests per exposure), we calculated q-values using the Benjamini–Hochberg method to control the FDR (36). Analyses were performed in SAS Version 9.4 (SAS Institute).

Results

Characteristics of the study population

Among never/former MHT users, 6% reported low-dose aspirin use, 17% reported regular-dose aspirin use, 17% reported non-aspirin NSAID use, and 9% reported acetaminophen use. Compared with non-aspirin users, aspirin users, and particularly low-dose users were older, more likely to have a history of diabetes, and less likely to report prior oral contraceptive use (**Table 1**). Among current MHT users, patterns in these covariate distributions were similar (Supplementary Table S1). NSAID and acetaminophen users had a higher BMI than non-users of these medications (Supplementary Table S2 and S3).

Associations between aspirin use and circulating hormone concentrations among never/former MHT users

Among the never/former MHT users, those who reported low-dose aspirin use had higher concentrations of parent estrogens than aspirin non-users (for estrone: GM 519.07 vs. 328.70 pmol/L, $P = 0.02$; for estradiol: GM 80.30 vs. 52.59 pmol/L, $P = 0.03$; **Table 2**). Differences were most evident for the conjugated forms of these estrogens. In contrast, estrogen concentrations did not differ between the regular-dose aspirin users and non-aspirin users (for estrone: GM 303.08 vs. 328.70 pmol/L, $P = 0.46$; for estradiol: GM 48.14 vs. 52.59 pmol/L, $P = 0.40$; **Table 2**). The same patterns were observed for the estrogen metabolites, with 2-, 4-, and 16-hydroxylation pathway estrogen metabolite concentrations higher among low-dose aspirin users, yet similar among regular-dose aspirin users as compared with non-aspirin users (**Table 2**; **Fig. 1**). The differences in estrogen metabolite concentrations between low-dose aspirin users and non-users persisted in models additionally adjusted for total estrone

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Table 1. Characteristics of postmenopausal women not currently using MHT from the WHI-OS, by dose of aspirin use ($n = 951$).

		No aspirin use			Low-dose aspirin use			Regular-dose aspirin use		
		No.	(%)	(Weighted %)	No.	(%)	(Weighted %)	No.	(%)	(Weighted %)
N		736	(77)	(79)	57	(6)	(6)	158	(17)	(15)
Age at blood draw	<55 yrs	64	(9)	(10)	4	(7)	(2)	11	(7)	(4)
	55–59 yrs	149	(20)	(20)	3	(5)	(7)	21	(13)	(9)
	60–64 yrs	178	(24)	(21)	16	(28)	(10)	33	(21)	(24)
	65–69 yrs	163	(22)	(23)	12	(21)	(22)	32	(20)	(25)
	70–74 yrs	117	(16)	(17)	11	(19)	(21)	38	(24)	(23)
	75–79 yrs	65	(9)	(8)	11	(19)	(39)	23	(15)	(16)
Blood draw year	1993–1996	446	(61)	(63)	22	(39)	(37)	108	(68)	(66)
	1997–1998	290	(39)	(37)	35	(61)	(63)	50	(32)	(34)
Race	White	637	(87)	(88)	54	(95)	(97)	142	(90)	(97)
	Non-White	99	(13)	(12)	3	(5)	(3)	16	(10)	(3)
Body mass index	<25 kg/m^2	274	(37)	(45)	21	(37)	(37)	53	(34)	(44)
	25–29.9 kg/m^2	219	(30)	(30)	20	(35)	(43)	53	(34)	(27)
	30+ kg/m^2	243	(33)	(25)	16	(28)	(20)	52	(33)	(29)
Smoking status	Never smoker	373	(51)	(51)	32	(56)	(62)	78	(49)	(38)
	Past smoker	300	(41)	(40)	21	(37)	(23)	72	(46)	(52)
	Current smoker	55	(7)	(8)	4	(7)	(14)	7	(4)	(10)
	Missing	8	(1)	(1)	0	(0)	(0)	1	(0)	(0)
Diabetes	No	697	(95)	(97)	52	(91)	(95)	142	(90)	(93)
	Yes	39	(5)	(3)	5	(9)	(5)	16	(10)	(7)
Cardiovascular disease	No	603	(82)	(83)	38	(67)	(59)	106	(67)	(66)
	Yes	119	(16)	(16)	17	(30)	(36)	48	(30)	(32)
	Missing	14	(2)	(1)	2	(4)	(5)	4	(3)	(2)
Time since menopause	<10 yrs	240	(33)	(34)	9	(16)	(6)	43	(27)	(22)
	10–<20 yrs	274	(37)	(35)	26	(46)	(42)	61	(39)	(44)
	20+ yrs	182	(25)	(27)	20	(35)	(45)	50	(32)	(29)
	Unknown	40	(5)	(4)	2	(4)	(7)	4	(3)	(5)
Prior oral contraceptive use	No	464	(63)	(59)	45	(79)	(81)	110	(70)	(64)
	Yes	272	(37)	(41)	12	(21)	(19)	48	(30)	(36)
Hormone therapy use	Never user	492	(67)	(60)	32	(56)	(45)	98	(62)	(48)
	Former user	244	(33)	(40)	25	(44)	(55)	60	(38)	(52)
	Current user	—	—	—	—	—	—	—	—	—
Non-aspirin NSAID use	No	614	(83)	(84)	48	(84)	(84)	133	(84)	(91)
	Yes	122	(17)	(16)	9	(16)	(16)	25	(16)	(9)
Acetaminophen use	No	673	(91)	(90)	51	(89)	(82)	141	(89)	(92)
	Yes	63	(9)	(10)	6	(11)	(18)	17	(11)	(8)

Abbreviation: yrs, years.

(**Table 2**), and the pattern of increased estrogen and estrogen metabolite concentrations among low-dose aspirin users was observed for all BMI subgroups (**Fig. 2**). Concentrations of adrenal androgens and their metabolites did not differ by the dose of aspirin use reported (**Table 2**; **Fig. 1**).

Though absolute concentrations of estrogen and estrogen metabolites were higher among low-dose aspirin users (**Table 2**; **Fig. 1**), the proportions of estrogens and pathway-specific metabolites relative to the total estrogen concentration were consistent across aspirin use categories (**Fig. 3**). Similarly, the ratio of unconjugated estradiol to testosterone did not differ by aspirin use (**Table 2**). The ratio of unconjugated estrone to androstenedione was higher for low-dose aspirin users as compared with non-users ($P = 0.02$; **Table 2**).

Patterns were consistent in analyses restricted to controls (Supplementary Table S4), never users of MHT (Supplementary Table S5), women without diabetes (Supplementary Table S6), and when extreme values were excluded (Supplementary Table S7). Patterns were also generally consistent in analyses stratified by history of CVD, though among women with a history of CVD, circulating levels of estrogens and estrogen metabolites were lower in regular-dose aspirin users as compared with non-users (Supplementary Fig. S1).

Associations between aspirin use and circulating hormone concentrations among current MHT users

For current users of MHT, patterns of estrogen and estrogen metabolite concentrations by dose of aspirin use were similar to patterns observed for never/former MHT users, with low-dose aspirin users tending to have higher concentrations of estrogens and estrogen metabolites than non-users of aspirin (Supplementary Table S8), though most differences were not statistically significant. Compared with non-users of aspirin, low-dose aspirin users had lower concentrations of DHEAS ($P = 0.03$) and regular-dose users had lower concentrations of DHTS ($P = 0.02$), but this pattern was not observed for the other androgens or androgen metabolites.

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Table 2. GMs and 95% confidence intervals of serum estrogens/estrogen metabolites (pmol/L) and androgens/androgen metabolites (nmol/L) by dose of aspirin use in postmenopausal women not currently using MHT in the WHI-OS.

	Model 1[a]							Model 1[a] + total estrone						
	No aspirin ($n = 736$)		Low-dose aspirin ($n = 57$)			Regular-dose aspirin ($n = 158$)		No aspirin ($n = 736$)		Low-dose aspirin ($n = 57$)			Regular-dose aspirin ($n = 158$)		
Hormone	GM	(95% CI)	GM	(95% CI)	P_{diff}[b]	GM	(95% CI)	P_{diff}[c]	GM	(95% CI)	GM	(95% CI)	P_{diff}[b]	GM	(95% CI)	P_{diff}[c]
Estrogens																
Estrone	328.70	(272.63–396.29)	**519.07**	**(352.98–763.32)**	**0.02**[d]	303.08	(235.05–390.8)	0.46								
Unconjugated	59.70	(52.41–68)	79.77	(59.32–107.26)	0.05	56.71	(46.91–68.56)	0.56								
Conjugated	261.70	(212.5–322.3)	**437.33**	**(289.16–661.43)**	**0.01**[d]	240.83	(182.26–318.21)	0.48								
Estradiol	52.59	(43–64.31)	**80.30**	**(54.01–119.38)**	**0.03**	48.14	(37.52–61.77)	0.40	57.48	(47.45–69.63)	**81.23**	**(59.86–110.22)**	**0.02**[d]	54.20	(43.94–66.85)	0.47
Unconjugated	14.20	(11.21–17.99)	16.92	(11.47–24.97)	0.32	12.83	(9.77–16.86)	0.32	15.28	(12.17–19.18)	17.08	(11.86–24.61)	0.47	14.15	(11.02–18.17)	0.37
Conjugated	34.59	(27.77–43.09)	**57.53**	**(37.32–88.69)**	**0.02**[d]	31.74	(23.88–42.18)	0.47	37.91	(30.67–46.86)	**58.21**	**(41.33–82)**	**0.01**[d]	35.86	(27.98–45.96)	0.56
2-Hydroxyestrone	73.22	(61.94–86.55)	**113.92**	**(82.16–157.94)**	**<0.01**[d]	63.88	(51.13–79.83)	0.12	79.25	(68.51–91.68)	**115.09**	**(87.23–151.85)**	**<0.01**[d]	70.99	(58.95–85.49)	0.12
2-Hydroxyestradiol	17.78	(14.96–21.13)	**27.54**	**(19.84–38.24)**	**0.01**[d]	16.17	(12.84–20.37)	0.29	19.15	(16.43–22.31)	**27.81**	**(20.93–36.94)**	**<0.01**[d]	17.85	(14.67–21.71)	0.33
2-Methoxyestrone	41.48	(36.98–46.53)	**59.43**	**(43.43–81.32)**	**0.02**	40.08	(33.48–47.97)	0.67	44.03	(39.34–49.27)	**59.89**	**(45.39–79.01)**	**0.02**[d]	43.38	(36.79–51.17)	0.83
Unconjugated	11.09	(9.6–12.82)	13.82	(9.84–19.43)	0.19	9.93	(7.92–12.44)	0.28	11.92	(10.33–13.74)	13.95	(10.13–19.21)	0.27	10.92	(8.93–13.35)	0.31
Conjugated	29.54	(26–33.56)	**44.47**	**(31.74–62.31)**	**0.02**[d]	29.41	(24.42–35.42)	0.96	31.20	(27.51–35.39)	**44.78**	**(33.23–60.36)**	**0.01**[d]	31.62	(26.49–37.75)	0.86
2-Methoxyestradiol	14.13	(12.14–16.43)	**20.27**	**(14.31–28.72)**	**0.04**	**11.66**	**(9.67–14.07)**	**0.01**	15.05	(13.04–17.37)	**20.44**	**(15.03–27.8)**	**0.03**	**12.69**	**(10.68–15.08)**	**0.01**
Unconjugated	2.21	(1.88–2.61)	2.55	(1.88–3.46)	0.31	1.91	(1.55–2.36)	0.09	2.34	(2–2.75)	2.57	(1.93–3.41)	0.45	2.06	(1.69–2.5)	0.08
Conjugated	11.42	(9.7–13.46)	**17.49**	**(12–25.5)**	**0.03**	**9.51**	**(7.76–11.65)**	**0.03**	12.18	(10.41–14.25)	**17.63**	**(12.6–24.67)**	**0.02**[d]	**10.36**	**(8.57–12.51)**	**0.03**
2-Hydroxyestrone-3-methyl ether	8.18	(7.13–9.38)	**11.72**	**(8.83–15.54)**	**0.01**[d]	7.57	(6.28–9.12)	0.34	8.64	(7.58–9.84)	**11.80**	**(9.08–15.33)**	**0.01**[d]	8.14	(6.86–9.65)	0.40
4-Hydroxyestrone	8.99	(7.59–10.66)	**13.88**	**(10.05–19.17)**	**<0.01**[d]	7.79	(6.2–9.78)	0.12	9.70	(8.35–11.28)	**14.02**	**(10.63–18.49)**	**<0.01**[d]	8.62	(7.1–10.46)	0.12
4-Methoxyestrone	4.46	(3.98–5)	5.88	(4.32–7.99)	0.07	4.21	(3.52–5.03)	0.47	4.71	(4.22–5.25)	5.92	(4.47–7.83)	0.09	4.52	(3.85–5.3)	0.57
4-Methoxyestradiol	1.95	(1.7–2.24)	2.24	(1.61–3.11)	0.40	**1.59**	**(1.33–1.9)**	**0.01**	2.08	(1.83–2.36)	2.25	(1.68–3.03)	0.57	**1.73**	**(1.47–2.04)**	**0.01**
16α-Hydroxyestrone	36.70	(30.87–43.62)	**55.94**	**(39.17–79.89)**	**0.01**[d]	31.94	(25.35–40.24)	0.13	39.79	(34.19–46.3)	**56.53**	**(41.62–76.78)**	**0.01**[d]	35.57	(29.29–43.19)	0.13
Estriol	156.29	(132.51–184.35)	**231.59**	**(166.91–321.35)**	**0.01**[d]	132.79	(106.09–166.21)	0.09	169.64	(146.45–196.49)	**234.06**	**(177.41–308.8)**	**0.01**[d]	148.10	(122.6–178.9)	0.08
Unconjugated	29.23	(25.24–33.85)	36.50	(26.45–50.36)	0.14	25.81	(21.02–31.69)	0.16	31.02	(27.01–35.61)	36.78	(27.31–49.53)	0.20	27.93	(23.2–33.63)	0.18
Conjugated	124.26	(104.02–148.44)	**192.69**	**(136.27–272.48)**	**0.01**[d]	105.92	(83.49–134.38)	0.11	135.41	(115.41–158.89)	**194.84**	**(145.19–261.47)**	**0.01**[d]	118.76	(97.1–145.25)	0.11
16-Ketoestradiol	40.57	(33.81–48.69)	**61.87**	**(43–89.02)**	**0.01**[d]	34.96	(27.51–44.43)	0.12	43.83	(37.15–51.71)	**62.49**	**(45.38–86.06)**	**0.02**[d]	38.74	(31.48–47.68)	0.13
16-Epiestriol	16.28	(13.94–19)	**24.96**	**(18.23–34.17)**	**<0.01**[d]	14.14	(11.57–17.29)	0.09	17.42	(15.18–20)	**25.18**	**(19.09–33.21)**	**<0.01**[d]	15.48	(13.02–18.4)	0.08
17-Epiestriol	12.11	(9.92–14.79)	**17.00**	**(11.97–24.15)**	**0.02**[d]	10.93	(8.67–13.78)	0.22	12.95	(10.8–15.53)	**17.15**	**(12.53–23.48)**	**0.02**[d]	11.95	(9.76–14.64)	0.25
Androgens																
DHEA	4.94	(4.13–5.91)	4.69	(3.58–6.15)	0.67	4.52	(3.68–5.56)	0.27	4.95	(4.13–5.94)	4.69	(3.57–6.17)	0.66	4.53	(3.68–5.58)	0.27
DHEAS	1,095.80	(880.81–1,363.27)	1,284.96	(897.5–1,839.69)	0.33	1,048.04	(823.14–1,334.38)	0.65	1,107.54	(890.22–1,377.92)	1,287.33	(896.61–1,848.31)	0.35	1,062.80	(834.66–1,353.29)	0.67
Androstenedione	1.38	(1.21–1.56)	1.30	(1.06–1.59)	0.53	1.26	(1.08–1.47)	0.16	1.38	(1.21–1.57)	1.30	(1.06–1.6)	0.53	1.26	(1.08–1.47)	0.16
Testosterone	0.52	(0.46–0.59)	0.55	(0.43–0.71)	0.62	0.51	(0.43–0.6)	0.79	0.52	(0.46–0.59)	0.55	(0.43–0.71)	0.62	0.51	(0.43–0.6)	0.79
5α-A	1.42	(1.23–1.63)	1.26	(0.98–1.62)	0.32	**1.22**	**(1.02–1.46)**	**0.02**	1.42	(1.23–1.63)	1.26	(0.98–1.62)	0.31	**1.22**	**(1.03–1.46)**	**0.02**
DHT	0.18	(0.16–0.2)	0.18	(0.15–0.21)	0.90	0.17	(0.15–0.2)	0.57	0.18	(0.16–0.2)	0.18	(0.15–0.21)	0.87	0.17	(0.15–0.2)	0.58
DHTS	1.03	(0.87–1.22)	1.03	(0.75–1.42)	0.98	1.12	(0.91–1.39)	0.28	1.04	(0.87–1.23)	1.03	(0.75–1.42)	0.97	1.14	(0.92–1.41)	0.26
ADT	0.59	(0.53–0.65)	0.54	(0.46–0.63)	0.17	0.59	(0.52–0.67)	0.98	0.59	(0.53–0.66)	0.54	(0.46–0.63)	0.15	0.59	(0.53–0.67)	0.99
ADT-G	21.02	(16.2–27.26)	24.48	(15.93–37.63)	0.46	18.24	(13.78–24.14)	0.16	21.21	(16.34–27.52)	24.53	(15.92–37.79)	0.49	18.46	(13.95–24.44)	0.17
3α-diol-3G	1.27	(1–1.61)	1.71	(1.15–2.54)	0.11	1.22	(0.95–1.56)	0.67	1.28	(1.01–1.62)	1.71	(1.16–2.54)	0.12	1.23	(0.96–1.57)	0.68
3α-diol-17G	1.25	(1.02–1.53)	1.18	(0.85–1.63)	0.71	1.20	(0.94–1.52)	0.64	1.24	(1.01–1.52)	1.18	(0.85–1.63)	0.72	1.19	(0.94–1.51)	0.64
Etio-G	30.57	(23.63–39.55)	36.18	(23.43–55.87)	0.42	27.96	(21.44–36.45)	0.37	30.86	(23.87–39.89)	36.25	(23.49–55.92)	0.44	28.30	(21.74–36.84)	0.38
Ratios																
Unconjugated estradiol to testosterone	26.95	(21.79–33.33)	30.41	(20.71–44.65)	0.51	24.82	(19.4–31.76)	0.39	29.11	(23.69–35.78)	30.80	(21.28–44.57)	0.73	27.46	(21.81–34.57)	0.47
Unconjugated estrone to androstenedione	42.80	(37.12–49.35)	**60.91**	**(45.31–81.86)**	**0.02**[d]	44.49	(36.78–53.82)	0.63	45.95	(39.88–52.95)	**61.62**	**(46.99–80.8)**	**0.02**[d]	48.83	(41.25–57.8)	0.34

Note: GMs and 95% CIs are bolded if they are statistically significantly different from the reference group (P_{diff} <0.05).

Abbreviations: 3α-diol-3G, 5α-androstane 3α,17b diol-3-glucuronide; 3α-diol-17G, 5α-androstane 3α,17b diol-17-glucuronide; 5α-A, 5α-androstane-3,17-dione; ADT, androsterone; ADT-G, ADT-glucuronide; CI, confidence interval; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; DHT, dihydrotestosterone; DHTS, dihydrotestosterone sulfate; etio-G, etiocholanolone-glucuronide.

[a]Adjusted for age at blood draw, year of blood draw, race, BMI, time since menopause, oral contraceptive use, diabetes, past use of menopausal hormone therapy.

[b]*P*-value estimated using the Wald test to compare low-dose aspirin use to no aspirin use.

[c]*P*-value estimated using the Wald test to compare regular-dose aspirin use to no aspirin use.

[d]Indicates an FDR *q*-value ≤0.05.

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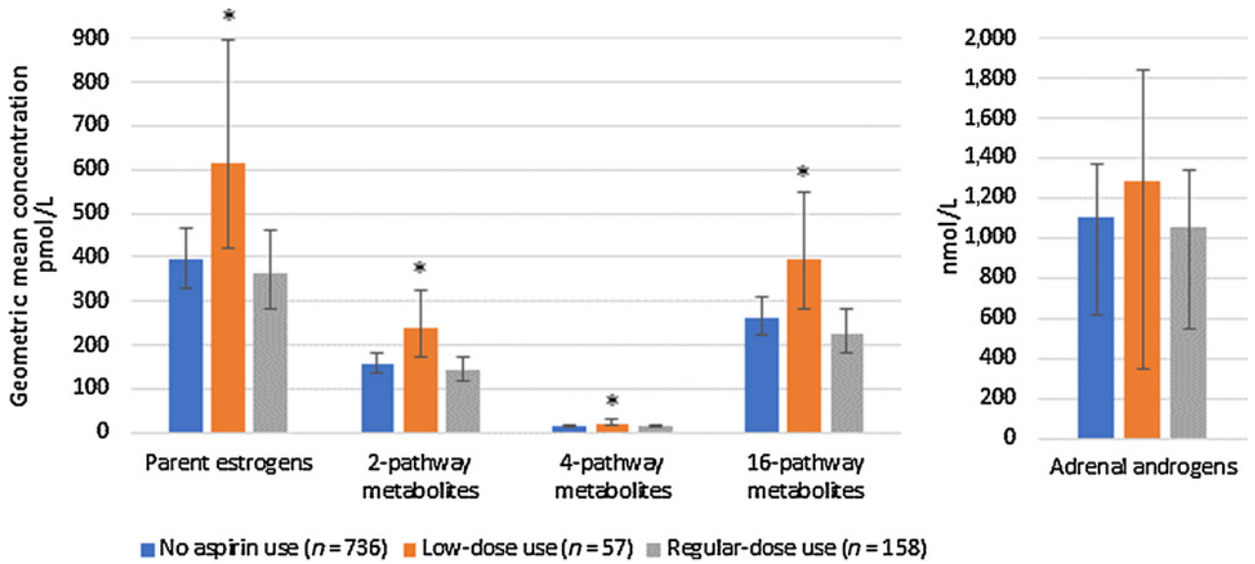

Figure 1.

Adjusted GM concentrations of parent estrogens, estrogen metabolites, and adrenal androgens[a] by dose of aspirin use in postmenopausal women not currently using MHT in the WHI-OS. [a]Adrenal androgens include DHEA, DHEAS, androstenedione, and testosterone. *indicates a statistically significant difference ($P < 0.05$) compared with no aspirin use.

Associations between non-aspirin NSAID and acetaminophen use and circulating hormone concentrations among never/former MHT users

Among never/former MHT users, there were no differences in the GM concentrations of estrogens, androgens, or their metabolites between non-aspirin NSAID users and non-users (Supplementary Table S9), or between acetaminophen users and non-users (Supplementary Table S10).

Discussion

In this study of postmenopausal women from the WHI-OS, we observed higher circulating concentrations of estrogens and their metabolites among low-dose aspirin users as compared with non-users of aspirin. Low-dose aspirin use was associated with an increase in the full cascade of estrogen metabolites, as estrogen metabolite concentrations from each pathway were statistically significantly higher among low-dose aspirin users even after adjustment for estrone concentration. Low-dose aspirin users also had more unconjugated estrone relative to androstenedione. Results were robust across several sensitivity analyses and did not appear to be modified by BMI, history of CVD, or MHT use. There were no differences in absolute hormone concentrations or in relative concentrations of estrogens to androgens between regular-dose aspirin users and non-users, or between users and non-users of non-aspirin NSAIDs or acetaminophen.

Our findings are contrary to our hypothesis that analgesic use would inhibit the aromatization of androgens to estrogens, leading to a lower estrogens-to-androgen ratio. Instead, our null results for regular-dose aspirin, non-aspirin NSAIDs, and acetaminophen suggest that most analgesics may not be associated with aromatase activity or subsequent estrogen metabolism, at least to an extent that impacts levels of these hormones systemically. Our results for regular-dose aspirin are consistent with results from a randomized controlled trial of postmenopausal women, which reported no difference in serum estrogen levels after six months of daily, regular-dose aspirin use (37).

Interestingly, in contrast to what we had anticipated, we observed positive associations between low-dose aspirin use and circulating estrogens and estrogen metabolites. Prior observational studies of postmenopausal women have reported inverse (21, 22) or null (23) associations between aspirin and other NSAID use and serum estrogen levels, but these studies did not differentiate between low- and regular-dose aspirin. Our divergent results for low- versus regular-dose aspirin underscore the importance of examining aspirin use by dose and, if replicated, suggest that low-dose aspirin may influence endogenous estrogen levels through a dose-specific mechanism. Differing usage patterns for low- versus regular-dose aspirin could also potentially explain these results. Low-dose aspirin is often taken daily or almost daily for prolonged periods while regular-dose aspirin may be used more sporadically, and it could be biologic effects specific to low-dose aspirin, the increased frequency or duration of use, or both underlying the association between low-dose aspirin use and higher circulating concentrations of estrogens and estrogen metabolites.

Although it is unclear how low-dose aspirin might increase endogenous estrogen concentrations, such a relationship could help explain why regular use of low-dose aspirin is more tenuously inversely associated with breast and endometrial

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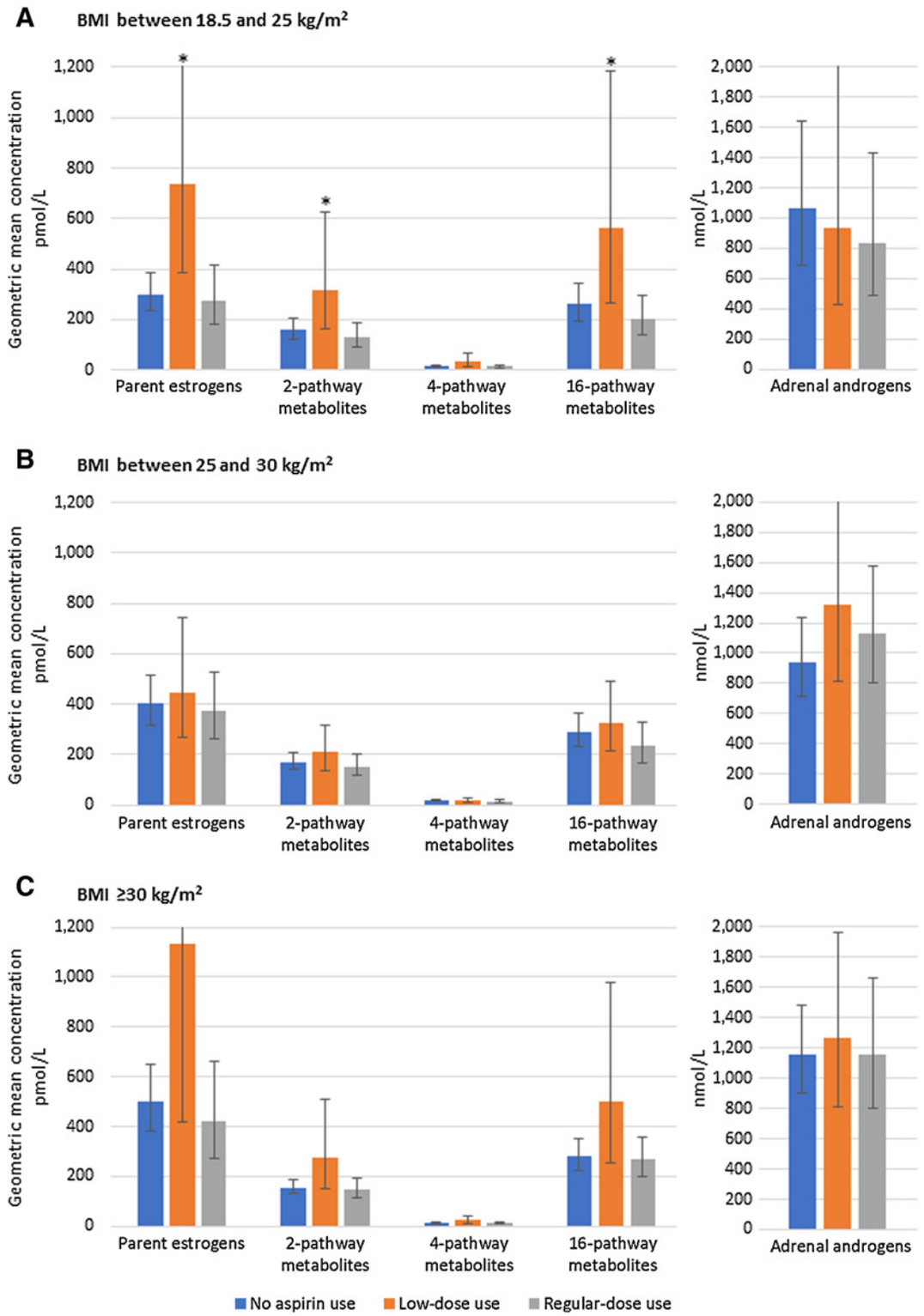

Figure 2.

Adjusted GM concentrations of parent estrogens, estrogen metabolites, and adrenal androgens[a] by dose of aspirin use in postmenopausal women not currently using MHT in the WHI-OS, among women with BMI between 18.5 and 25 kg/m^2 (**A**), BMI between 25 and 30 kg/m^2 (**B**), and BMI ≥30 kg/m^2 (**C**). [a]Adrenal androgens include DHEA, DHEAS, androstenedione, and testosterone. *indicates a statistically significant difference ($P < 0.05$) compared with no aspirin use.

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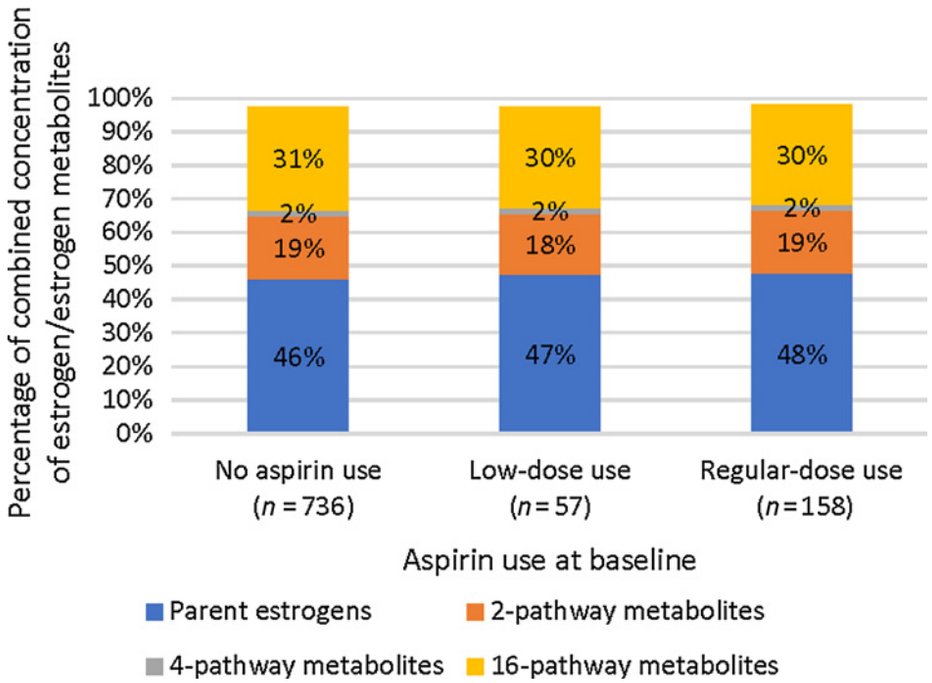

Figure 3.
Percentage of total adjusted GM estrogen/estrogen metabolite concentration for parent estrogens and estrogen metabolites by dose of aspirin use in postmenopausal women not currently using MHT in the WHI-OS.

cancer than regular-dose aspirin (1–3, 6, 38–48). Aspirin is hypothesized to reduce cancer risk via anti-inflammatory and antiplatelet mechanisms, but if low-dose aspirin use also increases endogenous estrogens, a strong risk factor for postmenopausal breast and endometrial cancers, this could partially or fully negate the purported beneficial effects. Such opposing mechanisms could help explain why most studies of low-dose aspirin and breast cancer risk have observed no benefit (38–43), in contrast to studies that looked at aspirin use regardless of dose (1–3). Other observational studies of low-dose aspirin have reported inverse associations with breast cancer (44–46), but these studies primarily observed associations only after long-term use, suggesting that future work should consider time-varying effects of low-dose aspirin on both circulating hormones and cancer risk. Similarly, for endometrial cancer risk, studies have reported stronger inverse associations with regular-dose aspirin than low dose (47, 48). A large pooled analysis of endometrial cancer studies also observed an inverse association among overweight and obese women who used aspirin 2–6 times per week, a frequency suggestive of regular-dose use, but not among overweight or obese women who used aspirin daily, a frequency suggestive of low-dose use (6).

This was a cross-sectional, observational study, and results must therefore be interpreted with caution. Though we carefully adjusted for confounding factors such as BMI and diabetes, other unobserved differences between the analgesic users and non-users, including those related to indications for analgesic use, may have still confounded the results. Notably, however, results were consistent when we restricted to women without a self-reported history of CVD, suggesting that the results were not confounded by this common indication for low-dose aspirin use. We did not have information on the frequency of analgesic use, and future studies are needed to disentangle the effects of low-dose versus frequent aspirin use. The serum estrogens, androgens, and their metabolites were also assessed at a single timepoint. Previous studies of postmenopausal women using the same assays have observed moderate 1-year ICCs for the parent estrogens (0.72 for estrone and 0.65 for estradiol), lower ICCs for the estrogen metabolites (range: 0.10–0.53; ref. 17), and moderate-to-high 2-year ICCs for the androgens and androgen metabolites (mean: 0.78; ref. 29), suggesting decent temporal stability, but future studies with repeat samples may help reduce measurement error and confirm whether our findings persist over time. Finally, there is potential for false positive results given the many simultaneous hypothesis tests that were conducted, although the consistent patterns of associations across analgesic types and classes of hormone and the fact that most associations had an FDR of 5% or lower support the robustness of our observations.

This study also has several notable strengths. To our knowledge, it is the first to examine aspirin use by dose in relation to circulating hormones. We used a well-validated LC/MS-MS assay with high reliability, sensitivity, and specificity to assess a comprehensive set of parent estrogens, androgens, and their metabolites, including conjugated and unconjugated forms in postmenopausal women. We accounted for exogenous hormone use, conducted several sensitivity analyses to carefully assess the effects of known determinants of endogenous

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hormone concentrations, and accommodated multiple testing in interpreting our findings.

In conclusion, this study does not support an association between use of regular-dose aspirin, non-aspirin NSAIDs, or acetaminophen and serum estrogen or androgen concentrations among postmenopausal women. Low-dose aspirin use was associated with higher concentrations of estrogens and estrogen metabolites among both current and non-current MHT users, though further studies are needed to confirm these associations and explore whether they may be causal. Given that low-dose aspirin use is common among older adults (49) and that endogenous estrogens have been linked to cancer (16–20) and other health outcomes (50, 51), elucidating the relationship between low-dose aspirin and endogenous estrogens is critical for understanding the balance of benefits and risks of low-dose aspirin use among postmenopausal women.

Authors' Disclosures

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Authors' Contributions

L.M. Hurwitz: Conceptualization, formal analysis, investigation, visualization, methodology, writing–original draft. **A.H. Shadyab:** Investigation, methodology, writing–review and editing. **F.K. Tabung:** Investigation, methodology, writing–review and editing. **G.L. Anderson:** Resources, data curation, supervision, investigation, methodology, writing–review and editing. **N. Saquib:** Investigation, methodology, writing–review and editing. **R.B. Wallace:** Investigation, methodology, writing–review and editing. **R.A. Wild:** Investigation, methodology, writing–review and editing. **R.M. Pfeiffer:** Investigation, methodology, writing–review and editing. **X. Xu:** Data curation, investigation, methodology, writing–review and editing. **B. Trabert:** Conceptualization, resources, data curation, formal analysis, supervision, investigation, visualization, methodology, project administration, writing–review and editing.

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