Published in final edited form as:

Breast Cancer Res Treat. 2013 September ; 141(2): 307–316. doi:10.1007/s10549-013-2673-0.

Plasma florescent oxidation products and breast cancer risk: repeated measures in the Nurses' Health Study

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Abstract

Reactive oxygen species (ROS), normally generated through biologic processes, may damage DNA, lipids, and proteins. ROS are balanced through enzymatic mechanisms and exogenous antioxidants; imbalance results in oxidative stress. Limited data suggest an association between oxidative stress and breast cancer. We evaluated pre-diagnostic plasma fluorescent oxidation products (FLOP), a global biomarker of oxidative stress, and breast cancer risk in a nested case-control study in the Nurses' Health Study. Participants provided two blood samples (1989–1990 and 2000–2002) (N = 18,743). 377 women developed breast cancer between the second collection and June 1, 2006. Cases were matched to 377 controls. Relative fluorescent intensity at three different excitation/emission wavelengths (FLOP_360, FLOP_320, FLOP_400) were quantified in both samples, providing distant (10 years before diagnosis) and proximate (6 years before diagnosis) measures of oxidative stress. We observed no association between FLOP and breast cancer risk in proximate or distant samples (e.g., proximate extreme quartiles: FLOP_360, RR 0.8, 95 % CI 0.5–1.3, $p_{\text{trend}} = 0.49$; FLOP_320, RR 1.1, 95 % CI 0.7–1.7, $p_{\text{trend}} = 0.53$; FLOP_400, RR 1.3, 95 % CI 0.8–2.0, $p_{\text{trend}} = 0.80$). In general no association was observed when cross-classifying or averaging proximate and distant exposure (e.g., extreme quartile of averages: FLOP_360, OR 0.9, 95 % CI 0.6–1.4, $p_{\text{trend}} = 0.82$; FLOP_400, OR 0.9, 95 % CI 0.6–1.4, $p_{\text{trend}} = 0.55$), with the exception of a significant trend for average FLOP_320 (extreme quartiles, OR 1.6, 95 % CI 1.0–2.4, $p_{\text{trend}} = 0.02$). We did not observe important associations between FLOP and breast cancer risk in this large prospective study, though our data suggest women with consistently high FLOP_320 may be at increased risk.

Keywords

Oxidative stress; Breast cancer; Fluorescent oxidation products

Introduction

Reactive oxygen species (ROS) result from both normal endogenous metabolic processes, including cellular metabolism and metabolism of endogenous estrogens [1-3], and exogenous exposures, such as alcohol and radiation [4, 5]. ROS generally are cleared through enzymatic destruction or exogenous antioxidants. Inadequate clearance of ROS results in a state of oxidative stress, which causes damage to lipids, proteins, and DNA, and thus may be related to carcinogenesis [6, 7]. Potential mechanisms include the creation of DNA lesions, which may result in genomic instability [8], as well as activation of transcription factors such as AP-1 and NF- κ B, which may lead to increased proliferation [9]. Given estrogens, alcohol intake, and ionizing radiation are all associated with breast cancer risk, it is possible that increased oxidative stress is a mechanism by which these risk factors influence breast carcinogenesis [1-3, 10-13]. In addition to evidence suggesting the carcinogenic potential of oxidative stress, recent studies suggest an inverse association between plasma carotenoids, which have antioxidant activity, and breast cancer risk [14, 15].

In prior epidemiologic studies of serum, plasma or urinary biomarkers of oxidative stress, the primary measures have been malondialdehyde (MDA) and 15-F_{2t}-isoprostanes (15-F_{2t}-IsoP), both measures of lipid peroxidation. Epidemiologic studies linking oxidative stress to breast cancer are primarily retrospective, with only two prospective studies to date [16, 17]. In several retrospective studies, many with fewer than 100 cases, higher MDA and 15-F_{2t}-IsoP levels were observed in cases relative to controls [18-26]. Both prospective studies to date were conducted in the Shanghai Women's Health Study (SWHS), and no association was observed between urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG), a measure of DNA oxidation, or MDA [16], or urinary 15-F_{2t}-IsoP and its metabolite 2, 3-dinor-5,6-dihydro-15-F_{2t}-isoprostanes (15-F_{2t}-IsoPM) [9, 17] and breast cancer risk overall. However, significantly different associations were observed by BMI, with an inverse association among women of normal weight and lower (BMI < 23 kg/m²) for 15-F_{2t}-IsoP and a positive association among overweight and obese women (BMI ≥ 29 kg/m²) for 15-F_{2t}-IsoPM [17].

A more global measure of oxidation stress, fluorescent oxidation products (FLOP) recently has been shown to be associated with cardiovascular disease in both men [27] and women [28]. FLOP quantifies the interaction of oxidative products with proteins, lipids and DNA and is thus broader than the lipid peroxidation-based biomarkers. Additionally, FLOP are stable in blood with delayed processing between collection and freezing, while MDA and 15-F_{2t}-IsoP are not [29], and therefore represent a measure of oxidative stress appropriate for studies where delayed processing of blood samples occurred.

Thus, there is limited literature on the association between oxidative stress and breast cancer risk, including no studies using a global measure of oxidative stress, and the potential importance of the timing of oxidative stress exposure has not been explored. Therefore, we conducted a nested case-control study within the Nurses' Health Study (NHS) among participants with two blood samples collected approximately 10 years apart to examine the association between plasma markers of oxidative stress both within 6 years and between 10 and 16 years of diagnosis, and the joint effects of exposure at those time points.

Methods

The NHS was initiated in 1976 when 121,700 registered nurses, ages 30–55, completed and returned a mailed questionnaire; participants have been followed since that time via biennial questionnaire [30, 31]. In 1989–1990, 32,826 participants provided a blood sample; in 2000–2002, a subset of 18,743 of these participants provided a second sample. Identical methods were used for both collections. Details have been described previously [12, 32]. Briefly, participants arranged to have their blood drawn and returned the sample to our laboratory via overnight courier with an ice pack. Upon receipt, samples were centrifuged and aliquoted into plasma, white blood cell, and red blood cell components. Samples have been stored since collection at -130°C in continuously monitored liquid nitrogen freezers. This investigation was approved by the Institutional Review Board of Brigham and Women's Hospital.

Case and control selection

Participants in this analysis contributed samples at both blood collections and were cancer-free at the time of the second collection. Cases were diagnosed with breast cancer after the second blood collection (2000–2002) and before June 1, 2006 (Fig. 1). A total of 377 cases of breast cancer were identified; 99 % of cases ($n = 373$) were confirmed via medical record review. The remaining 1 % of cases ($n = 4$) were confirmed by the nurse, and were included in this analysis given that 99 % of self-reported breast cancer cases in the cohort are confirmed upon medical review. Data on estrogen receptor (ER) and progesterone receptor (PR) status, as well as invasive versus in situ status, were abstracted from pathology records.

One control was matched to each case on factors at both blood collections, including age (± 1 year), month (± 1 month) and time of day (± 2 h) of blood collection, fasting status (> 10 h since a meal vs. < 10 h or unknown), and menopausal status and postmenopausal hormone (PMH) use (premenopausal, postmenopausal/no PMH use, postmenopausal/PMH use, unknown menopausal status); additionally cases and controls were matched on menopausal status at the time of the case diagnosis.

Laboratory assays

Case–control sets and samples from both blood collections were assayed together in random order; laboratory personnel were blinded to case–control status. Fluorescent oxidation products (FIOP) were measured at the University of Cincinnati by one of the coauthors (TW). The methods have been described in detail previously [33]. Briefly, plasma samples were mixed with ethanol/ether and with supernatant added for spectrofluorometric readings. Fluorescence was determined as relative fluorescent intensity per milliliter of plasma (FI/mL). Three FIOP measures were assayed: FIOP_360 (excitation 360 nm, emission 420 nm), FIOP_320 (excitation 320 nm, emission 420 nm), and FIOP_400 (excitation 400 nm, emission 475 nm). FIOP_360 represents the interaction between lipid oxidation products and protein, DNA, and carbohydrates [33]. FIOP_320 represents the interaction of lipid oxidative products, particularly lineolate, with DNA and metals [34, 35]. FIOP_400 represents the interaction between MDA, proteins, and phospholipids [35, 36]. The reproducibility of one FIOP measure over 3 years in NHS women, as measured by intraclass correlation coefficients (ICCs), ranged from 0.43 (FIOP_360) to 0.70 (FIOP_400) [28]; ICCs over 10 years ranged from 0.14 (FIOP_320) to 0.30 (FIOP_360). The three FIOP measures were moderately correlated (Spearman correlations: FIOP_360 and FIOP_320, $r = 0.55$, FIOP_360 and FIOP_400, $r = 0.75$, FIOP_320 and FIOP_400, $r = 0.41$). FIOP_360 has been the most commonly used FIOP measure in prior epidemiologic studies [29, 33].

Plasma total carotenoids were considered as a potential confounder and effect modifier. Carotenoids were assayed by reverse-phase high performance liquid chromatography [37] at the Micronutrient Analysis Laboratory in the Department of Nutrition at the Harvard School of Public Health. Total carotenoids were calculated as the sum of α -carotene, β -carotene, β -cryptoxanthin, lycopene, and lutein/zeaxanthin. Total carotenoids were not strongly correlated with FLOP (Spearman correlations: FLOP_360 and total carotenoids, $r = 0.15$; FLOP_320 and total carotenoids, $r = -0.12$; FLOP_400 and total carotenoids, $r = -0.06$).

Masked quality control samples (10 % of samples) were included. FLOP levels in these QC samples varied by batch resulting in across batch CVs ranging from 18 to 20 %. Therefore, we used the methods described by Rosner et al. [38] to adjust for batch-to-batch variation. Overall CVs for each FLOP were <10 % after recalibration. CVs for the individual carotenoids were <14 %.

Covariate data

Covariate data were collected on the biennial questionnaires and the questionnaire returned with blood samples. Covariates in this analysis included: family history of breast cancer, history of benign breast disease, age at menarche, age at first birth and parity, total physical activity, alcohol intake, and body mass index (BMI; kg/m²) at blood draw.

Statistical methods

We removed statistical outliers in FLOP measures using the extreme Studentized deviate many-outlier procedure [39] (range of N outliers excluded across FLOP measures: $N = 4$ –14 for 1st collection; $N = 1$ –16 for 2nd collection). Quartiles were formed based on the distribution in the controls, across both the 1990 and 2000 collections. Tests for trend were calculated modeling the quartile medians as continuous variables. FLOP levels were evaluated as proximate exposure (2000 blood collection, 6 years before diagnosis), distant exposure (1990 blood collection, 10–16 years before diagnosis), proximate and distant exposure mutually adjusted, average exposure (average of proximate and distant), and cross-classified by dichotomizing values from each collection at the median (distant/proximate: low/low, low/high, high/low, high/high). We additionally looked at percent change in FLOP levels between distant and proximate collections.

Relative risks (RR) and 95 % confidence intervals (95 % CI) were calculated using unconditional logistic regression, controlling for matching factors, as well as family history of breast cancer (yes/no), history of benign breast disease (yes/no), age at menarche (age <2, 12, 13, 14), age at first birth and parity (nulliparous, parity 1–2/age at first birth <25, parity 1–2/age at first birth >25, parity 3/age at first birth <25, parity 3/age at first birth >25), total physical activity (<3, 3 to <9, 9 to <18, 18 to <27, 27 MET-h/week), alcohol use (0, 0 to <5, 5 to <15, 15 g per day), and BMI at blood draw (kg/m²: <25, 25 to <30, 30). Results from unconditional regression models adjusted for matching factors were similar to those from conditional logistic regression models; we present results from unconditional models. When the proximate or distant FLOP values were assessed independently, covariate data from the questionnaire closest to that collection were used; when FLOP from 1990 and 2000 were considered in the same model, covariate data from both collections was used with the exception of BMI, because BMI was highly correlated between the two time points ($r = 0.83$). Therefore only BMI in 1990 was included in these models; results were similar when BMI in 2000 was included instead. We evaluated whether the association between FLOP and breast cancer risk varied by BMI, PMH use, and total plasma carotenoid levels by assessing the statistical significance of multiplicative interaction terms in our models with a Wald test. We conducted sensitivity analyses restricted to invasive and ER+/PR+ disease.

P values were considered statistically significant at <0.05 ; all statistical tests are two-sided. Analyses were conducted in SAS 9.3 (Cary, NC).

Results

Participants were on average 56 (SD 6.8) years old at the 1990 blood draw and 67 (SD 6.8) years old at the 2000 blood draw (Table 1). Cases and controls were similar with respect to most covariates (e.g., 1990 BMI cases: 25.6 kg/m², controls: 25.1 kg/m²; age at menarche 12.6 years in cases and controls), but cases had a higher prevalence of family history of breast cancer (14 vs. 10 % in 1990) and history of benign breast disease (49 vs. 40 % in 1990). The distributions of FLOP levels were similar between the two collections. 98 % of cases were postmenopausal at diagnosis.

Results from unadjusted and multivariable models were essentially the same, therefore all results presented are multivariable adjusted. We observed no significant associations between FLOP_360, FLOP_320, or FLOP_400 and breast cancer risk in samples collected distant from diagnosis (1990 collection; 10 years prior to diagnosis) or proximate to diagnosis (2000 collection; 6 years prior to diagnosis) (Table 2). Comparing the top to bottom quartiles from the proximate collection, the RRs (95 % CI) were 0.8 (0.5–1.3; $p_{\text{trend}} = 0.49$) for FLOP_360, 1.1 (0.7–1.7; $p_{\text{trend}} = 0.53$) for FLOP_320, and 1.3 (0.8–2.0; $p_{\text{trend}} = 0.80$) for FLOP_400. Results for the distant FLOP, as well as those mutually adjusted for proximate and distant levels, were similar.

We cross-classified and averaged the distant and proximate values to examine the joint effect of high oxidative stress at both collections. FLOP_360 and FLOP_400 were not associated with breast cancer risk in these analyses (e.g. high distant and proximate versus low distant and proximate FLOP_360, RR 0.9 (95 % CI 0.5–1.5)) (Table 3). Results were similar when we considered the average of distant and proximate FLOP_360 and FLOP_400. FLOP_320 was suggestively associated with breast cancer risk when comparing high distant and proximate to low distant and proximate (RR 1.5, 95 % CI 0.9–2.5) as well as the average of distant and proximate (4th vs. 1st quartile RR 1.6, 95 % CI 1.0–2.4, $p_{\text{trend}} = 0.02$).

In additional analyses, we observed no significant interaction (p for interaction = 0.09) by total carotenoids (low vs. high levels dichotomized at median), and results were similar after adjusting for total carotenoid levels. We conducted analyses cross-classifying average proximate and distant FLOP and average proximate and distant total carotenoids. There was significantly increased risk with high FLOP_360 and low carotenoids (e.g., high oxidative stress), relative to low FLOP_360 and high carotenoids (e.g., low oxidative stress), with similar results for FLOP_320 (high FLOP_360/low carotenoids vs. low FLOP_360/high carotenoids, RR 1.7, 95 % CI 1.1–2.9; high FLOP_320/low carotenoids vs. low FLOP_320/high carotenoids, RR 1.6, 95 % CI 1.0–2.4) (Table 4). We observed no association between FLOP_400 cross-classified with carotenoids and breast cancer risk (high FLOP_400/low carotenoids vs. low FLOP_400/high carotenoids, RR 1.3, 95 % CI 0.8–2.1).

In analyses stratified by BMI, results for FLOP_360 and FLOP_400 were similar for participants with BMI < 25 and BMI ≥ 25 at both collections. While not statistically significant ($p_{\text{interaction}} = 0.13$), results for average distant and proximate FLOP_320 appeared to differ by BMI (Table 5). Specifically, in women with BMI < 25 at both collections, high average FLOP_320 was associated with an increased risk of breast cancer (4th vs. 1st quartile RR 6.6, 95 % CI 2.6–16.9, $p_{\text{trend}} < 0.001$), with no association seen in overweight women (4th vs. 1st quartile RR 1.1, 95 % CI 0.5–2.4, $p_{\text{trend}} = 0.74$). We observed no significant interaction by postmenopausal hormone use (data not shown).

Results were similar when restricted to women fasting at least 8 h prior to blood collection, and when restricted to cases with invasive or (ER/PR) positive disease (data not shown).

Discussion

We did not observe significant associations between oxidative stress measured by plasma FLOP levels and breast cancer risk in this large nested case-control study evaluating both distant and proximate plasma FLOP levels. There was a suggested increased risk associated with high FLOP_320 when proximate and distant values were averaged, as well as a suggested difference in associations between FLOP_320 and breast cancer risk in women with BMI < 25 (under or normal weight) as compared to women with BMI ≥ 25; however this difference was not statistically significant. We observed a suggestion of increased risk associated with either high FLOP_360 or FLOP_320 and low average plasma carotenoid levels, suggesting women with presumably high oxidative stress marked by high FLOP and low plasma antioxidants are at higher risk than women with lower oxidative stress. To our knowledge, we are the first to examine the association between oxidative stress and breast cancer by level of total plasma carotenoids.

Prior retrospective case-control studies consistently have observed higher oxidative stress among breast cancer cases than controls [18-26]. However, with one exception [25], these studies were small (case $n < 100$) and due to the retrospective nature, higher levels of oxidative stress may be due to disease-related factors. In prospective analyses in the SWHS [16, 17], no associations were observed between urinary 8-OHdG and MDA [16] or urinary 15-F_{2t}-IsoP and its metabolite 15-F_{2t}-IsoPM in overall analyses [17]. However, 15-F_{2t}-IsoP was inversely associated with breast cancer risk in women with BMI < 23 (OR 0.46, 95 % CI 0.23–0.80, $p_{\text{trend}} = 0.006$) but not associated with risk in women with BMI ≥ 29 (OR 1.53, 95 % CI 0.52–4.51, $p_{\text{trend}} = 0.40$); 15-F_{2t}-IsoPM was not significantly associated with breast cancer risk in women with BMI < 23 (OR 0.79, 95 % CI 0.44–1.43, $p_{\text{trend}} = 0.49$), but was strongly positively associated with risk in women with BMI ≥ 29 (OR 10.27, 95 % CI 2.41–43.80, $p_{\text{trend}} = 0.003$) [17]. Similarly, we observed no association between plasma FLOP levels and breast cancer risk overall, with the suggestion of a difference in risk by BMI < 25 vs. ≥ 25. However, in contrast to SWHS, we observed the suggestion of increased risk among women with low BMI. Possible explanations for this difference include the differences in biomarkers of oxidative stress, as well as the quantification of oxidative stress markers in urine (SWHS) versus plasma (NHS). It is plausible that women who excrete high levels of markers of oxidative stress in urine have lower circulating levels; however, to our knowledge there are no data describing the relationship between urine and plasma measures of oxidative stress.

Oxidative stress may differentially impact risk of disease in women by BMI and by level of plasma carotenoids. Prior data support a positive association between BMI and measures of oxidative stress including isoprostanes [40, 41] and MDA [42], though an association has not been observed between BMI and plasma FLOP [27, 28] and BMI and the three FLOP measures are not correlated (Spearman $r < 0.09$) in this study. Given leaner women may have lower overall oxidative stress, it is plausible that oxidative stress quantified by FLOP, which does not capture BMI-related oxidative stress, is most relevant in relation to breast cancer in women with lower overall oxidative stress. We also observed suggestive associations between FLOP and breast cancer among women with low plasma carotenoids, suggesting that high oxidative stress in a low anti-oxidant environment may be important in the etiology of breast cancer. Further work is needed to explore these associations.

FLOP_360, FLOP_320 and FLOP_400 each represent different sources of oxidation. FLOP_360 and FLOP_400 are well correlated ($r = 0.75$) and are generated by reactions with

proteins and lipids, and, for FLOP_360, DNA. FLOP_320 represents interactions between lipid oxidation products and DNA in the presence of metals [34, 35] (FLOP_360 and FLOP_320 $r = 0.55$). We observed no associations between FLOP_360 or FLOP_400 and breast cancer, but the suggestion of an association with sustained high FLOP_320. Prior experimental data support associations between various metals and breast cancer [43-46], with metals activating the ER and stimulating proliferation of breast cancer cells [44, 46]. Higher levels of various metals, including iron, nickel, chromium, zinc, cadmium, mercury, and lead, have been detected in breast cancer tissue as compared to normal tissue [45], though data are limited. Given that FLOP_320 quantifies oxidation associated with metals, it is possible that this measure captures the oxidative stress most relevant to the etiology of breast cancer.

While oxidative stress as quantified by FLOP levels was not associated consistently with breast cancer risk in our study, these biomarkers have previously been associated with coronary heart disease (CHD) in both men [27] and women [28]. In the NHS, FLOP_360 was associated with a 1.64-fold increased risk of CHD, comparing extreme quintiles (95 % CI 1.06–2.53). Results were similar for FLOP_400 but attenuated for FLOP_320. FLOP levels previously have been shown to be positively correlated with smoking status, hypertension, and alcohol intake [28]. It is plausible that the oxidative pathways associated with CHD are different from the pathways associated with breast cancer risk.

Our study has several limitations. While we used a global measure to quantify oxidative stress in our study population, it is possible that more specific measures of oxidative stress, such as MDA, 15-F_{2t}-IsoP, or 15-F_{2t}-IsoPM are more relevant to the etiology of breast cancer. However, while a more broad measure of oxidative stress, plasma FLOP_360 is well correlated with both plasma 15-F_{2t}-IsoP ($r = 0.75$) and plasma MDA ($r = 0.94$) in fasting samples (Tianying Wu, personal communication).

Strengths of the current study include measurement of FLOP in both a proximate (6 years before diagnosis) and distant (10 years before diagnosis) plasma sample, a large sample size in a well-characterized population, and a global measure of oxidative stress allowing us to evaluate risk associated with a woman's burden of oxidative stress using a more comprehensive measure. Future experimental studies are needed to clarify the exact oxidation components captured by FLOP_320 and to investigate the potential underlying mechanism between FLOP_320 and breast cancer.

In summary, in this nested case-control study we did not observe significant associations between plasma fluorescent oxidative products and breast cancer risk regardless of whether the exposure was proximate or distant to disease diagnosis. Suggestive associations between FLOP_320 and breast cancer emerged when proximate and distant exposure were averaged, and when FLOP_360 and FLOP_320 were cross-classified with total plasma carotenoids. Future studies are needed to further investigate the role of oxidative stress in a low antioxidant environment. Given the suggestion of a differential effect by BMI, future studies should consider the role of oxidative stress in the etiology of breast cancer by BMI to better elucidate how oxidative stress and BMI interact to influence breast cancer risk.

Acknowledgments

The authors would like to thank Susan Hankinson, Sc.D. for her important contributions to this manuscript. This work was funded by National Institute of Health Grants R01 CA131218, P01 CA87969, R01 CA49449. RT Fortner is supported in part by T32 CA09001. We would like to thank the participants and staff of the Nurses' Health Study for their valuable contributions as well as the following state cancer registries for their help: AL, AZ, AR, CA, CO, CT, DE, FL, GA, ID, IL, IN, IA, KY, LA, ME, MD, MA, MI, NE, NH, NJ, NY, NC, ND, OH, OK, OR, PA, RI, SC, TN, TX, VA, WA, WY. In addition, this study was approved by the Connecticut Department of Public Health

(DPH) Human Investigations Committee. Certain data used in this publication were obtained from the DPH. The authors assume full responsibility for analyses and interpretation of these data.

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Fig. 1.
Study design: florescent oxidation products (FLOP) and breast cancer risk: Nurses' Health Study

Table 1

Characteristics of cases and controls at 1989–1990 (distant) and 2000–2002 (proximate) blood collections

	Case (n = 377) 1989–1990	Control (n = 377)	Case (n = 377) 2000–2002	Control (n = 377)
	Mean (SD)		Mean (SD)	
Age at blood draw ^a	56.1 (6.8)	56.1 (6.8)	67.1 (6.8)	67.2 (6.8)
Body mass index (kg/m ²)	25.6 (3.9)	25.1 (4.1)	27.0 (4.5)	26.4 (4.7)
Total activity (MET-h/week)	18.0 (24.7)	17.3 (18.0)	18.9 (17.8)	19.1 (20.3)
Alcohol consumption (g/day)	5.3 (8.2)	5.2 (7.7)	6.4 (9.9)	5.6 (8.7)
Age at menarche	12.6 (1.3)	12.6 (1.3)		
Parity	2.8 (1.5)	3.2 (1.8)		
Age at first birth	22.5 (7.3)	22.9 (6.4)		
Menopausal status at blood collection				
Postmenopausal, no PMH use, %	33	32	33	31
Postmenopausal, PMH use, %	35	35	65	67
Family history of breast cancer, %	14	10	23	15
Benign breast disease, %	49	40	64	54
	Median (IQR)		Median (IQR)	
FLOP_360 (fL/mL)	204 (170–254)	204 (168–259)	204 (174–245)	210 (180–246)
FLOP_320 (fL/mL)	388 (280–625)	360 (279–516)	412 (315–758)	414 (315–609)
FLOP_400 (fL/mL)	57.4 (46.9–74.4)	56.9 (46.9–73.1)	59.3 (50.0–73.2)	59.9 (49.4–72.9)

Values are means (SD) or percentages and are standardized to the age distribution of the study population *PMH* postmenopausal hormone^aValue is not age adjusted; matching factor

Table 2

Proximate (6 years prior to diagnosis) and distant (10 years prior to diagnosis) fluorescent oxidation products (FIOP) and breast cancer risk: Nurses' Health Study

	RR	95 % CI	RR	95 % CI	RR	95 % CI	RR	95 % CI	p trend
FIOP_360									
Quartile cutpoints, FI/mL	<174		174 to <207		207 to <251		251		
Case/control no.	89/68		99/99		82/109		82/80		
Proximate ^a Multivariate ^b RR (95 % CI)	1	(reference)	0.8	(0.5-1.3)	0.6	(0.4-0.9)	0.8	(0.5-1.3)	0.49
Proximate MV RR (95 % CI) adjusting for distant	1	(reference)	0.7	(0.5-1.2)	0.6	(0.4-1.0)	0.8	(0.5-1.3)	0.54
Case/control no.	103/109		85/84		84/71		95/100		
Distant ^a Multivariate ^b RR (95 % CI)	1	(reference)	1	(0.7-1.6)	1.2	(0.8-1.9)	1	(0.7-1.6)	0.86
Distant MV RR (95 % CI) adjusting for proximate	1	(reference)	1	(0.7-1.7)	1.3	(0.8-2.1)	1	(0.6-1.6)	0.93
FIOP_320									
Quartile cutpoints, FI/mL	<296		296 to <388		388 to <577		577		
Case/control no.	74/72		88/82		84/106		116/101		
Proximate ^a Multivariate ^b RR (95 % CI)	1	(reference)	1.0	(0.6-1.6)	0.8	(0.5-1.2)	1.1	(0.7-1.7)	0.53
Proximate MV RR (95 % CI) adjusting for distant	1	(reference)	1.2	(0.7-1.9)	0.8	(0.5-1.4)	1.1	(0.7-1.7)	0.82
Case/control no.	105/111		80/101		84/77		101/82		
Distant ^a Multivariate ^b RR (95 % CI)	1.0	(reference)	0.8	(0.5-1.2)	1.1	(0.7-1.7)	1.3	(0.9-2.0)	0.07
Distant MV RR (95 % CI) adjusting for proximate	1.0	(reference)	0.8	(0.5-1.2)	1.1	(0.7-1.8)	1.3	(0.8-2.0)	0.12
FIOP_400									
Quartile cutpoints, FI/mL	<47.8		47.8 to <57.8		57.8 to <73.0		73.0		
Case/control no.	63/80		104/87		98/101		90/88		
Proximate ^a Multivariate ^b RR (95 % CI)	1.0	(reference)	1.6	(1.0-2.5)	1.3	(0.8-2.1)	1.3	(0.8-2.0)	0.8
Proximate MV RR (95 % CI) adjusting for distant	1.0	(reference)	1.7	(1.1-2.8)	1.4	(0.9-2.3)	1.4	(0.8-2.4)	0.66
Case/control no.	102/100		86/94		83/80		97/93		
Distant ^a Multivariate ^b RR (95 % CI)	1.0	(reference)	0.9	(0.6-1.3)	1.0	(0.6-1.6)	1.0	(0.7-1.5)	0.80
Distant MV RR (95 % CI) adjusting for proximate	1.0	(reference)	0.8	(0.5-1.2)	0.9	(0.6-1.5)	0.8	(0.5-1.3)	0.73

^a Proximate sample: blood sample collected 6 years prior to diagnosis; Distant sample: blood sample collected 10 years prior to diagnosis

^b Multivariate models adjusted for age at blood draw, fasting status, menopausal status and postmenopausal hormone use at blood draw, time of blood draw, family history of breast cancer, history of BBD, body mass index at blood draw, age at menarche, age at first birth/parity, alcohol use, and total physical activity

Table 3

Florescent oxidation products (FIOP), proximate (6 years prior to diagnosis) and distant (10 years prior to diagnosis) levels crossclassified and breast cancer risk: Nurses' Health Study

	RR	95 % CI	RR	95 % CI	RR	95 % CI	RR	95 % CI
	Low distant/low proximate ^a		Low distant/high proximate		High distant/low proximate		High distant/high proximate	
Cross classification								
FIOP_360								
Case/control no.	230/233		52/43		65/63		30/37	
	1.0	(reference)	1.3	(0.8–2.0)	1.1	(0.7–1.6)	0.9	(0.5–1.5)
FIOP_320								
Case/control no.	206/228		70/67		55/48		46/34	
	1.0	(reference)	1.1	(0.7–1.6)	1.3	(0.8–2.1)	1.5	(0.9–2.5)
FIOP_400								
Case/control no.	229/226		51/58		58/63		39/30	
	1.0	(reference)	0.9	(0.5–1.3)	0.9	(0.6–1.4)	1.2	(0.7–2.0)
Average of proximate and distant								
	Quartile 1		Quartile 2		Quartile 3		Quartile 4	<i>P</i> trend
FIOP_360								
Quartile cutpoints, FI/mL	<181		1801 to <211		211 to <256		256	
Case/control no.	86/85		78/87		102/85		76/87	
	1.0	(reference)	0.9	(0.5–1.3)	1.1	(0.7–1.8)	0.9	(0.6–1.4) 0.82
FIOP_320								
Quartile cutpoints, FI/mL	<315		315 to <427		427 to <702		702	
Case/control no.	73/88		90/88		71/90		121/89	
	1.0	(reference)	1.2	(0.7–1.8)	0.9	(0.5–1.4)	1.6	(1.0–2.4) 0.02
FIOP_400								
Quartile cutpoints, FI/mL	<50.8		50.8 to <59.4		59.4 to <73.5		73.5	
Case/control no.	88/86		87/87		88/86		83/87	
	1.0	(reference)	1.0	(0.6–1.6)	1.0	(0.6–1.6)	0.9	(0.6–1.4) 0.55

Models adjusted for age at blood draw, fasting status, menopausal status and postmenopausal hormone use at blood draw, time of blood draw, family history of breast cancer, history of BBD, body mass index at blood draw, age at menarche, age at first birth/parity, alcohol use, and total physical activity

^aCross classification low and high categories defined by median

Table 4

Average proximate and distant FIOP and total carotenoids cross-classified and risk of breast cancer: Nurses' Health Study

	RR	95 % CI	RR	95 % CI	RR	95 % CI	RR	95 % CI
	Low FIOP/high carotenoids ^a		Low FIOP/low carotenoids		High FIOP/high carotenoids		High FIOP/low carotenoids	
FIOP_360								
Case/control no.	52/72		112/100		81/96		97/76	
	1.0	(reference)	1.6	1.0–2.6	1.3	0.8–2.1	1.7	1.1–2.9
FIOP_320								
Case/control no.	71/91		92/85		68/82		124/97	
	1.0	(reference)	1.4	0.9–2.2	1.1	0.7–1.7	1.6	1.0–2.4
FIOP_400								
Case/control no.	73/84		102/89		62/84		109/89	
	1.0	(reference)	1.4	0.9–2.1	0.9	0.5–1.4	1.3	0.8–2.1

Models adjusted for age at blood draw, fasting status, menopausal status and postmenopausal hormone use at blood draw, time of blood draw, family history of breast cancer, history of BBD, body mass index at blood draw, age at menarche, age at first birth/parity, alcohol use, and total physical activity

^a Cross classification low and high categories defined by median

Table 5
Average fluorescent oxidation products (FOP) and breast cancer risk by body mass index (BMI): Nurses' Health Study

	OR	95 % CI	OR	95 % CI	OR	95 % CI	OR	95 % CI	P _{trend}	P _{interaction}
FOP_360										
Quartile cutpoints, FI/mL	<181		181 to <211		211 to <256		256			0.43
BMI < 25										
Case/control no.	28/33		26/32		35/36		26/43			
	1.0	(reference)	1.5	(0.6–3.4)	1.8	(0.8–4.3)	1.1	(0.4–2.6)	0.97	
BMI ≥ 25										
Case/control no.	40/34		36/37		42/26		33/34			
	1.0	(reference)	0.8	(0.4–1.7)	1.3	(0.6–2.7)	1	(0.5–2.2)	0.75	
FOP_320										
Quartile cutpoints, FI/mL	<315		315 to <427		427 to <702		702			0.13
BMI < 25										
Case/control no.	17/40		32/34		24/41		43/33			
	1.0	(reference)	2.3	(0.9–5.6)	1.7	(0.7–4.3)	6.6	(2.6–16.9)	<0.001	
BMI ≥ 25										
Case/control no.	38/30		42/35		29/31		51/34			
	1.0	(reference)	1.2	(0.6–2.5)	0.8	(0.4–1.9)	1.1	(0.5–2.4)	0.74	
FOP_400										
Quartile cutpoints, FI/mL	<50.8		50.8 to <59.4		59.4 to <73.5		73.5			0.27
BMI < 25										
Case/control no.	33/40		28/32		34/35		20/38			
	1.0	(reference)	0.9	(0.4–2.1)	1.9	(0.8–4.1)	0.6	(0.2–1.4)	0.48	
BMI ≥ 25										
Case/control no.	34/24		39/40		41/31		39/31			
	1.0	(reference)	0.8	(0.4–1.8)	0.7	(0.3–1.7)	1.0	(0.4–2.3)	0.77	

Models adjusted for age at blood draw, fasting status, menopausal status and postmenopausal hormone use at blood draw, time of blood draw, family history of breast cancer, history of BBD, body mass index at blood draw, age at menarche, age at first birth/parity, alcohol use, and total physical activity