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## Estrogen metabolites and their relation to isoprostanes as a measure of oxidative stress

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### Abstract

**Objective:** Estradiol (E2) and its metabolites [2-hydroxyestrone (2-OHE1) and 16 $\alpha$ -hydroxyestrone (16 $\alpha$ -OHE1)] are believed to curtail greater oxidative stress found in the development and progression of disease conditions including atherosclerosis. We related estrogen levels to F<sub>2a</sub>-isoprostane levels, a biomarker of oxidative stress.

**Design and Participants:** Data were from 1647 women, aged 47-57 years, participating in the 5<sup>th</sup> annual follow-up of the Study of Women's Health Across the Nation (SWAN), a study of the menopausal transition.

**Measurements:** Serum E2 and urinary 2-OHE1 and 16 $\alpha$ -OHE1 concentrations were assayed by ELISA while urinary F<sub>2a</sub>-isoprostanes were assayed by EIA.

**Results:** F<sub>2a</sub>-isoprostane concentrations were elevated in women who smoked, a behavior associated with increased oxidative stress, but not in stages of the natural menopause. Mean F<sub>2a</sub>-isoprostane concentrations among premenopausal and postmenopausal women who smoked were 1082 and 1064 pg/mL, respectively, values double those in premenopausal (343 pg/mL) and postmenopausal (379 pg/mL) non-smoking women. 2-OHE1 and F<sub>2a</sub>-isoprostane concentrations were positively and highly related [partial correlations  $\rho_{Y|X} = 0.44$  and  $\rho_{Y|X} = 0.43$  in premenopausal and postmenopausal women, respectively]. Likewise, 16 $\alpha$ -OHE1 concentrations were positively and highly correlated with F<sub>2a</sub>-isoprostane concentrations [ $\rho_{Y|X} = 0.52$  and  $\rho_{Y|X} = 0.59$  in premenopausal and postmenopausal women, respectively]. E2 was significantly correlated with F<sub>2a</sub>-isoprostanes only in postmenopausal women [ $\rho_{Y|X} = 0.20$ ]. Associations were adjusted for age, body mass index, race/ethnicity, lipids, physical activity level, and alcohol consumption.

**Conclusions:** This study does not support the commonly-held hypothesis that levels of endogenous estradiol or its estrone metabolites favorably modify oxidative stress by decreasing F<sub>2a</sub>-isoprostane levels.

### Keywords

isoprostanes; estrogen; oxidative stress; antioxidant; smoking

## INTRODUCTION

It has been hypothesized that physiological levels of estradiol (E2) and estrogen metabolites, including 2-hydroxyestrone (2-OHE1) and 16 $\alpha$ -hydroxyestrone (16 $\alpha$ -OHE1), favorably modulate oxidative stress, potentially attenuating the development and progression of disease, including atherosclerosis and diabetes.<sup>1,2</sup> While the concept of estrogens as antioxidants is intriguing and might explain differential rates or timing of cardiovascular disease between men and women, the evidence remains controversial.

High, non-physiological levels of estradiol were associated with the *in vitro* inhibition of low-density lipoprotein (LDL) oxidation,<sup>3,4</sup> a prominent feature of atherosclerosis.<sup>5,6</sup> Some,<sup>7,8</sup> but not all studies<sup>9,10</sup> have shown that, in postmenopausal women, estrogen replacement diminished circulating oxidized LDL levels. Studies of oxidative stress according to sex difference or menopause transition to postmenopause are too few in number to clarify the role of estrogens as antioxidants. Ide et al. reported that urinary levels of F<sub>2a</sub>-isoprostanes are increased in healthy young men compared to premenopausal women;<sup>11</sup> however, two other studies<sup>12,13</sup> reported F<sub>2a</sub>-isoprostane levels were about 20% lower, not higher, in men as compared to women. Helmersson et al. reported increased levels in postmenopausal women compared to premenopausal women.<sup>14</sup>

Using F<sub>2a</sub>-isoprostane production as a marker for identifying oxidative stress,<sup>15,16</sup> we examined the potential antioxidant associations of estrogens considering stages of menopause. We hypothesized that an important antioxidant role for E2, 2-OHE1 and 16 $\alpha$ -OHE1 would be reflected in strong, inverse correlations with F<sub>2a</sub>-isoprostanes. We postulated that the associations between estrogens and the F<sub>2a</sub>-isoprostanes would be similar in premenopausal women and postmenopausal women using hormone therapy (HT), and dissimilar to the associations in postmenopausal women without HT use. These hypotheses were tested while adjusting for lipids, body mass index, race/ethnicity, smoking, exercise, vitamin E and iron supplementation and substantial alcohol consumption.<sup>17</sup>

## METHODS

### Sampling and Study Population

Data are from 1647 enrollees at the 5<sup>th</sup> annual follow-up visit of the Study of Women's Health Across the Nation (SWAN), a community-based, longitudinal study of the menopausal transition.<sup>18</sup> Eligible participants were from Boston MA, Chicago IL, the Detroit area MI, Los Angeles CA, Hudson County NJ, Oakland CA, and Pittsburgh PA. At baseline, eligibility criteria for the longitudinal cohort were: age 42 to 52 years; intact uterus and at least one ovary; no current use of estrogens or other medications known to affect ovarian function; at least one menstrual period in the 3 months before enrollment; and, self-identification as a member of one of the 5 eligible racial/ethnic groups. Sites enrolled a Caucasian and non-Caucasian sample including African-American women in Boston, Chicago, the Detroit area, and Pittsburgh, and Japanese, Chinese, and Hispanic women in Los Angeles, Oakland, and New Jersey, respectively. Two sites, Chicago and Newark, did not collect annual urine samples from enrollees so the only contribution for these sites comes from 44 women at the Chicago site

who provided urine samples for a SWAN substudy. There was Institutional Review Board approval for the study protocol at each study site and all subjects gave informed consent.

## Assays

This study is limited to specimens collected during the fifth annual follow-up examination and available through the SWAN Repository. When possible, early morning blood draws occurred in the fasted state during days 2-5 of the early follicular phase of the menstrual cycle. Urine specimens for the F<sub>2a</sub>-isoprostane analyses and estrone metabolites were collected on the days of blood draw (usually on days 2-5 of the early follicular phase and, by protocol, prior to 11 AM). At follow-up visit 05, 96% of samples were acquired fasting; 97% were acquired before 11 AM; and, 53% of samples were collected in days 2-5 of the follicular phase of the menstrual cycle. Assays were completed in the CLASS laboratory, University of Michigan.

Serum estradiol (E2) concentrations were measured with a modified, off-line ACS:180 (E2-6) immunoassay (Bayer Diagnostics Corp, Norwood, MA). The assay range for E2 was calibrated to address the lower values associated with the follicular phase or with the postmenopause. Inter- and intra-assay coefficients of variation were 10.6% and 6.4%, respectively, over the assay range 20-500 pg/ml.

2-OHE1 and 16 $\alpha$ -OHE1 were assayed by enzyme immunoassay (ESTRAMET™) in triplicate.<sup>19</sup> The inter- and intra-assay coefficients of variation were less than 10% for each analyte. Because urinary forms of 2-OHE1 and 16 $\alpha$ -OHE1 are found as 3-glucuronides or 3,3,16-glucuronides, it was necessary to remove these sugars to achieve recognition sites for the monoclonal antibodies. Therefore, the estrogens were deconjugated from glucuronic acid and sulfate using a mixture of  $\beta$ -glucuronidase and arylsulphatase enzyme isolated from the snail *Helix Pomatia*. The assay range was 0.6 to 40.0 ng/mL.

Samples were purified with F<sub>2a</sub>-isoprostane affinity columns according to the manufacturer's instructions (Cayman Chemical, Ann Arbor, MI). Samples were applied to the column, washed with buffer and eluted with 95% ethanol. Following evaporation of solvent, the dried samples were diluted 1:10 with 0.1 M phosphate buffer and assayed using the F<sub>2a</sub>-isoprostane enzyme immunoassay (EIA) kit (Cayman Chemical, Ann Arbor, MI). The standard curve range was 3.9-500 pg/mL. Samples were read at 405 nm in a 96-well microplate reader. The post-extraction intra- and inter-assay coefficients of variation were 14.4% (51.2 pg/mL, n=83 pairs) and 17.5% (51.2 pg/mL, n=85), respectively; the intra-assay coefficient of variation was 5.8% (n=1707 pairs).

Lipids were assayed at the Medical Research Laboratories, Highland Heights, Kentucky, USA, which is certified by the Centers for Disease Control Lipid Standardization Part III program<sup>20</sup>. All lipid and lipoprotein fractions were analyzed on EDTA treated plasma. Total cholesterol and triglycerides were analyzed by enzymatic methods and HDL-C was isolated using heparin-2M manganese chloride. Participants were classified as diabetic based on a fasting glucose  $\geq$  126 mg/dL [measured using a hexokinase-coupled reaction on a Hitachi 747-200 (Boehringer Mannheim Diagnostics, Indianapolis, IN)] or self-reported use of insulin or oral medications for diabetes.

## Physical and interview-based measures

Weight (kg) was measured using balance beam and electronic scales; height (m) was measured using stadiometers. The data were used to calculate body mass index (BMI; weight in kilograms divided by height squared in meters).

Women were designated as pre- or perimenopausal unless they had 12 months of amenorrhea and then they were designated as postmenopausal. Because of their limited number, women

who had surgical menopause were excluded from these data analyses. Use of exogenous hormones (HT) was self-reported.

Data on smoke exposure were obtained from a self-administered questionnaire incorporating American Thoracic Society questions<sup>21</sup> and validated questions on passive exposure.<sup>22</sup> Baseline leisure physical activity was based on the frequency, intensity, and duration of the two sports or exercise activities reported by the participant as occurring in the year prior to assessment.<sup>23</sup> Estimates of usual annual dietary data were obtained using a modified Block food frequency (FFQ) interviewer-assisted questionnaire,<sup>24</sup> administered in four languages, English, Spanish, Chinese, or Japanese. Serving sizes were reported with the assistance of food models. Genistein and daidzein intakes<sup>25</sup> and polyphenol levels were evaluated.<sup>26</sup> Total alcohol consumption per day was categorized as none, less than ½ serving (< 15 gm), and at least ½ serving (≥ 15 gm). Vitamin and mineral supplement use was self-reported.

### Statistical approach

Analyses were conducted using SAS Version 9.1 software. Information was excluded from the baseline enrollment of 3302 women for the following reasons: attrition by the 5<sup>th</sup> annual follow-up visit (n=696) and unavailable estrogen metabolite data because the protocol at two sites did not include annual urine collection (n=571). Of the remaining 2035 participants, 42 women had surgical menopause and 346 did not have repository specimens available for analyses. This final analytical sample consisted of 371 (23%) African-American, 837 (51%) Caucasian, 202 (12%) Chinese, and 237 (14%) Japanese women.

Median and interquartile ranges (IQR) were calculated for continuous variables, including 2-OHE1 and 16 $\alpha$ -OHE1, serum E2, F<sub>2a</sub>-isoprostanes, lipids, BMI, and age. *All continuous variables except for age* were log-transformed to satisfy the normality assumption in parametric testing. Analyses of covariance and multiple variable regression analyses were the primary statistical approaches. Type III sums of squares were used to estimate the adjusted correlations. Adjusted group means were identified using analyses of covariance and comparison of the pair-wise differences. *P*-values were two-sided at an  $\alpha < 0.05$ . Ninety-five percent confidence intervals (CI) were used to describe statistical probabilities of associations with ANCOVA models.

Data were stratified by current smoking behavior because smoking behavior had a strong main effect *and interaction with menopause status*. Further, a potential statistical interaction with race could not be tested because smoking was infrequently reported by Chinese or Japanese women, making full statistical adjustment or interaction testing inappropriate. Data were adjusted for age, BMI, race, physical activity level, and lipids as continuous variables. Adjustment also included vitamin E supplement use, iron supplement use, and diabetes as categorical variables. There were no associations with intakes of dietary fat, dietary genistein, daidzein, or polyphenol consumption.

## RESULTS

The overall median age at the 5th annual visit was 51 years, with premenopausal women slightly younger (50 years) and postmenopausal women slightly older (53 years). The median BMI was 26.5 kg/m<sup>2</sup> (Table 1). F<sub>2a</sub>-isoprostane concentrations were significantly and markedly different in smokers vs. non-smokers before and after adjusting for covariates. As seen in Figure 1, F<sub>2a</sub>-isoprostane concentrations in smokers were almost double the mean level of non-smokers in both African-American and Caucasian women, with few smokers among Chinese and Japanese women.

## Non-smokers

There were no statistically significant differences observed in mean  $F_{2a}$ -isoprostane concentrations in premenopausal vs. postmenopausal stages (343 vs. 379 pg/mL,  $P=0.09$ ) among women who did not smoke (Table 2). The mean  $F_{2a}$ -isoprostane concentration in women using HT (425 pg/mL) was 24% higher than the mean for premenopausal women ( $P=0.0002$ ).

In non-smokers, the mean E2 concentrations in premenopausal women and HT users were similar ( $P=0.26$ ) but were markedly lower ( $-62\%$ ,  $P<0.0001$ ) in postmenopausal women as compared to premenopausal women, seen in Table 2.

The mean 2-OHE1 concentration in postmenopausal women was 34% lower ( $P<0.0001$ ) than premenopausal women. Women using HT had a mean 2-OHE1 concentration 107% ( $P<0.0001$ ) higher than premenopausal women.

Compared to premenopausal women, the mean  $16\alpha$ -OHE1 concentration was 24% ( $P<0.0001$ ) lower in postmenopausal women and 76% ( $P<0.0001$ ) higher in women using HT.

## Smokers

Among smokers, mean  $F_{2a}$ -isoprostane concentrations for women who were premenopausal, postmenopausal, or using HT were 1082, 1064, and 871 pg/mL, respectively. These values were not statistically different from each other.

Among smokers, the mean E2 concentration in premenopausal women was greater than postmenopausal women (35.7 vs. 19.1 pg/mL,  $P<0.004$ ). E2 concentrations in women using HT were somewhat higher than premenopausal women (46.1 pg/mL,  $P=0.24$ ).

Among smokers, mean 2-OHE1 concentrations for women who were premenopausal, postmenopausal, or using HT were 12.5, 7.3 and 29.8 pg/ml, respectively. These values were significantly different from each other. Among smokers, mean  $16\alpha$ -OHE1 levels for women who were premenopausal, postmenopausal, or using HT were 8.8 pg/mL, 6.5 pg/mL, and 11.3 pg/mL, respectively, values that were not statistically significantly different from each other (see Table 2).

## Associations of estrogens with isoprostanes

As seen in Table 3, *among non-smoking women*, E2 concentrations were not significantly associated with  $F_{2a}$ -isoprostanes in premenopausal women or those that used HT. In postmenopausal women, the partial correlation was  $\rho_{Y|X}=0.20$  (95% CI 0.08, 0.31). This and all subsequent partial correlations include adjustment for age, BMI, race, lipids, menopausal status, exercise, alcohol consumption, and diabetes.

In women who did not smoke, consistent, substantial and positive associations were observed between 2-OHE1 and  $F_{2a}$ -isoprostane concentrations in women using hormones ( $\rho_{Y|X} = 0.39$ ), postmenopausal women ( $\rho_{Y|X} = 0.43$ ) and in premenopausal women ( $\rho_{Y|X} = 0.44$ ).  $16\alpha$ -OHE1 concentrations were highly and positively associated with  $F_{2a}$ -isoprostane concentrations in premenopausal women ( $\rho_{Y|X}=0.52$ ), postmenopausal women ( $\rho_{Y|X} = 0.59$ ), and women using HT ( $\rho_{Y|X} = 0.46$ ), see Table 3.

*Among women who smoked*,  $F_{2a}$ -isoprostane levels were not significantly associated with E2 concentrations.  $F_{2a}$ -isoprostane levels were positively associated with 2-OHE1 concentrations in premenopausal women and in women who reported HT use [ $\rho_{Y|X}=0.28$  and  $0.55$ , respectively], *as well as* in postmenopausal women [ $\rho_{Y|X}=0.39$ ] after adjustment for covariates.  $F_{2a}$ -isoprostane levels were positively associated with  $16\alpha$ -OHE1 concentrations in

premenopausal women ( $\rho_{Y|X} = 0.39$ ), postmenopausal women ( $\rho_{Y|X} = 0.64$ ), and women using HT ( $\rho_{Y|X} = 0.58$ ).

The patterns between estrogens and F<sub>2a</sub>-isoprostanes were similar even when data were stratified by race groups or BMI categories of obesity (data not shown).

## DISCUSSION

It has been argued<sup>9</sup> that estrogens could exert antioxidant actions in modifying F<sub>2a</sub>-isoprostane production due to the presence of an aromatic hydroxyphenol structure.<sup>27</sup> However, these data do not support that hypothesis; indeed, the opposite may be true. While there was a statistically significant correlation between endogenous E2 and F<sub>2a</sub>-isoprostanes in postmenopausal women, the direction of the correlation was positive, not negative, and circulating levels of E2 were low. The same direction was observed with exogenous hormone use. The correlations of the estrone metabolites with F<sub>2a</sub>-isoprostanes were positive, not negative, and quite strong. Further, the strengths of these associations were not diminished following adjustment for race, menopause status, BMI, lipids, smoking behavior, vitamin E and iron supplement use, and level of alcohol consumption. This evidence led us to conclude that higher levels of these estrone metabolites were associated with greater oxidative stress and did not support our a priori hypothesis. However, these three aspects of estrogen status do not reflect all estrogen metabolites and it is possible that a non-measured analyte may have a relationship different than the very striking relationships we observed. For example, Tang et al. reported that 4-hydroxysterone was a potent inhibitor of lipid peroxidation in a cell culture system<sup>28</sup> and Seeger et al. reported that 2-methoxyestrone and 2-hydroxyestrone inhibited lipid peroxidation better than estradiol and 16 $\alpha$ -hydroxyestrone in pooled samples from healthy individuals.<sup>29</sup> Evaluation of measures of the range of estrogen metabolites in association with health-related states has been limited by the availability of reproducible assays that target specific metabolites and that can be implemented in substantially-sized populations.

Our conclusion that E2 and estrone metabolites were not acting like potent antioxidants is contingent on the integrity of the F<sub>2a</sub>-isoprostanes to serve as informative markers of oxidative stress. F<sub>2a</sub>-isoprostanes, compounds produced by the free radical-induced peroxidation of arachidonic acid,<sup>30,31</sup> have been characterized as superior markers of the *in vivo* oxidative stress response.<sup>15,32-34</sup> Further, elevated levels of F<sub>2a</sub>-isoprostanes are associated with behaviors or conditions thought to include oxidative stress such as smoking,<sup>35,36</sup> alcohol consumption,<sup>17</sup> hypercholesterolemia,<sup>37,38</sup> diabetes,<sup>39</sup> liver disease,<sup>40</sup> breast cancer,<sup>41</sup> and atherosclerotic lesions.<sup>42</sup> Indeed, we showed that F<sub>2a</sub>-isoprostanes values were more than doubled in women who smoked. Additionally, F<sub>2a</sub>-isoprostanes levels were also correlated with HDL-c in pre- and post-menopausal women who did not smoke and with LDL in post-menopausal women who smoked (data not shown).

To better understand the relationship of estrogen and F<sub>2a</sub>-isoprostanes, we adjusted for or stratified by factors that had previously been identified as generating variation in F<sub>2a</sub>-isoprostane concentrations. In some<sup>7,8</sup> but not all<sup>9,10</sup> studies of postmenopausal women, estrogen therapy acted like an antioxidant by diminishing the level of circulating oxidized LDL. However, responses to HT may have been inconsistently observed because preparations included progestin products as well as estrogens. It has been reported that progesterone opposes the antioxidant actions of estrogen on plasma LDL oxidation in primates.<sup>33</sup>

Health-related and lifestyle behaviors may influence oxidative capacity<sup>34,43</sup>. We observed little modifying effect by diet of the association of estrogens with F<sub>2a</sub>-isoprostanes, based on intakes of genistein, daidzein, or total dietary fat. This is consistent with the findings of Block<sup>12</sup> who reported a very modest association with fruits, and none with vegetables, dairy foods, grains,

meats, or total fat intake. In contrast to diet, we identified a very strong association of oxidative stress with smoking behavior. A greater proportion of African-American and Caucasian women smoked, compared to the low proportion of smokers in Chinese and Japanese women, leading to greatly exaggerated mean  $F_{2a}$ -isoprostane values for African-American and Caucasian women. Though we evaluated the role of passive smoking, we found only an association with personal smoking behavior. Women who reported more physical activity had lower  $F_{2a}$ -isoprostane concentrations. Others have reported that endurance exercise is associated with increased plasma  $F_{2a}$ -isoprostanes in dogs,<sup>44</sup> horses,<sup>45</sup> and humans.<sup>46</sup>

This study had a number of strengths and limitations. A substantial number of women were evaluated with representation from four racial/ethnic groups. While this was a cross-sectional study, it was nested in a longitudinal study of the menopausal transition, so that menopausal status and HT use were definable within the context of those longitudinal observations. In spite of these strengths, the cross-sectional design precluded our ability to establish temporal sequence between single-time measures of estrogens and  $F_{2a}$ -isoprostanes index of oxidative stress. Study protocol dictated that serum and urine be collected in the early follicular phase of the menstrual cycle rather than in the later follicular phase or luteal phase when estrogen levels will be higher. As a result, we are describing that low estrogen levels did not suppress oxidative stress; however, this does not necessarily preclude the possibility that higher estrogen levels as seen later in the menstrual cycle might suppress oxidative stress. Initial criticism of the  $F_{2a}$ -isoprostane measurement by EIA has been largely overcome by the use of affinity columns to prepare the samples for analyses as well as by the improvement in the assays, a procedure we applied in our assay procedures.<sup>47</sup> EIA from eluted samples correlated very well with other methods, including gas chromatography-mass spectrometry.<sup>48,49</sup> A major advantage of the *urinary* measurement of  $F_{2a}$ -isoprostanes was that they are not formed by *ex vivo* autooxidation in urine and levels were stable even when urine was maintained at room temperature for as much as 10 days.<sup>50,51</sup> Further, the concentration of  $F_{2a}$ -isoprostanes was unchanged with storage at  $-20$  degrees C.<sup>52,53</sup> Values from repeated days of urinary excretion tended to be the same with low intra-subject coefficients of variation in persons whether they were healthy or diseased.<sup>11,32,36,51</sup> Little diurnal variation is observed, and therefore, measurement of  $F_{2a}$ -isoprostanes in a single early morning sample has been described as adequate to represent the daily isoprostane excretion in humans.<sup>52</sup>

In summary, these data indicate major differences in  $F_{2a}$ -isoprostanes between smoking and non-smoking women across the menopause transition. However, there was no evidence that the serum measure of early follicular phase E2 or urinary estrone metabolite measures were acting like antioxidants; indeed, the strong adjusted and unadjusted positive correlations between the estrone metabolites and  $F_{2a}$ -isoprostanes suggest an active metabolic environment associated with generation of free radicals. This study refutes the commonly-held hypothesis that levels of endogenous estradiol or its estrone metabolites favorably modify oxidative stress through a decrease in  $F_{2a}$ -isoprostanes.

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This report is based on samples from the SWAN Core Repository. If scientists are interested in developing studies based on this resource, a description of the SWAN Core and DNA Repositories and how to obtain access to the resources can be found at [www.swanrepository.org](http://www.swanrepository.org).

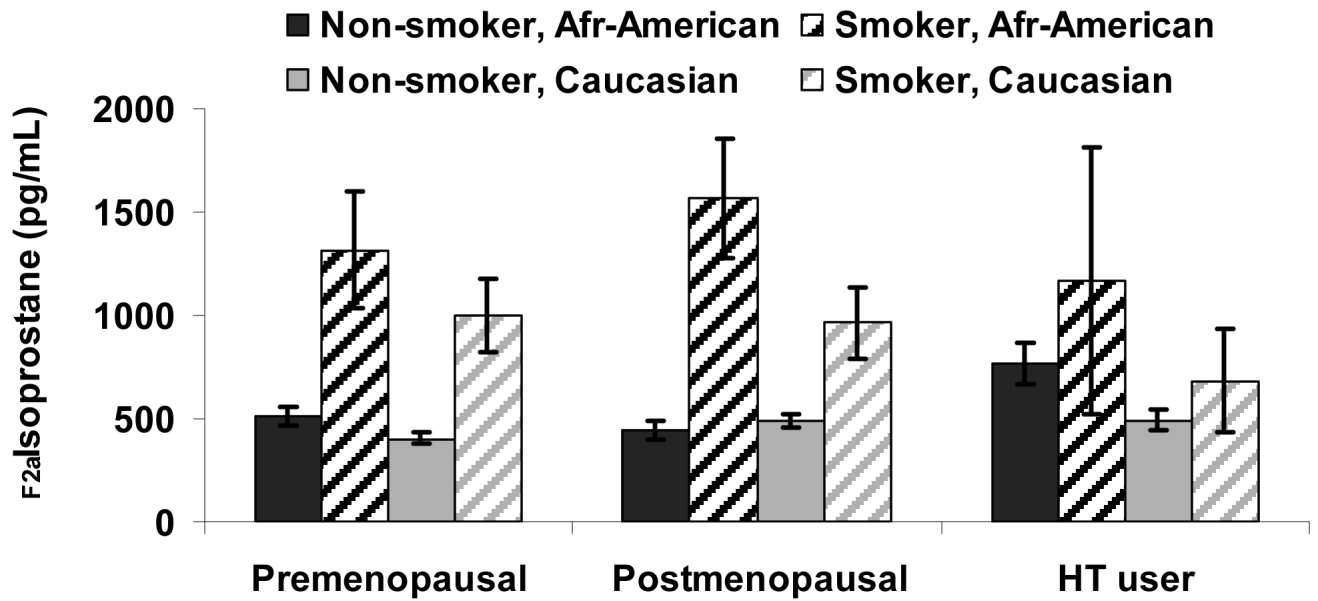
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**Figure 1.** F<sub>2a</sub>-isoprostanes concentrations among premenopausal women, postmenopausal women, and hormone therapy users, by race and smoking status at the 5<sup>th</sup> follow-up SWAN examination

**Table 1**  
 Characteristics of 1647 participants at the 5<sup>th</sup> follow-up SWAN examination (excluding women with hysterectomy)

Attribute	Pre- and Perimenopausal			Postmenopausal			HT users		
	Median	IQR*	N	Median	IQR	N	Median	IQR	N
Age (yr)	50.0	48 - 51	53.0	51 - 54	52.0	50 - 54	50 - 54	50 - 54	50 - 54
BMI (kg/m <sup>2</sup> )	26.5	23 - 32	27.0	23 - 32	26.0	23 - 31	23 - 31	23 - 31	23 - 31
Waist circumference (cm)	84.2	74 - 97	86.3	74 - 100	83.3	74 - 96	74 - 96	74 - 96	74 - 96
Estradiol (pg/mL)	38.4	20 - 79	13.2	10 - 21	39.5	22 - 68	22 - 68	22 - 68	22 - 68
2-hydroxyestrone (pg/mL)	8.7	5 - 15	5.3	3 - 9	17.3	8 - 41	8 - 41	8 - 41	8 - 41
16 $\alpha$ -hydroxyestrone (pg/mL)	6.1	4 - 10	4.3	3 - 6	9.0	5 - 18	5 - 18	5 - 18	5 - 18
F <sub>2<math>\alpha</math></sub> -isoprostanes (pg/mL)	433	235 - 785	413	231 - 747	495	262 - 872	262 - 872	262 - 872	262 - 872
Follicle-stimulating hormone (mIU/mL)	22.5	12 - 54	87.3	62 - 116	46.2	25 - 71	25 - 71	25 - 71	25 - 71
Race	N	%	N	%	N	%	N	%	%
African-American	205	22.7	99	26.2	66	18.1	66	18.1	18.1
Caucasian	445	49.3	168	44.4	223	61.1	223	61.1	61.1
Chinese	114	12.7	54	14.3	34	9.3	34	9.3	9.3
Japanese	138	15.3	57	15.1	42	11.5	42	11.5	11.5
Smoking									
Current	99	11.0	50	13.2	37	10.1	37	10.1	10.1
Not smoking	803	89.0	328	86.8	328	89.9	328	89.9	89.9
Exercise									
Lowest 2 quintiles	332	37.6	157	42.9	134	37.8	134	37.8	37.8
Middle 2 quintiles	373	42.2	136	37.2	144	40.7	144	40.7	40.7
Highest quintile	179	20.2	73	19.9	76	21.5	76	21.5	21.5
Alcohol consumption									
More than 1/2 serving / day	163	18.8	70	19.0	90	25.1	90	25.1	25.1
Less than 1/2 serving / day	268	31.0	94	25.5	105	29.3	105	29.3	29.3
None	434	50.2	204	55.5	163	45.6	163	45.6	45.6
Diabetes									

Attribute	Pre- and Perimenopausal		Postmenopausal		HT users	
	Median	IQR*	Median	IQR	Median	IQR
Yes	69	8.2	42	11.6	34	9.7
No	767	91.8	320	88.4	317	90.3

\* IQR = Interquartile range

Mean (SEM) of estrogen levels (pg/mL) by smoking and menopausal status at the 5<sup>th</sup> follow-up SWAN examination, adjusted for covariates\*

Table 2

	Non-Smokers			Smokers		
	Premenopausal N=803	Postmenopausal N=328	Using HT N=328	Premenopausal N=99	Postmenopausal N=50	Using HT N=37
<b>Estradiol (pg/mL)*</b>	38.9 (2.4)	14.7 (0.9)	36.0 (1.5)	35.7 (4.5)	19.4 (3.2)	46.1 (8.4)
<b>2-hydroxyestrone (pg/mL)*</b>	7.4 (0.3)	4.9 (0.3)	15.3 (0.9)	12.5 (1.6)	7.3 (1.2)	29.8 (5.5)
<b>16<math>\alpha</math>-hydroxyestrone (pg/mL)*</b>	5.4 (0.2)	4.1 (0.02)	9.5 (0.05)	8.8 (1.1)	6.5 (1.0)	11.3 (2.0)
<b>F<sub>2<math>\alpha</math></sub>-isoprostanes (pg/mL)*</b>	343 (12.4)	379 (19.5)	425 (21.9)	1082 (139.6)	1064 (174.1)	871 (159.6)

\* Adjusted for age, BMI, race, lipids, menopausal status, exercise, alcohol consumption, and diabetes

**Table 3**  
*Pearson* ( $\rho$ , 95% confidence intervals) and partial *Pearson* correlations ( $\rho_{Y|X}$ , 95% confidence intervals) between  $F_{2a}$ -isoprostane concentrations and estrogen measures, by menopause and smoking status

	Premenopausal			Postmenopausal			Using hormones		
	$\rho$	95% CI	N	$\rho$	95% CI	N	$\rho$	95% CI	N
<b>Nonsmokers</b>			N=778			N=323			N=325
<b>Unadjusted</b>									
Estradiol (pg/mL)	0.13	0.06, 0.20		0.31	0.21, 0.41		0.04	-0.07, 0.15	
2-hydroxyestrone (pg/mL)	0.43	0.37, 0.49		0.43	0.33, 0.51		0.34	0.24, 0.44	
16 $\alpha$ -hydroxyestrone (pg/mL)	0.57	0.52, 0.62		0.60	0.53, 0.67		0.44	0.35, 0.52	
<b>Adjusted*</b>									
Estradiol (pg/mL)	0.08	0.00, 0.15		0.20	0.08, 0.31		0.02	-0.09, 0.14	
2-hydroxyestrone (pg/mL)	0.44	0.38, 0.50		0.43	0.33, 0.52		0.39	0.29, 0.49	
16 $\alpha$ -hydroxyestrone (pg/mL)	0.52	0.47, 0.57		0.59	0.51, 0.66		0.46	0.36, 0.54	
<b>Smokers</b>			N=87			N=44			N=33
<b>Unadjusted</b>									
Estradiol (pg/mL)	-0.007	-0.22, 0.21		0.28	-0.03, 0.55		0.04	-0.31, 0.38	
2-hydroxyestrone (pg/mL)	0.22	0.01, 0.41		0.45	0.18, 0.66		0.49	0.18, 0.71	
16 $\alpha$ -hydroxyestrone (pg/mL)	0.36	0.16, 0.53		0.62	0.40, 0.78		0.43	0.10, 0.67	
<b>Adjusted*</b>									
Estradiol (pg/mL)	0.10	-0.15, 0.34		0.38	-0.01, 0.68		0.10	-0.43, 0.45	
2-hydroxyestrone (pg/mL)	0.28	0.04, 0.49		0.39	0.03, 0.66		0.55	0.16, 0.79	
16 $\alpha$ -hydroxyestrone (pg/mL)	0.39	0.16, 0.58		0.64	0.35, 0.81		0.58	0.19, 0.81	

\* Adjusted for age, BMI, race group, lipids, physical activity level, alcohol, and having diabetes.