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Oxidative stress and breast cancer risk in premenopausal women

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Abstract

Background—Detrimental effects of oxidative stress are widely recognized, but induction of apoptosis and senescence may also have benefits for cancer prevention. Recent studies suggest oxidative stress may be associated with lower breast cancer risk before menopause.

Methods—We conducted a nested case-control study (N=457 cases, 910 controls) within the NIEHS Sister Study cohort of 50,884 women. Premenopausal women ages 35–54 were eligible for selection. We matched controls 2:1 to cases on age and enrollment year and were breast cancer-free at the time of the corresponding case's diagnosis. Oxidative stress was measured by urinary F_2-isoprostane and metabolite (15-F_{2t}-Isoprostane-M) concentrations. Odds ratios (OR) and 95% confidence intervals (CI) were calculated with multivariable conditional logistic regression.

Results—After multivariable adjustment for body mass index (BMI) and other potential confounders, the OR for breast cancer comparing the >90th (≥2.94 ng/mgCr) to <25th percentile (1.01 ng/mgCr) was 1.1 (CI: 0.65–1.7) for F_2-isoprostane and 0.70 (CI: 0.43–1.1) for the metabolite. Higher metabolite concentrations were associated with lower breast cancer risk among women who were also premenopausal (353 cases, OR=0.59, CI: 0.34–1.0) or <46 years (82 cases, OR=0.15, CI: 0.06–0.42) at diagnosis. ORs for the metabolite and breast cancer were inverse among women with BMI 18.5–24.9 kg/m^2 (OR=0.47, CI, 0.18–1.2, 208 cases) and >30 kg/m^2 (OR=0.71, CI, 0.30–1.7, 107 cases), but not among women with BMI 25–29.9 kg/m^2 (OR=0.98, CI, 0.39, 2.5, 138 cases).

Conclusion—Together with other studies, our results support a possible inverse association between oxidative stress and premenopausal breast cancer risk.

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Date availability: Investigators may apply to access the study data through the National Institute of Environmental Health Sciences Sister Study Tracking and Review System (STaRS) website at: https://www.sisterstudystars.org

Keywords

Breast cancer; premenopausal; oxidative stress; nested case-control

Introduction

Oxidative stress describes an overabundance of reactive oxygen species, which interact with biomolecules including DNA, lipids, and protein. Oxidative stress has been associated with cardiovascular disease development [1,2] and its risk factors (e.g. age, smoking, and obesity). [3–6] Oxidative stress-induced DNA damage may also contribute to carcinogenesis with a positive association reported between oxidative stress levels and breast cancer among postmenopausal women. [7–9] Conversely, some effects of oxidative stress, including induction of apoptosis and senescence, may be beneficial for cancer prevention before menopause. [10,11] In two prospective studies of premenopausal women, higher oxidative stress was associated with an estimated 24%–42% lower breast cancer risk. [12,13]

The F_2-isoprostanes are secondary products of lipid peroxidation of arachidonic acid and were identified by the National Institutes of Health (NIH)-sponsored, multi-investigator Biomarkers of Oxidative Stress Study as an accurate measure of *in vivo* oxidative stress.[14,15] Analysis of F_2-isoprostanes by gas chromatography/ negative ion chemical ionization mass spectrometry provides stable, sensitive, and reliable measurements of oxidative stress. [16,17] Measurement in urine eliminates the potential for *ex vivo* oxidation that can occur in plasma and provides a time-integrated index of systemic oxidant stress.[17,18]

Although reports of an inverse association between oxidative stress and premenopausal breast cancer are counter to the expectation of oxidative stress and free radical-induced tissue damage, oxidative stress is necessary for p53 activation [19] and may increase TGF-β1 synthesis, [20,21] thereby increasing tumor suppressor activity and apoptotic signaling.[22] Accumulating evidence supports distinct biologic pathways for pre- versus postmenopausal breast cancer. Several risk factors, including childbirth, [23] obesity, [24] and cigarette smoking [25] are reported to have differential associations with breast carcinogenesis before and after menopause.

Identifying unique contributors to breast cancer risk in younger women is critical to prevention efforts. In recent decades, incidence rates of advanced breast cancer have increased among premenopausal women, whereas they have consistently decreased among women 60 and older during the same period.[26] To examine the relation between oxidative stress and breast cancer risk among premenopausal women, we prospectively measured urinary F_2-isoprostane and its primary metabolite in a case-control study nested within the National Institute of Environmental Health Sciences (NIEHS) Sister Study cohort of 50,884 women.

Methods

The Sister Study Prospective Cohort

The NIEHS Sister Study is a prospective observational study designed to identify environmental and genetic risk factors for breast cancer. From 2003 to 2009, 50,884 women from the U.S. and Puerto Rico were recruited through a national advertising campaign and a network of breast cancer professionals and recruitment volunteers. Women were ages 35 to 74 years, free of breast cancer at enrollment, and had a sister who had been diagnosed with breast cancer. Approval for the study was obtained from the Institutional Review Board of the NIEHS, the NIH, and the Copernicus Group. All participants provided informed written consent.

Information on demographics, medical and family history, and lifestyle factors was ascertained through telephone interview and written questionnaires at enrollment. Dietary intake and supplement use were ascertained via the Block food frequency questionnaire.[27] At enrollment, women provided first morning urine samples collected into a sterilized cup containing 125 mg of ascorbic acid and kept cold (0 to 4°C). Urine samples were then stored at −80°C at the study biorepository. During the home visit, current height, weight, and hip and waist circumferences were measured by trained study personnel.

Nested Case-Control Study

Eligibility criteria for the nested case-control study required women to be ages 35 to 54 years, premenopausal (defined as having at least one menstrual cycle in the previous 12 months), and to have at least one intact ovary and a blood and urine sample collected at baseline. Women ages 54 and younger were considered premenopausal if their only reason for not experiencing menses was hysterectomy (without bilateral oophorectomy).

Between enrollment and July 1, 2012, 461 self-reported incident breast cancer cases were identified. Two controls were matched to each case on age (within 5 months) and year of study enrollment and were breast cancer-free at the time of their matched case's diagnosis. At analysis, we further excluded cases whose diagnosis was later determined to have occurred pre-baseline (N=2) or after July 1, 2012 (N=1) or was not confirmed by medical records (N=1), and their matched controls. Further, four additional controls were excluded due to prophylactic bilateral mastectomy. Ultimately, 457 breast cancer cases and 910 controls contributed to these analyses.

Oxidative stress measurement

Urinary F_2-isoprostane and metabolite were measured using gas chromatography/negative ion chemical ionization mass spectrometry (GC/NICI MS) at the Eicosanoid laboratory at Vanderbilt University Medical Center. Protocols for these methods have been published in detail.[17,28–30] The GC/NICI-MS is carried out on an Agilent 5973 Inert Mass Selective Detector that is coupled with an Agilent 6890n Network GC system (Agilent Labs, Torrance, CA) that is interfaced with an Agilent computer. The lower limit of detection of F_2-isoprostane is in the range of 4 pg/mL using an internal standard with a blank of 3 parts per thousand. The precision of this assay in biologic fluids is $\pm$6% and the accuracy 94%.[28]

The lower limit of sensitivity for the metabolite is approximately 8 pg/mL with precision of ±7% and accuracy of 95%.[29]

Values of F_2-isoprostane and metabolite were adjusted for creatinine concentrations and are expressed as ng/mg of creatinine. All samples yielded numeric results—none were below the level of detection. In total, 77 batches were run, each containing 18 study participant samples and two quality control (QC) samples for a total of 20 samples. Six trios, each consisting of one case and two controls, were analyzed together within batches and distributed randomly across each batch. All sample labels blinded laboratory investigators to case-control or QC status. The coefficient of variation for QC duplicates included across batches was 16.0% for F_2-isoprostane and 12.5% for the metabolite.

Statistical analysis

We created categories of F_2-isoprostane and its metabolite based on the 25th, 50th, 75th, and 90th percentiles among controls. Body mass index (kg/m^2) was categorized according to WHO guidelines as <18.5 kg/m^2, 18.5–24.9 kg/m^2, 25–29.9 kg/m^2, and >30–34.9 kg/m^2.[31] Waist circumference categories were defined according to American Diabetes Association cutpoints for abdominal obesity as normal (≤80 cm), action level 1 (80.1–88cm), or action level 2 (>88cm).[32] Age-adjusted geometric means of natural log-transformed F_2-isoprostane and metabolite were calculated using generalized linear regression models according to enrollment characteristics among control participants.

To model the association between F_2-isoprostanes and metabolite concentrations and breast cancer, we used conditional logistic regression to calculate odds ratios and 95% confidence intervals accounting for the matching on age and enrollment year. We selected participant characteristics that were associated with both F_2-isoprostane and metabolite levels in age-adjusted models that could reflect health-conscious behaviors as potential confounders of breast cancer risk associations. Final multivariable models adjusted for BMI, waist circumference, smoking status, physical activity, fruit/vegetable consumption, total household income, alcohol consumption, and use of vitamin C or E supplements. For 33 observations with missing values for one or more covariates, we imputed data by multiple imputation. Linear tests for trend modeled the median values for the 1st, 2nd, and 3rd quartiles, the 75th–89th percentile, and ≥ 90th percentiles continuously. Sensitivity analyses were performed to assess the impact of additional adjustment for covariates that were associated with one oxidative stress marker or the other, but not both, including education, hysterectomy, dietary isoflavones, and non-steroidal anti-inflammatory drug (NSAID) use.

We performed stratified analyses by extent of disease, estrogen receptor (ER) status, menopausal status at diagnosis, and age at diagnosis to investigate potential effect modification by these factors. To test for statistical interaction, we also included cross product interaction terms in regression models. In all stratified analyses, each matched set was assigned to the value of the case in that set. Thus tests for interaction assessed whether the association between F_2-isoprostane or metabolite concentrations and breast cancer differed between matched sets in which the case was, for example, premenopausal versus postmenopausal.

All statistical analyses were performed with Sister Study Data Release 5.0.1 using SAS 9.4 (SAS Institute, Cary, NC).

Results

The average age at enrollment among cases and controls was 47.3 years (SD=4.4, range: 35–54) with a mean of 2.8 years (SD=1.9, range= <1–8.4) between urine collection and breast cancer diagnosis. Geometric means of F_2-isoprostane and metabolite, measures of oxidative stress, among controls are shown in Table 1. The geometric mean F_2-isoprostane and metabolite concentrations among controls were 1.44 ng/mg creatinine (SD=0.76) and 0.71 ng/mg creatinine (SD=0.32) respectively. Higher oxidative stress levels were associated with lower income, current smoking, higher BMI and waist circumference, fewer MET (metabolic equivalent)-hours of weekly physical activity, low fruit and vegetable consumption, and not taking Vitamin C or E supplements. Counter to expectation, alcohol consumption was inversely related to oxidative stress. No association was observed between F_2-isoprostane or metabolite levels and race/ethnicity, age at menarche, oral contraceptive use, or parity. Lower education, prior hysterectomy, and use of NSAIDs were associated with higher oxidative stress for the metabolite, but not F_2-isoprostane measurements. Dietary isoflavones also showed an inconsistent relation across F_2-isoprostane and metabolite measurements (Table 1).

In the combined sample of cases and controls, the correlation between F_2-isoprostane and metabolite was 0.51 (). The geometric mean urinary excretion levels for F_2-isoprostane and metabolite among cases were 1.43 ng/mg creatinine (median=1.42) and 0.67 (median=0.66) ng/mg creatinine, respectively. Corresponding values among controls were 1.44 ng/mg creatinine (median=1.39) and 0.71 ng/mg creatinine (median=0.69).

Overall, we observed no association between F_2-isoprostane and odds of breast cancer. The OR for breast cancer comparing the >90th (≥2.94 ng/mg creatinine) to <25th percentile (1.01 ng/mg creatinine) of F_2-isoprostane was 1.1 (CI: 0.65–1.7). This was similar within subgroups defined by ER expression, extent of disease, and menopausal status. Multivariable adjustment did not substantially change estimates overall or within subgroups (Tables 2–3).

Compared to the lowest quartile, women with metabolite values at or above the 90th percentile had an OR for total breast cancer of 0.70 (95% CI: 0.43–1.1) (P_{trend}=0.2). In analyses stratified by ER status, associations among ER positive tumors (84%) were similar to the overall results. There were too few ER negative tumors (N=66) to produce stable estimates in multivariable models (Table 2). The OR for invasive breast cancer was 0.54 (95% CI: 0.30–0.99) among women with metabolite levels at or above 90th percentile compared to the lowest quartile (P_{trend}=0.05). Odds of DCIS did not appear to vary according to metabolite concentrations (P_{trend}=0.6); however, formal interaction tests did not indicate a different association between invasive disease and DCIS ($P_{interaction}$=0.3) (Table 2).

All women were classified as premenopausal at enrollment; however, we also conducted analyses stratified according to menopausal status at diagnosis. Among the 457 cases, 353

(77%) remained premenopausal at diagnosis. We observed a clear negative trend (P_{trend}=0.05) of decreasing breast cancer odds with increasing metabolite concentrations. Compared to the lowest quartile, women with metabolite concentrations ≥ 90th percentile had an OR of 0.59 for developing breast cancer (95% CI: 0.34–1.0) (Table 3). This pattern was not observed among women who were postmenopausal at diagnosis ($P_{interaction}$=0.01).

When restricted to trios where the case participant was diagnosed with breast cancer at ages 35–45, inverse associations with breast cancer odds were apparent for both F_2-isoprostane and its metabolite. Due to small numbers, the 75th–89th and ≥90th percentile categories were combined. Comparing 4th to 1st quartile levels, the OR for breast cancer was 0.31 (95% CI: 0.10–0.92) for F_2-isoprostane and 0.15 (95% CI: 0.06–0.42) for the metabolite in the 35–45 age group. F_2-isoprostane and its metabolite did not appear to be inversely associated with breast cancer risk among women ages 46–50 or 51–60 at diagnosis (Table 3). Additional adjustment for education, hysterectomy, dietary isoflavones, or NSAID use in sensitivity analyses did not influence these findings.

We further analyzed results according to the duration between urine collection and diagnosis; calendar year of collection (as a proxy for storage time); body mass index, and familial predisposition to breast cancer. We observed no meaningful variation between estimates for urine samples collected within 3 years of diagnosis compared to longer periods (eTable 1). In analyses stratified by calendar year (2003–2005 vs. 2006–2009), the magnitude of the point estimates appeared more strongly inverse for 2006–2009, which would correspond to a shorter sample storage time (eTable 2). However, confidence intervals for corresponding calendar year estimates were overlapping. Odds ratios for 15-F_{2t}-IsoP-M ≥ 90th compared to <25th percentiles were highly similar among women with BMIs within 18.5–24.9 kg/m^2 (OR=0.47; 95% CI: 0.18–1.2, N=208 cases) and ≥ 30.0 kg/m^2 (OR=0.71; 95% CI: 0.30–1.7, N=107 cases). In women with BMI of 25.0–29.9 kg/m^2, we did not observe an inverse association above the 90th percentile (compared to <25th) for 15-F_{2t}-IsoP-M (OR=0.98; 0.39–2.5); however, the OR for the 75th–89th percentile was 0.66 (95% CI: 0.29–1.5). Finally, after exclusion of women with two or more first-degree relatives with a breast cancer diagnosis (including 150 cases), a known mutation in the *BRCA1* or *BRCA2* genes (29 cases), or a history of ulcerative colitis or Crohn's disease (5 cases), our interpretations remained unchanged (eTable 3).

Discussion

In our analysis, menopausal status at diagnosis modified the association between oxidative stress (as measured by F_2-isoprostane and its metabolite) and breast cancer risk. We did not observe a strong or consistent pattern between oxidative stress and breast cancer risk among women who transitioned through menopause prior to diagnosis. However, our findings supported an inverse association between oxidative stress and breast cancer risk before menopause. These results warrant replication and should be interpreted with caution as they were based on relatively small numbers. Our results contribute to a growing body of prospective studies[12,13] with similar findings. In addition, the lower odds of breast cancer associated with higher metabolite concentrations persisted after careful adjustment for

numerous factors that influence oxidative stress levels, including smoking, dietary factors, socioeconomic characteristics, and physical activity.

Most previous studies that evaluated oxidative stress using plasma or urinary F_2-isoprostane levels were traditional case-control studies where biologic samples were obtained after diagnosis.[9,33–36] Despite conscientious efforts to analyze pretreatment samples separately to assess potential changes due chemotherapy or radiation,[9,37] these studies cannot exclude the possibility that differences in F_2-isoprostane levels were a consequence of cancer development rather than a precursor.

One prior study prospectively evaluated urinary levels of F_2-isoprostane and metabolite in relation to breast cancer risk.[12] In a case-control analysis (N=436 cases, 852 controls) nested within the prospective Shanghai Women's Health Study, 3rd vs. 1st tertile F_2-isoprostane and metabolite values were associated with a lower risk of breast cancer among premenopausal women (OR=0.58, 95% CI: 0.35–0.98 and OR=0.68, 95% CI: 0.41–1.14, respectively), and a higher risk of breast cancer among postmenopausal women (OR=1.33, 95% CI: 0.83–2.13 and OR=1.47, 95% CI: 0.86–2.53, respectively).

In models that combined pre- and postmenopausal women, the authors also reported differential associations according to BMI. Among women with a BMI ≥ 29 kg/m^2 (N=40 cases, 77 controls), 3rd tertile vs. 1st tertile metabolite values were associated with 10-fold higher breast cancer odds (OR=10.27; 2.41–43.80). This positive association contrasted to that observed among women with a BMI <23 kg/m^2 (N=158 cases, 293 controls) where higher levels were associated with lower breast cancer odds.

It is not clear to what extent BMI and menopausal status overlapped in the Shanghai cohort; however, the authors state that the positive association among higher BMI women was present irrespective of menopausal status. In the Shanghai study, the average BMI was 24 kg/m^2, active smoking was rare among women (<3%), and passive smoking was common (~80%). These characteristics vary substantially from U.S. populations and influence both baseline oxidative stress levels and breast cancer risk, making direct comparison across populations difficult. While we did not observe variation in metabolite associations according to BMI, associations among premenopausal women and mean metabolite concentrations among controls (0.71 ng/mg creatinine in our study and 0.71 in Shanghai [12]) were highly similar. In our study, premenopausal status at urine collection was an eligibility requirement. Therefore, we cannot address potential variation by postmenopausal status at enrollment—however, we did see suggested evidence of a positive association among women who were postmenopausal by the time of diagnosis. Of note, the F_2-isoprostane and metabolite measurements in the Shanghai study were performed with the same methods and laboratory used in our report.

Other oxidative stress markers, including fluorescent oxidation products (FlOPs), 8-hydroxy-2deoxyguanosine (8-oxoG), and malondialdehyde (MDA) have more often been evaluated in prospective nested case-control studies. In the Nurses' Health Studies I and II, a positive association was seen for postmenopausal breast cancer risk with FlOP_320, but not FlOP_360 or FlOP_400.[7] Conversely, in women who were premenopausal at blood draw,

plasma FlOP_320 and FlOP_360 appeared inversely associated with breast cancer risk. Comparing highest to lowest quartiles, the RR for breast cancer was 0.76 (0.55–1.06) for FlOP320 and 0.68 (0.50–0.95) for FlOP_360—results were not further stratified by menopausal status at diagnosis..[13] In analyses of ER- negative breast cancer (that did not stratify by menopausal status), FlOP_360 (RR_{Q4vsQ1}=0.40; 95% CI: 0.20–0.81) and FlOP_400 (RR_{Q4vsQ1}=0.42; 95% CI: 0.22–0.82) were inversely related to ER- breast cancer risk among women with BMI <25 kg/m^2, but not in higher BMI groups (FlOP_360 RR_{Q4vsQ1}=1.10; 95% CI: 0.54–2.24 and FlOP_400 RR_{Q4vsQ1}=0.96; 95% CI: 0.46–1.99).[38]

In the Danish Diet, Cancer, and Health Study, 8-oxodG levels were positively associated with ER-positive breast cancer risk; however, all women were ages 50–64 and postmenopausal at urine collection.[8] This association was not replicated for 8-oxodG or MDA in the Shanghai Women's Health Study, although the primary analysis combined both pre- and postmenopausal women. Subgroup analyses according to menopausal status were described as not statistically significant and the direction of estimates was not shown.[39]

A basal level of reactive oxygen species generation and oxidative stress is necessary for normal physiologic functioning. Reactive oxygen species are involved in cell signaling, cell generation and degeneration, cellular homeostasis, microorganism defense, and human pregnancy. The term "oxidative strain" has been proposed to describe changes in F_2-isoprostane levels that potentially promote physiologic functions that are beneficial to health, to contrast with the destructive connotation of "oxidative stress".[40] In premenopausal women, such effects may include enhanced tumor suppressor activity and apoptosis through p53 activation [19] and TGF-β1 synthesis,[20,21] with benefits for cancer surveillance and prevention. After menopause, it is possible that the net effect of oxidative stress on cancer risk reflects greater cumulative exposure to oxidative stress-induced genetic damage and longer-latency carcinogenic processes.

Key strengths of our analysis include the prospective collection of biologic samples and detailed questionnaire information to address potential confounding by reproductive, anthropometric, lifestyle, and socioeconomic characteristics and the use of novel and highly accurate markers of oxidative stress. However, some limitations must be considered. Our analysis represents the largest sample to date of premenopausal women; however, sample sizes were insufficient for analyses of ER negative tumor subtypes. Our oxidative stress assessment was based on a single urine collection with an average 2.8 year follow-up to diagnosis. The similar estimates for samples collected within 2–3 years of diagnosis, compared to further from diagnosis, provides reassurance that our findings are not due to changes induced by preclinical disease.

Modulation of oxidative stress levels has been an active area of debate in the context of cancer treatment—where antioxidant use could be potentially counterproductive during chemotherapies that work, in part, by inducing oxidative tissue damage.[41] Our findings do not support antioxidant supplement use for cancer prevention, especially among younger women.[42] In our study, supplement use was associated with lower urinary oxidative stress levels among premenopausal women—however, lower levels did not translate to reduced breast cancer risk.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Davies SS, Roberts LJ 2nd. F2-isoprostanes as an indicator and risk factor for coronary heart disease. Free Radic Biol Med. 2011; 50(5):559–66. [PubMed: 21126576]
2. Gross M, Steffes M, Jacobs DR Jr, Yu X, Lewis L, Lewis CE, Loria CM. Plasma F2-isoprostanes and coronary artery calcification: the CARDIA Study. Clin Chem. 2005; 51(1):125–31. [PubMed: 15514100]
3. Morrow JD, Frei B, Longmire AW, Gaziano JM, Lynch SM, Shyr Y, Strauss WE, Oates JA, Roberts LJ 2nd. Increase in circulating products of lipid peroxidation (F2-isoprostanes) in smokers. Smoking as a cause of oxidative damage. N Engl J Med. 1995; 332(18):1198–203. [PubMed: 7700313]
4. Taylor AW, Bruno RS, Traber MG. Women and smokers have elevated urinary F(2)-isoprostane metabolites: a novel extraction and LC-MS methodology. Lipids. 2008; 43(10):925–36. [PubMed: 18751751]
5. Dorjgochoo T, Gao YT, Chow WH, Shu XO, Yang G, Cai Q, Rothman N, Cai H, Li H, Deng X, Shrubsole MJ, Murff H, Milne G, Zheng W, Dai Q. Obesity, age, and oxidative stress in middle-aged and older women. Antioxid Redox Signal. 2011; 14(12):2453–60. [PubMed: 21043829]
6. Il'yasova D, Wang F, Spasojevic I, Base K, D'Agostino RB Jr, Wagenknecht LE. Urinary F2-isoprostanes, obesity, and weight gain in the IRAS cohort. Obesity (Silver Spring). 2012; 20(9): 1915–21. [PubMed: 21959342]
7. Fortner RT, Tworoger SS, Wu T, Eliassen AH. Plasma florescent oxidation products and breast cancer risk: repeated measures in the Nurses' Health Study. Breast Cancer Res Treat. 2013; 141(2): 307–16. [PubMed: 24046001]
8. Loft S, Olsen A, Moller P, Poulsen HE, Tjonneland A. Association between 8-oxo-7,8-dihydro-2′-deoxyguanosine excretion and risk of postmenopausal breast cancer: nested case-control study. Cancer Epidemiol Biomarkers Prev. 2013; 22(7):1289–96. [PubMed: 23658396]
9. Rossner P Jr, Gammon MD, Terry MB, Agrawal M, Zhang FF, Teitelbaum SL, Eng SM, Gaudet MM, Neugut AI, Santella RM. Relationship between urinary 15-F2t-isoprostane and 8-oxodeoxyguanosine levels and breast cancer risk. Cancer Epidemiol Biomarkers Prev. 2006; 15(4): 639–44. [PubMed: 16614103]
10. Finkel T. Oxygen radicals and signaling. Curr Opin Cell Biol. 1998; 10(2):248–53. [PubMed: 9561849]
11. Nemoto S, Finkel T. Ageing and the mystery at Arles. Nature. 2004; 429(6988):149–52. [PubMed: 15141200]
12. Dai Q, Gao YT, Shu XO, Yang G, Milne G, Cai Q, Wen W, Rothman N, Cai H, Li H, Xiang Y, Chow WH, Zheng W. Oxidative stress, obesity, and breast cancer risk: results from the Shanghai Women's Health Study. J Clin Oncol. 2009; 27(15):2482–8. [PubMed: 19380446]
13. Sisti JS, Lindstrom S, Kraft P, Tamimi RM, Rosner BA, Wu T, Willett WC, Eliassen AH. Premenopausal plasma carotenoids, fluorescent oxidation products, and subsequent breast cancer risk in the nurses' health studies. Breast Cancer Res Treat. 2015; 151(2):415–25. [PubMed: 25917867]

14. Kadiiska MB, Gladen BC, Baird DD, Germolec D, Graham LB, Parker CE, Nyska A, Wachsman JT, Ames BN, Basu S, Brot N, Fitzgerald GA, Floyd RA, George M, Heinecke JW, Hatch GE, Hensley K, Lawson JA, Marnett LJ, Morrow JD, Murray DM, Plastaras J, Roberts LJ 2nd, Rokach J, Shigenaga MK, Sohal RS, Sun J, Tice RR, Van Thiel DH, Wellner D, Walter PB, Tomer KB, Mason RP, Barrett JC. Biomarkers of oxidative stress study II: are oxidation products of lipids, proteins, and DNA markers of CCl4 poisoning? Free Radic Biol Med. 2005; 38(6):698–710. [PubMed: 15721980]
15. Milne GL, Dai Q, Roberts LJ 2nd. The isoprostanes--25 years later. Biochim Biophys Acta. 2015; 1851(4):433–45. [PubMed: 25449649]
16. Milne GL, Musiek ES, Morrow JD. F2-isoprostanes as markers of oxidative stress in vivo: an overview. Biomarkers. 2005; 10(Suppl 1):S10–23. [PubMed: 16298907]
17. Milne GL, Sanchez SC, Musiek ES, Morrow JD. Quantification of F2-isoprostanes as a biomarker of oxidative stress. Nat Protoc. 2007; 2(1):221–6. [PubMed: 17401357]
18. Dorjgochoo T, Gao YT, Chow WH, Shu XO, Yang G, Cai Q, Rothman N, Cai H, Li H, Deng X, Franke A, Roberts LJ, Milne G, Zheng W, Dai Q. Major metabolite of F2-isoprostane in urine may be a more sensitive biomarker of oxidative stress than isoprostane itself. Am J Clin Nutr. 2012; 96(2):405–14. [PubMed: 22760572]
19. Liu B, Chen Y, St Clair DK. ROS and p53: a versatile partnership. Free Radic Biol Med. 2008; 44(8):1529–35. [PubMed: 18275858]
20. Montero A, Munger KA, Khan RZ, Valdivielso JM, Morrow JD, Guasch A, Ziyadeh FN, Badr KF. F(2)-isoprostanes mediate high glucose-induced TGF-beta synthesis and glomerular proteinuria in experimental type I diabetes. Kidney Int. 2000; 58(5):1963–72. [PubMed: 11044216]
21. McGowan TA, Dunn SR, Falkner B, Sharma K. Stimulation of urinary TGF-beta and isoprostanes in response to hyperglycemia in humans. Clin J Am Soc Nephrol. 2006; 1(2):263–8. [PubMed: 17699215]
22. Chang CF, Westbrook R, Ma J, Cao D. Transforming growth factor-beta signaling in breast cancer. Front Biosci. 2007; 12:4393–401. [PubMed: 17485383]
23. Schedin P. Pregnancy-associated breast cancer and metastasis. Nature reviews Cancer. 2006; 6(4): 281–91. [PubMed: 16557280]
24. White AJ, Nichols HB, Bradshaw PT, Sandler DP. Overall and central adiposity and breast cancer risk in the sister study. Cancer. 2015; 121(20):3700–8. [PubMed: 26193782]
25. Johnson KC, Miller AB, Collishaw NE, Palmer JR, Hammond SK, Salmon AG, Cantor KP, Miller MD, Boyd NF, Millar J, Turcotte F. Active smoking and secondhand smoke increase breast cancer risk: the report of the Canadian Expert Panel on Tobacco Smoke and Breast Cancer Risk (2009). Tobacco control. 2011; 20(1):e2.
26. Johnson RH, Chien FL, Bleyer A. Incidence of breast cancer with distant involvement among women in the United States, 1976 to 2009. JAMA. 2013; 309(8):800–5. [PubMed: 23443443]
27. Block G, Hartman AM, Dresser CM, Carroll MD, Gannon J, Gardner L. A data-based approach to diet questionnaire design and testing. Am J Epidemiol. 1986; 124(3):453–69. [PubMed: 3740045]
28. Morrow JD, Roberts LJ 2nd. Mass spectrometric quantification of F2-isoprostanes in biological fluids and tissues as measure of oxidant stress. Methods Enzymol. 1999; 300:3–12. [PubMed: 9919502]
29. Morales CR, Terry ES, Zackert WE, Montine TJ, Morrow JD. Improved assay for the quantification of the major urinary metabolite of the isoprostane 15-F(2t)-Isoprostane (8-iso-PGF(2alpha)) by a stable isotope dilution mass spectrometric assay. Clin Chim Acta. 2001; 314(1–2):93–9. [PubMed: 11718683]
30. Milne GL, Gao B, Terry ES, Zackert WE, Sanchez SC. Measurement of F2- isoprostanes and isofurans using gas chromatography-mass spectrometry. Free Radic Biol Med. 2013; 59:36–44. [PubMed: 23044261]
31. WHO Technical Report Series. Geneva, Switzerland: 2000. Obesity: preventiong and managing the global epidemic.
32. Ardern CI, Janssen I, Ross R, Katzmarzyk PT. Development of health-related waist circumference thresholds within BMI categories. Obes Res. 2004; 12(7):1094–103. [PubMed: 15292473]

33. Kedzierska M, Olas B, Wachowicz B, Jeziorski A, Piekarski J. The lipid peroxidation in breast cancer patients. Gen Physiol Biophys. 2010; 29(2):208–10. [PubMed: 20577033]

34. Mannello F, Tonti GA, Pagliarani S, Benedetti S, Canestrari F, Zhu W, Qin W, Sauter ER. The 8-epimer of prostaglandin F(2alpha), a marker of lipid peroxidation and oxidative stress, is decreased in the nipple aspirate fluid of women with breast cancer. Int J Cancer. 2007; 120(9):1971–6. [PubMed: 17266038]

35. Shen J, Gammon MD, Terry MB, Wang Q, Bradshaw P, Teitelbaum SL, Neugut AI, Santella RM. Telomere length, oxidative damage, antioxidants and breast cancer risk. Int J Cancer. 2009; 124(7): 1637–43. [PubMed: 19089916]

36. Yeon JY, Suh YJ, Kim SW, Baik HW, Sung CJ, Kim HS, Sung MK. Evaluation of dietary factors in relation to the biomarkers of oxidative stress and inflammation in breast cancer risk. Nutrition. 2011; 27(9):912–8. [PubMed: 21353477]

37. Il'yasova D, Spasojevic I, Wang F, Tolun AA, Base K, Young SP, Marcom PK, Marks J, Mixon G, DiGiulio R, Millington DS. Urinary biomarkers of oxidative status in a clinical model of oxidative assault. Cancer Epidemiol Biomarkers Prev. 2010; 19(6):1506–10. [PubMed: 20501773]

38. Hirko KA, Fortner RT, Hankinson SE, Wu T, Eliassen AH. Plasma fluorescent oxidation products and risk of estrogen receptor-negative breast cancer in the Nurses' Health Study and Nurses' Health Study II. Breast Cancer Res Treat. 2016; 158(1):149–55. [PubMed: 27294610]

39. Lee KH, Shu XO, Gao YT, Ji BT, Yang G, Blair A, Rothman N, Zheng W, Chow WH, Kang D. Breast cancer and urinary biomarkers of polycyclic aromatic hydrocarbon and oxidative stress in the Shanghai Women's Health Study. Cancer Epidemiol Biomarkers Prev. 2010; 19(3):877–83. [PubMed: 20160264]

40. Basu S. F2-isoprostanes in human health and diseases: from molecular mechanisms to clinical implications. Antioxid Redox Signal. 2008; 10(8):1405–34. [PubMed: 18522490]

41. Lawenda BD, Kelly KM, Ladas EJ, Sagar SM, Vickers A, Blumberg JB. Should supplemental antioxidant administration be avoided during chemotherapy and radiation therapy? J Natl Cancer Inst. 2008; 100(11):773–83. [PubMed: 18505970]

42. Martinez ME, Jacobs ET, Baron JA, Marshall JR, Byers T. Dietary supplements and cancer prevention: balancing potential benefits against proven harms. J Natl Cancer Inst. 2012; 104(10): 732–9. [PubMed: 22534785]

Table 1

Geometric means of urinary isoprostane levels by characteristics among 910 controls in the Sister Study

	N	F_2-Isoprostane, ng/mgCr (mean ± SD)	15-F_{2t}-Isoprostane metabolite, ng/mgCr (mean ± SD)
Race/ethnicity			
Non-Hispanic white	792	1.5 ± 0.77	0.72 ± 0.32
Non-Hispanic black	60	1.3 ± 0.70	0.66 ± 0.30
Hispanic	35	1.5 ± 0.74	0.76 ± 0.31
Other	23	1.3 ± 0.50	0.66 ± 0.30
Education			
Less than Bachelor's Degree	377	1.5 ± 0.81	0.78 ± 0.35
Bachelor's Degree	291	1.4 ± 0.67	0.68 ± 0.28
Higher than Bachelor's Degree	242	1.4 ± 0.77	0.65 ± 0.30
Total household income			
Less than $50,000	149	1.6 ± 0.91	0.82 ± 0.41
$50,000 to $99,999	368	1.6 ± 0.83	0.76 ± 0.34
$100,000 or greater	371	1.3 ± 0.62	0.63 ± 0.26
Don't know/refused	22	1.1 ± 0.53	0.76 ± 0.29
Alcohol Drinking Status			
Never	24	1.7 ± 0.80	0.78 ± 0.31
Former	107	1.6 ± 0.87	0.81 ± 0.40
Current	779	1.4 ± 0.74	0.70 ± 0.31
Smoking status			
Never	557	1.4 ± 0.71	0.69 ± 0.31
Former	275	1.4 ± 0.79	0.71 ± 0.31
Current	78	1.7 ± 0.91	0.88 ± 0.40
Body mass index (kg/m^2)			
<18.5	15	1.1 ± 0.68	0.70 ± 0.33
18.5–24.9	404	1.3 ± 0.64	0.62 ± 0.26
25.0–29.9	250	1.5 ± 0.68	0.70 ± 0.30
30.0+	240	1.7 ± 0.98	0.91 ± 0.39
Waist circumference (cm)			
≤80	441	1.3 ± 0.65	0.63 ± 0.25
81–88	167	1.5 ± 0.73	0.70 ± 0.32
>88	300	1.6 ± 0.90	0.87 ± 0.38
Current physical activity (MET-hrs/wk)			
<28.02	226	1.7 ± 0.89	0.81 ± 0.36
28.02–43.82	226	1.4 ± 0.74	0.70 ± 0.31
43.83–65.94	227	1.4 ± 0.73	0.70 ± 0.30
≥65.95	226	1.3 ± 0.65	0.65 ± 0.29
Age at menarche (years)			
<12	176	1.5 ± 0.84	0.76 ± 0.37
12	244	1.5 ± 0.76	0.73 ± 0.33

	N	F_2-Isoprostane, ng/mgCr (mean ± SD)	15-F_{2t}-Isoprostane metabolite, ng/mgCr (mean ± SD)
13	250	1.4 ± 0.67	0.69 ± 0.29
14+	240	1.5 ± 0.78	0.70 ± 0.31
Current oral contraceptive use			
No	847	1.5 ± 0.76	0.72 ± 0.32
Yes	63	1.3 ± 0.65	0.67 ± 0.31
Parity			
0	201	1.4 ± 0.78	0.69 ± 0.32
1	118	1.5 ± 0.77	0.73 ± 0.34
2	374	1.5 ± 0.75	0.71 ± 0.31
3+	216	1.5 ± 0.73	0.73 ± 0.33
Prior hysterectomy			
No	786	1.4 ± 0.74	0.70 ± 0.31
Yes	124	1.6 ± 0.87	0.77 ± 0.36
Fruits and Vegetables (servings/day)			
<3	287	1.6 ± 0.88	0.77 ± 0.33
3–4.9	263	1.5 ± 0.69	0.72 ± 0.32
≥5	341	1.3 ± 0.67	0.66 ± 0.30
Vitamin C supplement use			
No	699	1.5 ± 0.75	0.73 ± 0.32
Yes	191	1.3 ± 0.73	0.65 ± 0.30
Vitamin E supplement use			
No	726	1.5 ± 0.75	0.73 ± 0.33
Yes	164	1.3 ± 0.73	0.64 ± 0.27
Dietary isoflavones (mg)			
<0.74	224	1.4 ± 0.71	0.72 ± 0.31
0.74–1.25	222	1.5 ± 0.89	0.74 ± 0.33
1.26–2.76	223	1.5 ± 0.70	0.74 ± 0.33
≥2.77	222	1.4 ± 0.71	0.66 ± 0.31
NSAIDs (total pill-years)			
<0.75	556	1.4 ± 0.71	0.69 ± 0.30
0.75–13.9	133	1.5 ± 0.73	0.74 ± 0.31
14.0–48.9	138	1.5 ± 0.86	0.75 ± 0.35
≥49	83	1.5 ± 0.92	0.78 ± 0.40

Table 2

Odds ratios (OR) and 95% confidence intervals (CI) for breast cancer overall, and by ER status and extent of disease, according to F_2-Isoprostane and 15-F_{2t}-Isoprostane metabolite concentrations categorized at the approximate 25th, 50th, 75th, and 90th percentiles.

F_2-Isoprostane (ng/mgCr)	N Cases	Minimally adjusted model, OR (95% CI)[a]	Fully adjusted model, OR (95% CI)[b]	15-F_{2t}-Isoprostane metabolite (ng/mgCr)	N Cases	Minimally adjusted model, OR (95% CI)[a]	Fully adjusted model, OR (95% CI)[b]
All							
<1.01	105	1.0 (ref)	1.00 (ref)	<0.53	136	1.0 (ref)	1.0 (ref)
1.01–1.38	112	1.1(0.78, 1.5)	1.1 (0.78, 1.5)	0.53–0.68	112	0.82 (0.60, 1.1)	0.82 (0.60, 1.1)
1.39–1.95	125	1.2 (0.88, 0.67)	1.3 (0.93, 1.8)	0.69–0.95	111	0.80 (0.58, 1.1)	0.82 (0.59, 1.1)
1.96–2.93	76	1.2 (0.82, 1.7)	1.3 (0.89, 2.0)	0.96–1.27	64	0.79 (0.55, 1.1)	0.84 (0.56, 1.3)
≥2.94	39	0.94 (0.59, 1.5)	1.1 (0.65, 1.7)	≥1.28	34	0.60 (0.38, 0.95)	0.70 (0.43, 1.1)
		P_{trend}=1.0	P_{trend}=0.6			P_{trend}=0.03	P_{trend}=0.2
ER positive							
<1.01	77	1.00 (ref)	1.00 (ref)	<0.53	104	1.0 (ref)	1.0 (ref)
1.01–1.38	88	1.1 (0.76, 1.6)	1.2 (0.79, 1.7)	0.53–0.68	88	0.7 (0.51, 1.0)	0.74 (0.51, 1.1)
1.39–1.95	101	1.2 (0.84, 1.7)	1.3 (0.91, 2.0)	0.69–0.96	83	0.76 (0.53, 1.1)	0.81 (0.55, 1.2)
1.96–2.93	52	1.0 (0.68, 1.6)	1.2 (0.74, 1.9)	0.97–1.27	47	0.69 (0.45, 1.1)	0.75 (0.47, 1.2)
≥2.94	28	0.95 (0.55, 1.6)	1.1 (0.61, 2.0)	≥1.28	24	0.55 (0.32, 0.94)	0.64 (0.36, 1.1)
		P_{trend}=0.8	P_{trend}=0.8			P_{trend}=0.03	P_{trend}=0.2
ER negative[c]							
<1.01	11	1.0 (ref)		<0.53	15	1.0 (ref)	
1.01–1.38	14	1.5 (0.58, 3.8)		0.53–0.68	16	2.6 (1.0, 6.9)	
1.39–1.95	20	2.2 (0.88, 5.7)		0.69–0.96	23	2.5 (1.0, 6.3)	
1.96–2.93	16	2.0 (0.73, 5.3)		0.97–1.27	8	0.85 (0.32, 2.3)	
≥2.94	5	0.81 (0.22, 3.0)		≥1.28	4	0.71 (0.20, 2.6)	
		P_{trend}=0.9				P_{trend}=0.4	
DCIS							
<1.01	29	1.0 (ref)	1.00 (ref)	<0.53	30	1.0 (ref)	1.0 (ref)
1.01–1.38	25	0.85 (0.46, 1.6)	0.88 (0.46, 1.7)	0.53–0.68	27	0.85 (0.46, 1.6)	0.81 (0.42, 1.6)
1.39–1.95	30	1.0 (0.53, 1.9)	1.0 (0.51, 2.0)	0.69–0.96	31	1.1 (0.55, 2.1)	1.2 (0.60, 2.4)

F_2-Isoprostane (ng/mgCr)	N Cases	Minimally adjusted model, OR (95% CI)[a]	Fully adjusted model, OR (95% CI)[b]	15-F_{2t}-Isoprostane metabolite (ng/mgCr)	N Cases	Minimally adjusted model, OR (95% CI)[a]	Fully adjusted model, OR (95% CI)[b]
1.96–2.93	17	0.88 (0.42, 1.9)	0.85 (0.39, 1.9)	0.97–1.27	17	0.96 (0.46, 2.0)	1.2 (0.54, 2.6)
≥2.94	11	1.2 (0.49, 3.1)	1.4 (0.53, 3.9)	≥1.28	7	0.85 (0.30, 2.4)	1.2 (0.38, 3.6)
		P_{trend}=0.7	P_{trend}=0.6			P_{trend}=0.9	P_{trend}=0.6
Invasive							
<1.01	68	1.0 (ref)	1.0 (ref)	<0.53	99	1.0 (ref)	1.0 (ref)
1.01–1.38	79	1.2 (0.80, 1.8)	1.2 (0.81, 1.9)	0.53–0.68	78	0.82 (0.56, 1.2)	0.80 (0.54, 1.2)
1.39–1.95	90	1.3 (0.89, 1.9)	1.45 (0.98, 2.3)	0.69–0.96	73	0.74 (0.51, 1.1)	0.72 (0.48, 1.1)
1.96–2.93	54	1.3 (0.82, 2.0)	1.5 (0.91, 2.5)	0.97–1.27	43	0.74 (0.48, 1.2)	0.75 (0.46, 1.2)
≥2.94	24	0.82 (0.46, 1.5)	0.91 (0.48, 1.7)	≥1.28	22	0.52 (0.30, 0.89)	0.54 (0.30, 0.99)
		P_{trend}=0.6	P_{trend}=1.0			P_{trend}=0.02	P_{trend}=0.05

Abbreviations: ER=estrogen receptor, CR=creatinine, DCIS=ductal carcinoma in situ

[a]Adjusted for age and enrollment year

[b]Adjusted for age, enrollment year, fruits/vegetable servings per day, BMI, waist circumference, smoking status, physical activity, income, alcohol, vitamin C supplements, vitamin E supplements

[c]Multivariable models not reported due to small number of ER negative cases

Table 3

Odds ratios (OR) and 95% confidence intervals (CI) for breast cancer by menopausal status and age at diagnosis according to F_2-Isoprostane and 15-F_{2t}-Isoprostane metabolite concentrations categorized at the approximate 25th, 50th, 75th, and 90th percentiles.

F_2-Isoprostane (ng/mgCr)	N Cases	Minimally adjusted model, OR (95% CI)[a]	Fully adjusted model, OR (95% CI)[b]	15-F_{2t}-Isoprostane metabolite (ng/mgCr)	N Cases	Minimally adjusted model, OR (95% CI)[a]	Fully adjusted model, OR (95% CI)[b]
Premenopausal							
<1.01	87	1.0 (ref)	1.0 (ref)	<0.53	115	1.0 (ref)	1.00 (ref)
1.01–1.38	83	0.94 (0.66, 1.3)	0.98 (0.68, 1.4)	0.53–0.68	87	0.81 (0.57, 1.2)	0.82 (0.57, 1.2)
1.39–1.95	101	1.2 (0.86, 1.7)	1.4 (0.94, 2.0)	0.69–0.96	82	0.70 (0.49, 1.0)	0.72 (0.50, 1.1)
1.96–2.93	55	0.98 (0.64, 1.5)	1.1 (0.71, 1.8)	0.97–1.27	44	0.69 (0.45, 1.1)	0.76 (0.47, 1.2)
≥2.94	27	0.87 (0.51, 1.5)	1.1 (0.60, 1.9)	≥1.28	25	0.51 (0.30, 0.86)	0.59 (0.34, 1.0)
		P_{trend}=0.7	P_{trend}=0.7			P_{trend}=0.006	P_{trend}=0.05
Postmenopausal							
<1.01	15	1.00 (ref)	1.00 (ref)	<0.53	18	1.0 (ref)	1.0 (ref)
1.01–1.38	27	2.2 (0.98, 4.9)	2.1 (0.88, 4.9)	0.53–0.68	22	1.1 (0.54, 2.3)	1.0 (0.47, 2.2)
1.39–1.95	22	1.5 (0.65, 3.3)	1.4 (0.57, 3.3)	0.69–0.96	28	1.7 (0.81, 3.8)	2.0 (0.84, 4.6)
1.96–2.93	21	3.3 (1.4, 8.2)	3.0 (1.1, 8.0)	0.97–1.27	20	1.6 (0.74, 3.5)	1.5 (0.60, 3.7)
≥2.94	12	1.7 (0.66, 4.6)	1.4 (0.49, 4.2)	≥1.28	9	1.4 (0.52, 3.8)	1.2 (0.37, 4.0)
		P_{trend}=0.2	P_{trend}=0.4			P_{trend}=0.3	P_{trend}=0.5
35–45 years							
<1.01	18	1.0 (ref)	1.0 (ref)	<0.53	32	1.0 (ref)	1.0 (ref)
1.01–1.38	25	0.74 (0.34, 1.6)	0.54 (0.21, 1.4)	0.53–0.68	19	0.58 (0.27, 1.3)	0.39 (0.15, 1.0)
1.39–1.95	22	0.70 (0.31, 1.6)	0.61 (0.23, 1.6)	0.69–0.96	18	0.39 (0.18, 0.85)	0.24 (0.09, 0.63)
≥1.96	17	0.42 (0.17, 1.0)	0.31 (0.10, 0.92)	≥0.97	13	0.26 (0.12, 0.58)	0.15 (0.06, 0.42)
		P_{trend}=0.05	P_{trend}=0.06			P_{trend}=0.001	P_{trend}<0.001
46–50 years							
<1.01	43	1.0 (ref)	1.0 (ref)	<0.53	50	1.0 (ref)	1.0 (ref)
1.01–1.38	38	0.89 (0.53, 1.5)	0.94 (0.54, 1.6)	0.53–0.68	46	0.96 (0.58, 1.6)	0.97 (0.58, 1.6)
1.39–1.95	50	1.4 (0.85, 2.3)	1.5 (0.84, 2.5)	0.69–0.96	43	0.83 (0.50, 1.4)	0.80 (0.46, 1.4)
≥1.96	42	1.3 (0.72, 2.2)	1.5 (0.78, 2.8)	≥0.97	34	0.77 (0.45, 1.3)	0.80 (0.43, 1.5)
		P_{trend}=0.3	P_{trend}=0.1			P_{trend}=0.3	P_{trend}=0.4

F_2-Isoprostane (ng/mgCr)	N Cases	Minimally adjusted model, OR (95% CI)[a]	Fully adjusted model, OR (95% CI)[b]	15-F_{2t}-Isoprostane metabolite (ng/mgCr)	N Cases	Minimally adjusted model, OR (95% CI)[a]	Fully adjusted model, OR (95% CI)[b]
51–60 years							
<1.01	44	1.0 (ref)	1.0 (ref)	<0.53	54	1.0 (ref)	1.0 (ref)
1.01–1.38	48	1.3 (0.81, 2.2)	1.4 (0.84, 2.4)	0.53–0.68	46	0.83 (0.52, 1.3)	0.83 (0.51, 1.4)
1.39–1.95	53	1.3 (0.77, 2.1)	1.4 (0.81, 2.3)	0.69–0.96	50	1.0 (0.63, 1.7)	1.1 (0.67, 1.9)
≥1.96	56	1.3 (0.82, 2.2)	1.6 (0.91, 2.7)	≥0.97	51	1.0 (0.63, 1.7)	1.2 (0.68, 2.1)
		P_{trend}=0.4	P_{trend}=0.2			P_{trend}=0.7	P_{trend}=0.3

[a] Adjusted for age and enrollment year

[b] Adjusted for age, enrollment year, fruits/vegetable servings per day, BMI, waist circumference, smoking status, physical activity, income, alcohol, vitamin C supplements, vitamin E supplements