Dietary Fat Subgroups, Zinc, and Vegetable Components Are Related to Urine F₂a-Isoprostane Concentration, a Measure of Oxidative Stress, in Midlife Women¹ ²

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Abstract

Smoking, diet, and physical activity may impact chronic diseases in part by promoting or attenuating oxidative stress. We evaluated associations between lifestyle factors and urine F₂a-isoprostanes, a marker of oxidative stress in 1610 participants of the Study of Women’s Health Across the Nation (SWAN). Dietary intake and physical activity were assessed at baseline and the 5th year 05 (Y05). These data were related to Y05 urinary F₂a-isoprostane concentration with regression analyses. Median urine F₂a-isoprostane concentration was 433 ng/L overall, 917 ng/L in smokers (inter-quartile range (IQR): 467, 1832 ng/L), and 403 ng/L in nonsmokers (IQR: 228, 709 ng/L; P < 0.0001 for difference). Higher trans fat intake was associated with higher urine F₂a-isoprostane concentration; partial Spearman correlations (rᵦₓ) between Y05 urine F₂a-isoprostane concentration and trans fatty acids was 0.19 (P = 0.03) in smokers and 0.13 (P < 0.0001) in nonsmokers. Increased log trans fat intake from baseline to Y05 was associated with higher concentration of log urine F₂a-isoprostanes in nonsmokers (β = 0.131, SE = 0.04, P = 0.0003). In nonsmokers, the partial correlation (rᵦₓ) between lutein and urine F₂a-isoprostane concentration was −0.13 (P < 0.0001). Increased intake of lutein from baseline to Y05 was also associated with lower log urine F₂a-isoprostane concentration (β = −0.096, SE = 0.03, P = 0.0005) in nonsmokers. Increased zinc intake from baseline to Y05 was associated with lower log urine F₂a-isoprostane concentration in smokers and nonsmokers (β = −0.346, SE = 0.14, P = 0.01), and −0.117, 0.04 (P = 0.001), respectively. In conclusion, diet (fat subtypes, zinc, and vegetable components) and smoking were associated with urine F₂a-isoprostanes, a marker of oxidative stress. J. Nutr. 137: 2412–2419, 2007.

Introduction

Oxidative stress, an unfavorable balance between free radical generation and level of antioxidants, has been implicated in the pathogenesis of cancers, atherosclerosis, and diabetes (1,2). Stable biomarkers of this oxidative stress include F₂a-isoprostanes, the prostaglandin-like compounds formed in vivo from the free radical catalyzed peroxidation of arachidonic acid. (3–5). F₂a-isoprostanes have been shown to be reliable biomarkers of lipid peroxidation. They are continuously formed under normal physiological conditions and increased production has been observed with co-occurrence of smoking, alcohol intake, exercise, and drug treatment. There is decreased production with dietary antioxidant supplementation and fruit and vegetable intake (6).

Lifestyle factors, including dietary intake and physical activity, are thought to influence free radical proliferation. Antioxidants mediate oxidative damage by mitigating the progression of unpaired electron transfer associated with free radical formation (7) and they are identified as constituents responsible for the protective effects of fruits and vegetables (8–13).

Polyphenols may be effective in preventing oxidative stress-associated diseases (14,15), but long-term investigations of their efficacy are sparse. Studies have focused on β-carotene, ascorbic acid, and vitamin E, and although some evidence supports a protective effect against oxidative damage (16,17), results from randomized, controlled trials in healthy populations are less convincing (18–20). Lutein, from sources including spinach and greens, has
been associated with favorable levels of oxidative stress markers (21). Although human studies are scarce, intake of saturated (22) and trans fats has been implicated in higher oxidative stress levels (23).

Cigarette smoking and environmental tobacco smoke in nonsmokers has been associated with higher levels of oxidative stress (24–26). Cigarette smoke has been shown to increase the requirements for several antioxidants (27, 28); however, smokers generally consume lower levels of antioxidants and higher amounts of saturated and trans fats than nonsmokers (29, 30). Thus, it is clear that associations between dietary intake and oxidative stress are not comparable in smokers vs. nonsmokers.

Physical activity has also been associated with oxidative stress, with indications that this association is apparently related to the intensity and duration of the activity. Physical activity of moderate intensity and duration has been associated with a favorable effect or no increase in F2α-isoprostanes (31, 32). The higher oxygen required for muscle activity in higher intensity or endurance exercise may lead to an accumulation of excess reactive oxygen species in anaerobic respiration and increased oxidative stress (33).

We evaluated the association between oxidative stress and lifestyle factors in women being studied longitudinally through the menopausal transition. We postulated that antioxidant intake would be inversely correlated with urine F2α-isoprostanes, a measure of oxidative stress, and that these relationships would differ by cigarette smoking status. We also hypothesized that low or moderate physical activity would be inversely associated with urine F2α-isoprostanes, whereas more vigorous activity would be linked to higher levels of oxidative stress. Although rates of certain cancers and cardiovascular disease differ by race (34, 35), no research on lifestyle factors and oxidative stress has focused on ethnically diverse, midlife women. Inclusion of diverse women in our analysis adds a key strength, providing a wide range in nutrient intakes and physical activity and differing rates of smoking.

Materials and Methods

Sampling and study population

This report includes data from the baseline and 5th annual follow-up examination (Y05)7 of the Study of Women’s Health Across the Nation (SWAN), a community-based, longitudinal study of the menopausal transition (36). The analysis includes 1610 participants with baseline and Y05 dietary intake and physical activity data who were located in Boston, MA; Chicago, IL; the Detroit, MI area; Los Angeles, CA; Oakland, CA; or Pittsburgh, PA. Observations from the New Jersey SWAN site were excluded because of incomplete data at Y05. Study eligibility criteria at baseline for the SWAN longitudinal cohort were: age 42–52 y; intact uterus and at least 1 ovary; no current use of estrogens or other medications known to affect ovarian function; at least 1 menstrual period in the 3 mo before enrollment; and self-identification as a member of a site-eligible racial/ethnic group. Caucasians were recruited at all study sites. African-American women were recruited in Boston, Chicago, Pittsburgh, and the Detroit area, while Japanese and Chinese women were recruited at the Los Angeles and Oakland sites, respectively. The Chicago site only provided urine samples for 44 women for a SWAN substudy; these samples were analyzed for F2α-isoprostanes and represent the Chicago site contribution. Institutional Review Board approval was secured for the study protocol and participants gave informed consent at each study site.

Assays

F2α-isoprostanes were assayed in urine specimens collected prior to 0900 during the Y05 visit and made available through the SWAN Repository (n = 1606). Assays were completed in the Clinical Ligand Assay Service Satellite laboratory at the University of Michigan.

Samples were applied to the F2α-isoprostane affinity column, washed with buffer, and eluted with 95% ethanol. Following evaporation of the solvent, the dried samples were diluted 1:10 with 0.1 mol/L phosphate buffer and assayed using the F2α-isoprostane enzyme immunoassay kit (Cayman Chemical). The range of the standard curve was 3.9–500 ng/L. Samples in a 96-well microplate were read at 405 nm. The postextraction intra-assay CV was 14.4% (51.2 ng/L, n = 83 pairs) and the inter-assay CV was 17.5% (51.2 ng/L, n = 85). The intra-assay CV for the concentration of F2α-isoprostanes was 5.8% (n = 1707 pairs).

Diet and lifestyle data

Dietary and supplement data were obtained at baseline and Y05 from a modified interviewer-assisted FFQ. Originally developed by Block (37), the questionnaire was designed to obtain “usual” dietary patterns for the previous year and was administered in 3 languages (English, Chinese, or Japanese). All FFQ contained a 103-item core food list; the Chinese and Japanese language versions included additional culturally-specific foods.

Numbers of daily servings of Brassica vegetables, including cabbage, broccoli, cauliflower, Brussels sprouts, and kale, were specifically assessed due to their association with lower F2α-isoprostane concentration, independent of micronutrient intake (8). For most fruit, a daily serving was quantified as 1 medium piece, except watermelon (one slice), cantaloupe (1/4 medium), and mangos or papayas (1/2 medium). The serving size for prunes and strawberries was 1/2 cup. Whereas most daily vegetable servings consisted of 1/2 cup, the exceptions were green salad (1 medium bowl), French fries (3/4 cup), and white potatoes (1/2 cup). Polyphenol values from Manach (38) were assigned to foods and beverages by using the midpoint of the published range (in milligrams of the particular polyphenol per serving) for a mean daily serving estimated via FFQ. The contribution of coffee and caffeine were evaluated independently and not included in the total polyphenol value or in hydroxyxynamic acids. Genistein and daidzein intakes, based on database information from Reinli and Block (39), were evaluated separately.

Baseline and Y05 dietary data were excluded from this report under the following conditions: too few or too many solid foods (or food groups) consumed per day (<4 or >17, respectively; n = 104), missing data for >10 foods (n = 4), and daily energy intake considered too low or too high for usual intake [<500 or >20920 kJ/d (5000 kcal/d), respectively; n = 14].

Physical activity was assessed using an adaptation of the Kaiser Physical Activity Survey (40). This 38-question survey is a self-administered questionnaire with established test-retest reliability and validity (40, 41). Total possible physical activity scores ranged from 3 to 14, with higher numbers indicating greater activity.

Other lifestyle variables. Weight (kilograms) was measured using balance beam scales and height (meters) was measured using stadiometers. Data on cigarette smoking and environmental tobacco smoke exposure were obtained from a self-administered questionnaire incorporating American Thoracic Society questions (42) and validated questions on environmental tobacco smoke exposure (43).

Statistical approach

SAS version 9.1 statistical software was used for data management and analyses. The univariate distributions of continuous measures were examined and natural log transformations were employed, as appropriate, to meet the assumptions of normality and to reduce skewness.

Created variables. Due to almost negligible intakes of daidzein and genistein by Caucasian and African-American participants, only Chinese and Japanese participants were included in analyses of these variables. Daily intakes of daidzein and genistein in Chinese and Japanese women were categorized into tertiles. The 33rd and 66th percentile cut-off

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7 Abbreviations used: IQR, interquartile range; SWAN, Study of Women’s Health Across the Nation; Y05, 5th annual follow-up examination.

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8 1 kcal = 4.184 kJ.
Values for daidzein intake were 906 μg and 3071 μg, respectively, in Chinese, and 2845 μg and 8992 μg, respectively, in Japanese women. The 33rd and 66th percentile cut-off values for genistin were 1757 μg and 6118 μg, respectively, for Chinese and 4039 μg and 13286 μg, respectively, in Japanese participants.

Most analyses were stratified based on a smoking status variable that classified participants as active smokers or not. A 4-level variable that considered environmental tobacco smoke was used to compare the least squared means of urine F2\textsubscript{alpha}-isoprostane concentration. The levels were: 1) nonsmokers with no active or environmental tobacco smoke exposure; 2) smokers with >1 h/wk of environmental tobacco smoke exposure; 3) smokers without environmental tobacco smoke exposure; or 4) smokers with >1 h/wk of environmental tobacco smoke exposure.

Physical activity variables were categorized to test the hypothesis that more intense physical activity would have a positive association with urine F2\textsubscript{alpha}-isoprostanes and moderate physical activity would have an inverse association. The categories were generated from the total physical activity questionnaire scores at Y05 (cut-offs: 40th and 80th percentiles, 7.25 and 9.4 points, respectively) Additionally, Y05 scores for the questionnaire’s “sport” subsection that measured the frequency, intensity, and duration of 2 sports or exercise activities in the year prior to assessment had cut-offs at the 33rd and 66th percentiles, 2.25 and 3.5 points, and ranged from 1–5 points.

**Statistical analyses.** Medians and quartile values (Q1 and Q3) were calculated for continuous measures according to smoking status stratum. The Wilcoxon’s rank-sum, Kruskal-Wallis, and chi-square tests were used to test differences in medians between race/ethnic groups and between smokers and nonsmokers.

Partial Spearman correlations (\( \rho_{xy} \)) were estimated to describe the statistical associations between Y05 urine F2\textsubscript{alpha}-isoprostanes and dietary/ supplemental intakes after controlling for age, BMI, physical activity, clinical site, and race/ethnicity. Additionally, given that antioxidants attenuate oxidative damage caused by high dietary fat intake and because the beneficial effect of antioxidants is dependent on the amount of dietary fat a person consumes, partial Spearman correlations between urine F2\textsubscript{alpha}-isoprostanes and antioxidants were also adjusted for Y05 total dietary fat intake.

Multiple variable regression analyses, with log-transformed urine F2\textsubscript{alpha}-isoprostane concentration as the dependent variable, were used to estimate prospective associations with changes in dietary consumption over time. Significant P-values were 2-sided at \( p < 0.05 \). Dietary variables were evaluated singly rather than simultaneously to avoid collinearity.

Associations between individual dietary variables and urine F2\textsubscript{alpha}-isoprostane concentration were stratified by smoking status for 3 reasons. First, there was a strong relationship in our data between smoking status and urine F2\textsubscript{alpha}-isoprostane concentration, which is supported by others (24,44); second, the dietary intake of smokers differed from that of nonsmokers in our data and in nationally representative data (30); and finally, associations between dietary intake variables and F2\textsubscript{alpha}-isoprostanes differed in smokers vs. nonsmokers in our results and in other investigations (45,46). Because smoking was a behavior rarely reported by Chinese or Japanese women, those few Asian women who reported smoking were excluded from selected analyses, as appropriate.

When total environmental smoke exposure was evaluated, there was a graded increase in the least squared means of urine F2\textsubscript{alpha}-isoprostane concentration according to cigarette smoke exposure. However, after adjustment for age, clinical site, BMI, physical activity, and race, a significant difference in least squared means of urine F2\textsubscript{alpha}-isoprostane concentration was only observed between active smokers and nonsmokers, regardless of environmental tobacco smoke exposure status. Thus, the analysis is stratified based on active smoking.

### Results

The overall median concentration of urine F2\textsubscript{alpha}-isoprostanes was 433 ng/L. Because cigarette smoking status differed by race, dietary intake, and urine F2\textsubscript{alpha}-isoprostane concentration, results are reported by smoking status. Median urine F2\textsubscript{alpha}-isoprostane concentration was more than twice as high in women who smoked [917 ng/L, inter-quartile range (IQR): 467, 1832 ng/L] as in nonsmokers (403 ng/L, IQR: 228, 709 ng/L; \( P < 0.0001 \) for difference). Twenty-one percent of African-American women, 10% of Caucasian women, 1% of Chinese women, and 8% of Japanese women reported smoking (Table 1). In nonsmokers, Chinese and Japanese women had lower median urine F2\textsubscript{alpha}-isoprostane concentrations compared with the other 2 racial/ethnic groups (\( P < 0.0001 \); Table 1).

**Dietary intake and physical activity score at Y05 by smoking status.** In general, women who smoked had dietary intakes lower in micronutrients, polyphenols, fruits, and vegetables and higher in fat than nonsmokers (Table 2). Women who smoked reported somewhat lower levels of physical activity than nonsmokers [median physical activity questionnaire scores: 7.0 (IQR: 5.9, 8.5) vs. 7.9 (IQR: 6.5, 9.2), respectively; \( P < 0.0001 \) for difference].

Overall, ~52–61% women reported use of 1 of the following supplements: \( \beta \)-carotene, vitamins A, C, D, E, or zinc; women

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**Table 1** Characteristics of SWAN participants according to smoking status and race

<table>
<thead>
<tr>
<th></th>
<th>Smokers</th>
<th>Nonsmokers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>African American</td>
<td>Caucasian</td>
</tr>
<tr>
<td></td>
<td>African American</td>
<td>Caucasian</td>
</tr>
<tr>
<td>n</td>
<td>76 (48, 53)</td>
<td>50 (48, 52)</td>
</tr>
<tr>
<td></td>
<td>283</td>
<td>747</td>
</tr>
<tr>
<td>( \text{Age, y} )</td>
<td>51</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>51 (49, 53)</td>
<td>51 (49, 53)</td>
</tr>
<tr>
<td>( \text{BMI, kg/m}^2 )</td>
<td>31</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>33 (27, 39)</td>
<td>27</td>
</tr>
<tr>
<td>Physical activity score</td>
<td>6.7</td>
<td>6.0</td>
</tr>
<tr>
<td></td>
<td>7.3</td>
<td>6.0</td>
</tr>
<tr>
<td>Urine F2\textsubscript{alpha}-isoprostane level, ng/L</td>
<td>1226 (721, 2074)</td>
<td>803 (470, 1676)</td>
</tr>
<tr>
<td>Economic stress, % frequency</td>
<td>13 (12)*</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Very hard to pay for basics</td>
<td>41 (37)</td>
<td>32 (31)</td>
</tr>
<tr>
<td>Somewhat hard to pay for basics</td>
<td>57 (51)</td>
<td>68 (67)</td>
</tr>
</tbody>
</table>

1 Values are medians and quartiles (Q1, Q3) collected at Y05, except economic stress, for which data collected at y 6 are presented; information is presented for those with urine F2\textsubscript{alpha}-isoprostanes data. *Values different between race/ethnicity among smokers, \( P < 0.05 \) (Wilcoxon’s rank-sum or chi-square test) or nonsmokers, \( P < 0.05 \) (Kruskal-Wallis or chi-square test).

2 Values not estimated due to small cell sizes.
TABLE 2 Dietary intake of SWAN participants by smoking status

<table>
<thead>
<tr>
<th>Smokers</th>
<th>Nonsmokers</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>143</td>
</tr>
<tr>
<td>Energy, kcal/d</td>
<td>7824 [5908, 10305]</td>
</tr>
<tr>
<td>Total fat, g/d</td>
<td>73.4 [54.4, 98.8]</td>
</tr>
<tr>
<td>SFA, g/d</td>
<td>25.4 [17.3, 34.8]</td>
</tr>
<tr>
<td>Trans fatty acid, g/d</td>
<td>5.9 [3.4, 8.9]</td>
</tr>
<tr>
<td>Linoleic acid, g/d</td>
<td>1.0 [0.6, 2.0]</td>
</tr>
<tr>
<td>Oleic acid, g/d</td>
<td>23.4 [20.1, 37.8]</td>
</tr>
<tr>
<td>n-3 fatty acid, g/d</td>
<td>1.4 [1.0, 1.7]</td>
</tr>
<tr>
<td>Vitamin A, retinol, µg/d</td>
<td>6703 [3782, 10573]</td>
</tr>
<tr>
<td>α-Carotene, µg/d</td>
<td>11.6 [3.1, 29.4]</td>
</tr>
<tr>
<td>β-Carotene, µg/d</td>
<td>2537 [1311, 4647]</td>
</tr>
<tr>
<td>Lutein, µg/d</td>
<td>1633 [872, 3546]</td>
</tr>
<tr>
<td>Lycopene, µg/d</td>
<td>1011 [439, 1570]</td>
</tr>
<tr>
<td>β-Cryptoxanthin, µg/d</td>
<td>96.4 [50.4, 157.3]</td>
</tr>
<tr>
<td>Vitamin C, mg/d</td>
<td>136.1 [71.0, 226.1]</td>
</tr>
<tr>
<td>Vitamin D, µg/d</td>
<td>4.3 [2.3, 11.9]</td>
</tr>
<tr>
<td>Vitamin E, µ-TE/d</td>
<td>15.5 [8.7, 31.6]</td>
</tr>
<tr>
<td>Zinc, mg/d</td>
<td>12.4 [7.6, 23.7]</td>
</tr>
<tr>
<td>Animal zinc, mg/d</td>
<td>5.5 [4.2, 7.6] A</td>
</tr>
<tr>
<td>Fruit A servings/d</td>
<td>1.0 [0.5, 1.4]</td>
</tr>
<tr>
<td>Vegetables A servings/d</td>
<td>1.5 [0.8, 2.1]</td>
</tr>
<tr>
<td>Brassica vegetables, servings/d</td>
<td>0.3 [0.2, 0.6]</td>
</tr>
<tr>
<td>Broccoli, servings/wk</td>
<td>0.1 [0.25, 0.2]</td>
</tr>
<tr>
<td>Cauliflower, Brussels sprouts, servings/wk</td>
<td>0.25 [0.0, 0.6]</td>
</tr>
</tbody>
</table>

1 Values are medians and quartiles (Q1, Q3) collected at Y05. *Different from nonsmokers, P < 0.05 (Wilcoxon’s rank-sum test).
2 Includes African Americans and Caucasians, n = 143; Japanese and Chinese smokers were excluded due to small cell sizes.
3 Includes all ethnic groups, n = 1385.
4 1 kcal = 4.184 kJ.
5 A 1/2-cup serving of fruit is ~123 g.
6 A 1/2-cup serving of chopped vegetables is ~90 g.

who smoked were less likely to report dietary supplement (~46–55%) use than nonsmokers (~56–67%; comparison of smokers vs. nonsmokers taking at least 1 supplement vs. none, P = 0.001).

Concurrent associations between nutrients, foods, and urinary F2α-isoprostane concentration. Among both smokers and nonsmokers, there were significant positive partial Spearman correlations between the urine F2α-isoprostane concentration and trans fatty acid intake (ρ_y = 0.19 (P = 0.03) and 0.13 (P < 0.0001), respectively). These partial correlations reflect the direction and strength of the association of interest after accounting for the variation from age, race, BMI, physical activity, and clinical site. We observed a significant partial correlation among nonsmokers between saturated fat intake and the urine F2α-isoprostane concentration (ρ_y = 0.07; P = 0.007). Although the partial correlation between saturated fat and the urine F2α-isoprostane concentration among smokers was the same direction and similar magnitude as that for trans fats (ρ_y = 0.14), it was not significant (P = 0.1). Higher vitamin E combined diet and supplement intake was associated with lower concentration of urine F2α-isoprostane concentration in both smokers and nonsmokers (ρ_y = -0.2 (P = 0.02) and -0.12 (P < 0.0001), respectively).

In nonsmokers, higher intakes of lutein (ρ_y = -0.13; P < 0.0001) and β-carotene (ρ_y = -0.13; P < 0.0001), along with more daily vegetable servings (ρ_y = -0.12; P < 0.0001) and Brassica vegetable servings (ρ_y = -0.11; P = 0.0001), were associated with lower concentration of urine F2α-isoprostanes.

Among smokers, we found inverse partial correlations between concentration of urine F2α-isoprostanes and vitamin D (ρ_y = -0.18; P = 0.04), total polyphenols (ρ_y = -0.18; P = 0.04), and isoavonones (ρ_y = -0.2; P = 0.02), indicating lower oxidative stress with higher intakes. There was a borderline concurrent association with zinc (ρ_y = -0.17; P = 0.05) in smokers.

In the Japanese women, there were inverse partial correlations with urine F2α-isoprostane concentration for daidzein (ρ_y = -0.15 (P = 0.03)) and genistein (ρ_y = -0.15 (P = 0.04)) intakes, whereas in Chinese women, there was a borderline association with genistein (ρ_y = -0.15 (P = 0.05)).

Coffee or caffeine intake and urine F2α-isoprostane concentrations were not associated.

Nutrient intake change (baseline to Y05) and concentration of urinary F2α-isoprostanes at Y05. Increased trans fat intake between baseline and Y05 follow-up was associated with significantly higher concentration of urine F2α-isoprostanes (Table 3) among nonsmokers. Increased lutein and vitamin C consumption between baseline and Y05 follow-up was associated with significantly lower concentration of urine F2α-isoprostanes in nonsmokers. Increased zinc consumption was associated with significantly lower concentration of urine F2α-isoprostanes in smokers and nonsmokers.

In African-American and Caucasian women who smoked, increased total isoflavone intake (not including genistein or daidzein) from baseline to Y05 was associated with significantly lower concentration of urine F2α-isoprostanes (Table 4). High baseline intake of daidzein was significantly associated with lower urine F2α-isoprostane concentration in nonsmoking Chinese and Japanese women, respectively, compared with their respective counterparts with the lowest intake. Higher baseline intake of genistein was also significantly associated with lower urine F2α-isoprostane concentrations in nonsmoking Chinese women compared with Chinese women with the lowest intake.

In nonsmokers, higher Brassica vegetable consumption at baseline and increased consumption of Brassica vegetables from baseline to Y05 was associated with significantly lower urine F2α-isoprostane concentration. Also in nonsmokers, women in the highest category of baseline broccoli consumption (>1.5 servings/wk) had significantly lower urine F2α-isoprostane concentration than women from the lowest category (≥0.625 servings/wk).

Physical activity. We examined physical activity as a continuous variable, dichotomized as a categorical variable (vigorous vs. not vigorous), and as an ordinal variable in regression analyses. There were no significant (P < 0.05) associations with urine F2α-isoprostane concentration.

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High baseline daidzein intake\(^6\) trans fatty acids have been associated with elevated coronary heart disease risk (47).

In animal studies, \(\text{trans}\) fat intake is associated with increased oxidative stress (23). In human studies, higher intakes of \(\text{trans}\) fatty acids have been associated with elevated coronary heart disease risk (47).

Trans fats, found in some margarines, crackers, salad dressings, and other foods, are a result of food processing when hydrogen is added to unsaturated fats in vegetable oils to increase shelf-life. Thus, the link between higher \(\text{trans}\) fat intake and higher \(F_{2\alpha}\)-isoprostanes, a measure of arachidonic acid peroxidation, is logical because \(\text{trans}\) fats are partially hydrogenated polyunsaturated fats. After ingestion, \(\text{trans}\) fats can be incorporated into cellular membrane phospholipids, often displacing \(\text{cis}\)-PUFA, leading to decreased cell membrane fluidity (48). This reduced fluidity allows for increased activity of free radicals in the phospholipid bilayer, precipitating oxidative damage. \(F_{2\alpha}\)-isoprostanes and several prostaglandins arise from the peroxidation of arachidonic acid (48), a metabolic process thought to contribute to heart disease through altered platelet aggregation and the promotion of insulin resistance (49–51).

We also found an association between urine \(F_{2\alpha}\)-isoprostanes and saturated fats in nonsmokers. We were unable to resolve whether the association with urine \(F_{2\alpha}\)-isoprostanes was only with \(\text{trans}\) fat, with saturated fats, or both. In our analyses, the types of fat intake were highly correlated, precluding their simultaneous inclusion in a regression model. Further, the 2 types of fats are not readily distinguished biologically. Relatively greater proportions of both saturated and \(\text{trans}\) fatty acids are stored in the phospholipid bilayer leading to increased cell membrane

**TABLE 3** Associations between log urine \(F_{2\alpha}\)-isoprostanes and change in nutrient intake from baseline to Y05 in regression analyses according to smoking classification

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Smokers(^2) (\beta \pm \text{SE} )</th>
<th>Partial (r^2)</th>
<th>P-value</th>
<th>Nonsmokers(^3) (\beta \pm \text{SE} )</th>
<th>Partial (r^2)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change in trans fat intake</td>
<td>0.188 ± 0.14</td>
<td>NS(^4)</td>
<td>1.4</td>
<td>0.131 ± 0.04</td>
<td>0.0003</td>
<td>1.0</td>
</tr>
<tr>
<td>Change in saturated fat intake</td>
<td>0.292 ± 0.2</td>
<td>NS</td>
<td>1.7</td>
<td>0.047 ± 0.06</td>
<td>NS</td>
<td>0.1</td>
</tr>
<tr>
<td>Change in lutein intake</td>
<td>-0.214 ± 0.09</td>
<td>NS</td>
<td>1.7</td>
<td>-0.096 ± 0.03</td>
<td>0.0005</td>
<td>0.9</td>
</tr>
<tr>
<td>Change in lycopene intake</td>
<td>-0.014 ± 0.1</td>
<td>NS</td>
<td>0.0</td>
<td>-0.003 ± 0.03</td>
<td>NS</td>
<td>0.0</td>
</tr>
<tr>
<td>Change in (\beta)-carotene intake from diet/supplements</td>
<td>-0.094 ± 0.11</td>
<td>NS</td>
<td>0.6</td>
<td>-0.055 ± 0.03</td>
<td>NS</td>
<td>0.2</td>
</tr>
<tr>
<td>Change in vitamin C intake</td>
<td>-0.166 ± 0.09</td>
<td>NS</td>
<td>2.6</td>
<td>-0.074 ± 0.03</td>
<td>0.007</td>
<td>0.6</td>
</tr>
<tr>
<td>Change in zinc intake</td>
<td>-0.346 ± 0.14</td>
<td>0.01</td>
<td>5.0</td>
<td>-0.117 ± 0.04</td>
<td>0.001</td>
<td>0.8</td>
</tr>
</tbody>
</table>

\(^{1}\) Independent variables in regression models were constructed as log (Y05 variable) – log (baseline variable).

\(^{2}\) Includes African-Americans and Caucasians, \(n = 136\); models were adjusted for site, baseline value of independent variable of interest, along with baseline values and change from baseline to Y05 values for fat intake, age, BMI, and physical activity score; Japanese and Chinese smokers were excluded due to small cell sizes.

\(^{3}\) Includes all ethnic groups, \(n = 1321\); models were adjusted for site, race/ethnicity, baseline value of independent variable of interest, along with baseline values and change from baseline to Y05 values for fat intake, age, BMI, and physical activity score.

\(^{4}\) NS = nonsignificant, \(P > 0.05\).

**Discussion**

We evaluated whether nutritional factors, physical activity, and smoking behaviors were associated with increased oxidative stress as assessed by urine \(F_{2\alpha}\)-isoprostane concentrations in a large multi-race/ethnic sample of midlife women. This, has important public health implications because oxidative stress is believed to contribute to risk for cardiovascular disease, diabetes, and cancers (1,2) and lifestyle behaviors are potentially modifiable.

Our results show associations between higher intake of \(\text{trans}\) fats and higher concentration of urine \(F_{2\alpha}\)-isoprostanes in smokers and nonsmokers concurrently and in nonsmokers prospectively. In animal studies, \(\text{trans}\) fat intake is associated with increased oxidative stress (23). In human studies, higher intakes of \(\text{trans}\) fatty acids have been associated with elevated coronary heart disease risk (47).

**TABLE 4** Associations of log urine \(F_{2\alpha}\)-isoprostanes with phytoestrogen intakes in regression models according to smoking classification and race/ethnicity\(^1\)

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Smokers(^2) African American and Caucasian (\beta \pm \text{SE} )</th>
<th>Partial (r^2)</th>
<th>Nonsmokers Chinese(^4) (\beta \pm \text{SE} )</th>
<th>Partial (r^2)</th>
<th>Japanese(^4) (\beta \pm \text{SE} )</th>
<th>Partial (r^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change in isoflavones intake(^5)</td>
<td>-0.265 ± 0.11*</td>
<td>5.3</td>
<td>-0.008 ± 0.02</td>
<td>0.01</td>
<td>-0.049 ± 0.05</td>
<td>0.6</td>
</tr>
<tr>
<td>High baseline daidzein intake(^6)</td>
<td>-0.334 ± 0.14*</td>
<td>3.2</td>
<td>-0.319 ± 0.16*</td>
<td>2.3</td>
<td>-0.03 NS</td>
<td>0.2</td>
</tr>
<tr>
<td>High baseline genistein intake(^6)</td>
<td>-0.435 ± 0.14*</td>
<td>5.3</td>
<td>-0.231 ± 0.16</td>
<td>1.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^{1}\) \(\ast P < 0.05\).

\(^{2}\) \(n = 136\). Model adjusted for site, baseline value of independent variable of interest, along with baseline values and change from baseline to Y05 values for fat intake, age, BMI, and physical activity score; Japanese and Chinese smokers were excluded due to small cell sizes.

\(^{3}\) \(n = 960\). Model adjusted for site, baseline value of independent variable of interest, along with baseline values and change from baseline to Y05 values for fat intake, age, BMI, and physical activity score.

\(^{4}\) Chinese, \(n = 178\); Japanese, \(n = 185\). Models adjusted for baseline value of independent variable of interest, along with baseline values and change from baseline to Y05 values for fat intake, age, BMI, and physical activity score.

\(^{5}\) Independent variables in regression models were: log (Y05 variable) – log (baseline variable).

\(^{6}\) High intake was highest tertile; the reference group was the lowest tertile of intake.
rigidity compared with less saturated fats. Finally, both fat subtypes are found in many of the same foods.

Fairly consistent inverse associations were found with lutein intake; however, less consistent inverse associations were found for β-carotene and lycopene. Block (16) reported that lutein, α- and β-carotene, and lycopene were inversely related to F2α-isoprostanes. However, in multiple variable analyses adjusting for sex, age, race, smoking status, and BMI, only β-carotene remained significant in their analysis.

Although lutein, β-carotene, and lycopene are found in many of the same foods, there is more commonality in good dietary sources of lutein and β-carotene. Structurally, all 3 molecules contain long polyene chains; however, β-carotene and lutein have ring structures at either end of the chains, whereas the lycopene chain contains uncyclized ends. The double bonds on the polyene chains of all 3 molecules can contribute to oxidation/reduction reactions, thus limiting peroxidation of membrane phospholipids. It is noteworthy that the hydroxyl functional group on lutein’s β-ring provides an additional target for oxidation/reduction that the others do not possess.

We observed inverse associations between both daidzein and genistein intakes and urine concentration of F2α-isoprostanes in Chinese and Japanese women. The association between soy intake and coronary heart disease has been reported (52,53); however, intervention studies with intermediate, oxidative stress marker endpoints have provided mixed results (54–56). Due to the relative absence of smoking in Chinese and Japanese women, it was not possible to ascertain whether high genistein and daidzein intake would minimize the impact of smoking on the isoprostanes. The inclusion of other broad classes of polyphenols and their evaluation in all women indicated that polyphenols, apart from genistein and daidzein, could be important contributors to the amelioration of oxidative stress.

Several associations between dietary intake and urine F2α-isoprostanes differed in smokers and nonsmokers, supporting the idea that smoking modifies these associations. Because smokers have higher concentration of isoprostanes and consume lower amounts of antioxidants and more trans and saturated fats than nonsmokers, they may be more responsive to the beneficial effect of antioxidants (57). Among the strongest inverse concurrent associations observed were in smokers for vitamins C and E. Research indicates that vitamin C has the capacity for regeneration by other antioxidants and subsequent reenlistment for oxidation/reduction actions (58,59). Although vitamin E has been reported to disappear in response to oxidative stressors such as cigarette smoke (59), evidence is mixed and a protective effect for cardiovascular disease has not been established (18,60).

Concurrent relationships between lower zinc and higher urinary F2α-isoprostane concentration were noteworthy in both smokers and nonsmokers and increased zinc intake over time was also related to lower urinary F2α-isoprostane concentration in both groups. The mechanisms of action for zinc’s antioxidant effects are an area of active research. Zinc’s antioxidant effects are thought to occur through displacement of pro-oxidant metals, such as iron and copper, upon binding with cell membranes, thus decreasing free radical production at the ligand-binding site (61). The zinc-containing enzyme Cu-Zn superoxide dismutase is thought to be a critical enzyme for oxygen free radical defense (62).

Environmental tobacco smoke exposure has been proposed as a risk factor for cardiovascular disease (63). Some evidence shows that it is associated with oxidative stress markers similar to that of active smokers (26). In our investigation, although we observed a graded increase in least squared means of urine F2α-isoprostanes according to increased levels of total smoke exposure, significant differences based on environmental tobacco smoke exposure disappeared in analyses adjusted for other relevant variables.

In models adjusted for covariates, physical activity was not associated with urine F2α-isoprostanes. Our inability to detect an association could have been due to the small observed range of physical activity scores in our sample and lack of a sizable subgroup of women with regular intense physical activity. The absence of a significant association has also been reported in older persons (32) in whom intense physical activity is less common.

Our investigation had strengths and limitations. Our diverse sample provided a wide range in lifestyle exposures, including intake of soy products and other food sources of polyphenols. A major advantage of this investigation was the availability of exposure data at 2 points in time. The use of F2α-isoprostanes as a marker of oxidative stress was a strength due to their specificity for lipid peroxidation and chemical stability (64,65). Little diurnal variation was observed and, therefore, measurement of urinary F2α-isoprostanes in a single early morning sample has been described as adequate to represent the daily isoprostane excretion in humans (66). Nonetheless, nutrient values obtained from FFQ inevitably involve some misclassification error due to the difficulty in recalling and quantifying items consumed during the past year and recall of physical activity is susceptible to misclassification error for similar reasons. Whereas the level of Type I error was set at 0.05 for each nutrient or food component, the numerous statistical tests may increase the likelihood of delineating false positive findings; thus, it is important to evaluate the findings also on the basis of the existing literature and biological plausibility. Further, analyses of Chinese and Japanese women who smoked were not possible due to the small numbers of such smokers.

This investigation consistently showed that dietary trans fatty acid intakes were associated with oxidative stress, as measured by urine F2α-isoprostanes, while we observed a protective association for lutein and zinc intakes. Our results suggest that oxidative stress levels may be modifiable by changes in intake of these nutrients over time. Furthermore, the magnitude of concurrent and prospective associations in women who smoke suggest that those with elevated oxidative stress or cancer and cardiac disease risk may particularly benefit from decreased trans fatty intake and increased intakes of lutein and other antioxidants (47,57). Finally, this study provided no evidence to consider physical activity as a primary mediator of oxidative stress.

**Literature Cited**


Dietary intake and oxidative stress in women 2417


