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Plasma Protein Carbonyls and Breast Cancer Risk in Sisters Discordant for Breast Cancer from the New York Site of the Breast Cancer Family Registry

Jennifer Zipprich[1], **Mary Beth Terry**[2], **Yuyan Liao**[2], **Meenakshi Agrawal**[1], **Irina Gurvich**[1], **Ruby Senie**[2], and **Regina M. Santella**[1]

1 *Department of Environmental Health Sciences, Mailman School of Public Health, Columbia University, New York, NY*

2 *Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, NY*

Abstract

Reactive Oxygen Species (ROS) are important in the pathogenesis of many diseases, including breast cancer. Several population-based case-control studies have demonstrated that various biomarkers of oxidative stress are associated with an increase in breast cancer risk. We selected sisters discordant for breast cancer (n=645) from the New York site of the Breast Cancer Family Registry to explore factors that contribute to variation in plasma protein carbonyls, and to determine whether this biomarker is associated with an increase in breast cancer risk among those with a family history. Late age at menarche, HRT use, and Hispanic race were significantly associated with lower plasma protein carbonyl levels in unaffected sisters. Plasma protein carbonyls were associated with an increase in breast cancer risk (Q2 OR = 1.4, 95% CI=0.8–2.7; Q3 OR= 2.4, 95% CI = 1.1–4.9, Q4 OR= 1.9, 95% CI=0.8–4.2), though not in a dose-dependent manner. These data suggest that oxidative damage is a risk factor for breast cancer in high risk women.

Keywords

Breast cancer; Oxidative stress; Biomarkers

INTRODUCTION

Reactive oxygen species (ROS) are produced by metabolic processes such as oxidative phosphorylation, and the cytochrome p450 monoxygenase system, but can also be generated by radiation or other environmental exposures. An imbalance between the generation of ROS and the capacity of antioxidant systems can result in oxidative damage to proteins, lipids, and DNA, which, if unrepaired, is important in the pathogenesis of many diseases, including breast cancer.

Biological markers of oxidative stress have been associated with increased breast cancer risk in several population-based studies. We previously reported that plasma protein carbonyls, and urinary 15-f2t-isoprostanes, were associated with an increase in breast cancer risk in a large case-control study (1,2). Several smaller studies have shown that plasma or serum

Author for correspondence: Regina M. Santella, Department of Environmental Health Sciences, Mailman School of Public Health, Columbia University, 630 West 168th Street, P & S 19-418, New York, NY 10032, USA, Phone: 212-305-8158, Fax: 212-305-5328, Email: rps1@columbia.edu.

malondialdehyde, urinary 8-oxodG, and plasma $O_2^{\cdot -}$ production are higher in breast cancer cases compared with controls (3–6). Additionally, higher levels of 8-oxodG adducts have been detected in breast tumor tissue compared with adjacent non-tumor tissue, and with normal breast tissue from controls (7–9). Thus, oxidative stress has been identified as a risk factor for breast cancer in many population-based studies.

Family history is one of the most significant risk factors for breast cancer. Breast cancer in a first degree relative is associated with a 2-fold or greater increase in risk of breast cancer; this risk may increase depending upon factors such as age at diagnosis, the presence of genetic mutations, and the number of first and second degree relatives with breast cancer (10,11). Though increased risk due to family history is well described in the literature, little is known about the factors underlying this risk. High penetrance mutations in genes such as *BRCA1* and *BRCA2, ATM*, and *CHEK2* have been identified but these explain only 20% of familial breast cancer risk; the remaining 80% of risk is likely attributable to other unknown genetic and/or environmental factors (12). Therefore, it is a high priority to identify potentially modifiable factors that may reduce breast cancer risk in individuals at increased risk due to their family history.

Since oxidative stress has emerged as a risk factor for breast cancer in population-based studies, we aimed to determine whether oxidative damage is associated with an increase in breast cancer risk among women with a family history of breast cancer. We additionally explored whether reproductive or lifestyle characteristics influence oxidative damage levels, and the nature of these relationships. Our study was comprised of sisters discordant for breast cancer who are participating in the New York site of the Breast Cancer Family Registry (BCFR). We measured plasma protein carbonyl levels as a biological marker of ROS exposure. Protein carbonyls are formed on the amino acid side chains of proteins and can be detected in various biological specimens including plasma using a well established ELISA method (13,14).

MATERIALS AND METHODS

Subjects

The subjects for this study were selected from families participating in the New York site of the BCFR. The study was approved by Columbia University's Institutional Review Board; written informed consent was obtained from all subjects, and strict quality controls and safeguards were used to protect confidentiality. The description of the resources, the recruitment, and data collection methods of the BCFR are detailed elsewhere (15). Briefly, BCFR is a multi-center resource designed to address questions related to gene-environment interactions relevant to breast cancer. Families participating in the New York site were recruited 1995 to 2006 from health clinics, hospitals, and community organizations in the New York metropolitan area. Recruitment was initially through an individual, with or without cancer, whose family met one of the following criteria: 1) one first or second degree relative with breast or ovarian cancer diagnosed before age 45 years, 2) one first or second degree relative with both breast and ovarian cancer regardless of age at diagnosis, 3) two first or second degree relatives with either breast or ovarian cancer diagnosed after age 45 years, 4) one male family member with a history of breast cancer, and 5) a family known to carry a *BRCA* mutation.

A total of 1,336 families including 4,871 individuals were recruited from New York City and the surrounding area (New Jersey and Connecticut). Of these families there are 342 where at least two sisters discordant for breast cancer participated in the New York BCFR (n=842 individuals). 90% of these sisters also donated a blood sample (754 individuals, 313 sister sets). We included 268 sister sets that were available at the time this study was initiated; 192 sister sets had 1 case and 1 control, 59 sister sets had 1 case and 2 controls or 2 cases and 1 control, and the remaining 17 sister sets had higher multiples of either or both cases and controls.

Subjects were administered a questionnaire that collected information on demographics, personal history of cancer, pertinent lifestyle and environmental factors (ionizing radiation exposure, smoking, and alcohol consumption), and reproductive factors known to be significant in cancer development (pregnancy history, breast feeding, hormone use). The family history of all cancers (excluding non-melanoma skin cancers, and cervical carcinoma *in situ*) were self reported as was treatment for breast and ovarian cancers.

Plasma protein carbonyl measurement

Protein carbonyls were measured by non-competitive ELISA based on the method developed by Winterbourne and Buss, and described in detail by Rossner et. al. (2,14). Oxidized BSA standards were prepared as in Winterbourne and Buss (2,14). Plasma protein concentration was quantitated using the Bicinchoninic Acid Kit according to the manufacturer's protocol (Sigma Aldrich, St. Louis, MO). After quantitation plasma samples were diluted to 4 mg/ml in 10 mM PBS pH 7.0 for the detection of carbonyls.

Plasma samples and oxidized BSA standards were derivitized with 10 mM dinitrophenylhydrazine (DNP), 6.0 M guanidine hydrochloride, 0.5M potassium phosphate, pH 2.5. ELISA plates (Corning Costar, Lowell, MA) were coated with 1μg of the derivitized samples (100ul) and standards and were incubated overnight at 4°C. Plates were blocked with 0.1% BSA in 10 mM PBS pH 7.0 for 1 hr at room temperature. Biotinylated anti-DNP antibody (Molecular Probes, Carlsbad, CA), 1:1500 in 10 mM PBS, 0.1%BSA, 0.1% Tween-20, was added and plates were incubated for 1 hr at 37°C. Streptavidin-Biotin Horse Radish Peroxidase (HRP, Amersham, Piscataway, NJ), 1:4000 in 10 mM PBS, 0.1% BSA, 0.1% Tween-20 was added and plates were incubated for 1 hr at room temperature. The HRP was reacted with TMB substrate (Sigma) and the reaction was stopped by adding 2.5 M H_2SO_4. Absorbance was read at 450nm on a Molecular Devices Microplate Spectrophotometer (Sunnyvale, CA).

Sample values were interpolated against the standard curve created from oxidized BSA standards ranging from approximately 0.10–0.60 nmol/mg using Microsoft Excel XP. Test plasma carbonyl concentrations below the lowest standard were considered non-detectable (n=9), and a concentration corresponding to half of the lowest standard was input for those samples. The carbonyl concentrations ranged from 0.10 nmol/mg to 0.64 nmol/mg and the plasma protein concentrations ranged from 28 mg/ml to 107 mg/ml. Each sample was analyzed in duplicate, and a pooled plasma sample was included in every plate to estimate the inter-assay coefficient of variation (11%, n=18). To determine stability of the biomarker, plasma protein carbonyls were measured in a pooled plasma sample that was repeatedly frozen and thawed; there was no significant difference in protein carbonyl levels after up to four freeze-thaw cycles.

Statistical analysis

Our analysis included age at time of specimen collection, BMI (weight/height^2), race, menopausal status, age at menarche, hormone replacement therapy use, oral contraceptives use, parity, smoking, and alcohol consumption. In the conditional logistic regression analysis, BMI was categorized into quartiles based upon the distribution in the unaffected sisters (Q1: <22.0, Q2: 22.0–24.3, Q3: 24.4–27.9, Q4:≥ 28.0), age at menarche was divided based upon the median age at menarche among unaffected sisters (Median age = 13) and age was categorized by decade (18–39, 40–49, 50–59, >60). HRT use, OC use, parity, smoking, and alcohol consumption were categorized as ever/never, and menopausal status was categorized as premenopausal or postmenopausal. Smoking status was first parsed into 4 categories (never smoked, light smoker defined as <100 cigarettes over a lifetime, former regular smoker defined as ≥ 1 cigarette per day for more than 3 months, and current smoker). However, since there was no evidence that carbonyl levels varied by the distinctions among current and former

smokers we collapsed smoking into a dichotomous ever/never variable for the multivariable analysis. These questionnaire data were collected at or close to the time of blood donation.

Plasma protein carbonyls were log-transformed to approximate a normal distribution. ANOVA and multiple linear regression were used to assess associations between plasma protein carbonyls and the reproductive and lifestyle factors in the unaffected sisters. Stratified analysis was used to evaluate the effect of menopausal status, a potential modifier, on the prediction of plasma protein carbonyl levels.

A conditional logistic regression model was used to evaluate the association between plasma protein carbonyl levels and breast cancer risk. Plasma protein carbonyl levels were categorized by quartiles based on the distribution in the unaffected sisters. Two models were developed, an age-adjusted model, and a multivariable model. The multivariable model contained only variables that were significantly associated with the outcome or confounded the relationship between the biomarker and breast cancer. Confounding was assessed by a 10% change in the β-estimate observed upon the addition of the potential confounder into the model. As a secondary analysis, we stratified the models by menopausal status or time since diagnosis (>3 yrs or ≤3 yrs) to determine whether these factors modified the relationship between our biomarkers and breast cancer risk.

SAS statistical software version 9.1.2 was used for all statistical analyses.

RESULTS

We first evaluated the log-transformed mean levels of plasma protein carbonyl by age, BMI, cigarette smoking status, alcohol consumption, oral contraceptive use, parity, age at menarche, menopausal status and HRT use in cases and unaffected sisters. The mean log-protein carbonyl values in cases and unaffected sisters are shown in Table 1. Significant decreases in plasma protein carbonyl levels were associated with an older age at menarche ($p=0.01$), a younger age at first live birth ($p=0.03$), and the use of hormone replacement therapy ($p<0.01$) among unaffected sisters (Table 1). Since around 20% of the women in our study were nulliparous, age at first live birth was excluded from subsequent multivariable analysis.

A multivariable regression model was developed to predict plasma protein carbonyl levels among unaffected sisters (Table 2). Older age at menarche, HRT use and Hispanic race were found to significantly predict plasma protein carbonyl levels. When the model was stratified by menopausal status (Table 3), we found that high BMI (≥ 28.0) was associated with a decrease in plasma protein carbonyl levels in premenopausal women ($p=0.03$). In contrast, high BMI (≥ 28.0) in postmenopausal women was associated with an increase in plasma protein carbonyl levels ($p=0.02$). A late age at menarche (≥13 yrs) was associated with a decrease in plasma protein carbonyl levels in premenopausal women ($p=0.005$), but was not significant in postmenopausal women ($p=0.76$). Finally, parity was associated with a decrease in plasma protein carbonyl levels in postmenopausal women ($p=0.01$), but no relationship was detected in premenopausal women ($p=0.39$). Since it was possible that several controls from the same family were included in these models, we also analyzed the data using a mixed model (16). The results were similar (data not shown), therefore only the multiple linear regression results are presented.

The conditional logistic regression models revealed an increase in breast cancer risk associated with plasma protein carbonyls: Q2: OR 1.4, 95% CI: 0.8–2.7, Q3: OR 2.4, 95% CI: 1.1–4.9, and Q4: OR 1.9, 95% CI: 0.8–4.2 (Table 4). Since others have shown that menopausal status is associated with changes in ferritin levels, circulating estradiol/estrone levels, antioxidant enzyme expression, and body fat amount/distribution, all factors that may influence ROS production, we also stratified our risk models by menopausal status (17,18). The trends in risk

were similar between premenopausal women and postmenopausal women, though the risk was slightly higher among postmenopausal women (data not shown).

To determine whether the disease may have impacted our biomarker, we also stratified the plasma protein carbonyl model by years from breast cancer diagnosis. We found that the trends and the level of association with breast cancer risk was similar between women with a cancer diagnosis greater than three years from specimen collection and women diagnosed within 0–3 years of specimen collection (data not shown).

DISCUSSION

Molecular and biochemical studies have shown that ROS stimulate epigenetic changes and interact with cellular macromolecules (DNA, proteins, and lipids), both of which can result in shifts in homeostasis that are important in cellular transformation (19). The most common DNA lesion produced by ROS, 8-oxo-2-deoxyguanosine (8-OxodG), promotes G to T transversion mutations (20). Further, lipid peroxidation products such as malondialdehyde can also react with DNA to form a number of pro-mutagenic lesions (21). Metabolism of estrogen, a well established risk factor for breast cancer, generates ROS, indicating that oxidative stress may be an important mediator of estrogen dependent breast carcinogenesis (22,23). There is a growing body of epidemiological evidence that oxidative stress biomarkers such as urinary 15-f2t-isoprostanes, malondialdehyde, and urinary and tissue 8-oxodG, are elevated in breast cancer cases compared with controls in population-based studies (1,3,5–9).

We selected sisters discordant for breast cancer who are participating in the New York Site of the BCFR to determine whether oxidative damage is also associated with breast cancer in high risk women. Breast cancer in a first degree relative is associated with a 2-fold or more increased risk of breast cancer (10,11). Since there is limited information about potentially modifiable factors that could reduce breast cancer risk in those at increased risk due to their family history, we evaluated whether oxidative damage could explain part of this risk.

Carbonyl groups formed by ROS on amino acid side chains are chemically stable moieties making them useful in detecting oxidative damage (24). Carbonyl modifications are not repaired and the extent of carbonylation depends upon factors that influence oxidant status. Thus, the plasma protein carbonyl biomarker captures the net of pro-oxidant exposures and antioxidant status. The most commonly oxidized amino acids are Arg, Lys, Pro, Thr, and the plasma protein, fibrinogen, is highly susceptible to free radical attack (24,25). Intracellular oxidized proteins are rapidly degraded by the 20S proteosome (26). However, it is unclear whether extracellular oxidized proteins are actively degraded or simply eliminated by normal turnover. Plasma protein carbonyl levels have been shown to increase with fat overload and exercise, and decrease with weight loss, and vitamin treatment (27–30).

In our analysis of factors associated with plasma protein carbonyl levels in unaffected sisters, we found that early age of menarche (<13 yrs) was significantly associated with an increase in plasma protein carbonyl levels. Others have reported differences in girls with early menarche (< 12 yrs) such as higher serum estradiol levels, and earlier onset of regular ovulatory cycles (31). Further, a combination of early age at menarche and increased waist circumference was associated with higher 17-β estradiol levels in adult women indicating that ones' age at menarche can contribute to lasting impacts on hormone levels (32)

Contrary to our expectation, HRT use was associated with a decrease in plasma protein carbonyls. Others have also reported a decrease in plasma protein carbonyl levels as well as other biomarkers of ROS exposure such as isoprostanes, plasma total thiol levels, and lipid peroxides, and an increase in total antioxidant status and reduced sulfhydryl groups among women supplemented with HRT (33–36). Although the mechanisms through which HRT could

reduce ROS are not entirely clear, it has been suggested that upregulation of downstream antioxidant systems, such as an increase in glutathione levels, leads to a net reduction in ROS (36).

Finally, Hispanic race was associated with a decrease in plasma protein carbonyl level. Race-associated differences in pro-oxidant or antioxidant exposures may have contributed to the differences that we observed in plasma protein carbonyls. For example, in our study 53% of Caucasian versus 33% of Hispanic unaffected sisters reported ever smoking. In addition, racial or ethnic differences in diet have previously been shown to impact on serum antioxidant levels. An analysis of NHANES data found that Mexican-Americans had significantly higher levels of some nutritional biomarkers including β-cryptoxanthin and lutein compared with Caucasians (37)

We did not find an association between alcohol consumption and plasma protein carbonyl level. Although ethanol metabolism has been shown to generate free radicals, components of some alcoholic beverages, such as resveratrol in red wine, have antioxidant properties (38). Since our questionnaire did not collect information on the various types of alcohol consumed, we cannot draw firm conclusions about the relationship between plasma protein carbonyls and alcohol consumption.

Cigarette smoke contains free radicals, which have been shown experimentally to increase protein carbonyl content of plasma in a dose-dependent manner (39). In humans, smoking has also been associated with a slight but non-significant increase in oxidized plasma proteins in current versus former or never smokers, however, this increase was not found to be dose-dependent (2,40). We did not detect an association between smoking and plasma protein carbonyls based on never, former, and current smoking status. The lack of association could have resulted from misclassification due to under-reporting of current smoking or exposure to environmental tobacco smoke.

Age-related increases in a variety of biomarkers of ROS exposure were observed in human lens, brain tissue, fibroblasts, and skeletal muscles (41). We found a decrease in mean plasma protein carbonyl levels as age increased; however when HRT users were excluded from the analysis mean plasma protein carbonyl levels were similar for cases and unaffected sisters from 18–59 yrs, and increased in those ≥ 60 yrs (data not shown).

When we stratified the plasma protein carbonyl prediction model by menopausal status we found that race, HRT use, parity, BMI, and age at menarche and were predictive of plasma protein carbonyls but the direction and strength of the effects were not the same in pre-and postmenopausal women. The effects of race and HRT use in the stratified model were consistent with the main model. Parity was negatively associated with plasma protein carbonyl levels in postmenopausal women. Parous women were previously reported to have lower circulating estrogen and prolactin levels and higher sex-hormone-binding globulin when compared with nulliparous women (42). High BMI decreased plasma protein carbonyls in premenopausal women and increased plasma protein carbonyls in postmenopausal women. Though this relationship could have been observed by chance, it is interesting that high BMI in premenopausal women is protective against breast cancer while high BMI in postmenopausal women increases risk (43). Perhaps part of this risk is mediated through ROS.

There is evidence that menarche, pregnancy, lactation, and menopause are associated with significant and long-term changes in estrogen and other hormone levels. Estrogen, and its metabolites have been shown to generate ROS, and to be strongly associated with another ROS biomarker, F_{2a}-isoprostane, in premenopausal and/or postmenopausal women depending upon the metabolite (44). While we did not find a large mean difference in plasma protein carbonyls between premenopausal and postmenopausal women, there are other changes that occur during

menopause that could influence oxidant status. Changes in ferritin levels, antioxidant enzyme expression, and body fat amount/distribution may either increase or decrease ROS production (17,18). Therefore, it is possible that other factors not included in our analysis impacted on plasma protein carbonyl levels in postmenopausal women. In fact, our stratified model demonstrates that the predictors of plasma protein carbonyls are different depending on menopausal status.

Another aim was to investigate whether oxidative damage was associated with breast cancer in those with a family history. Plasma protein carbonyl levels were also associated with an increase in breast cancer risk, an increase that was significant only in the 3rd quartile. Individuals with the highest level of plasma protein carbonyls had a slightly attenuated risk of breast cancer. This result may reflect a saturation effect or could be due to other attributes of the unaffected sisters that promote health despite having high levels of ROS. Our finding is in agreement with a recent publication from our laboratory that describes an increase in breast cancer risk associated with plasma protein carbonyl levels in a population-based case-control study of breast cancer in women on Long Island, NY (2). The strength of the association was higher in the present study, likely because we used a family design, the conditional analysis of which would provide better control for unmeasured genetic or environmental variables. Elevated plasma protein carbonyls have also been observed in bladder, and lung cancer (45, 46).

There were several limitations to the current study. First, our breast cancer cases were prevalent and biospecimens were collected months to years after diagnosis. Thus, the plasma protein carbonyl biomarker could have been impacted upon by factors associated with the disease or treatment. However, this limitation is not exclusive to our study, as most of the population-based studies that analyze oxidative stress markers are also case-control designs with cases diagnosed within 6–12 months of study enrollment. Our analysis indicated that there was no impact of treatment or disease on the plasma protein carbonyl biomarker. However, replication in a prospective study is necessary. Finally, we were primarily concerned with reproductive factors and did not include other potentially important variables such as exercise and diet-related variables in the analysis.

Our study also had several strengths. First, this study focused on the association of a potentially modifiable risk factor among women with a family history of breast cancer. Since relatively little is known about familial aggregation of breast cancer this data fills an important niche. Second, our study was well designed to investigate factors that influence oxidative stress levels since our questionnaire data was collected concurrently with biospecimen donation, thereby providing accurate and timely exposure information.

In sum, we found that plasma protein carbonyls can be modified by several factors including BMI, parity, and HRT use. Although we expected to detect associations between plasma protein carbonyls and other factors known to induce oxidative stress such as cigarette smoking and alcohol consumption, exposure misclassification may have hindered detection of these relationships. Finally, our data suggest that oxidative damage is a risk factor for breast cancer even among women with a family history.

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Table 1

Log-plasma protein carbonyls by age, BMI, cigarette smoking status, alcohol consumption, age at menarche, oral contraceptive use, parity, age at first birth, menopausal status, and HRT use in breast cancer cases and their unaffected sisters.

		Cases			Controls		
Risk Factor		**N**	**Log-Carbonyl**	**P**	**N**	**Log-Carbonyl**	**P**
Age (years)	18–39	60	2.60		90	2.58	
	40–49	111	2.59		138	2.56	
	50–59	79	2.57		88	2.50	
	≥60	37	2.63	0.88	42	2.61	0.07
BMI (kg/m2)	< 22.0	71	2.55		88	2.58	
	22.0–24.3	69	2.60		84	2.52	
	24.4–27.9	73	2.53		91	2.54	
	≥28.0	72	2.66	0.09	90	2.54	0.47
Cigarette Smoking Status	Never	160	2.58		204	2.55	
	Former	113	2.61		110	2.56	
	Current	14	2.45	0.21	44	2.52	0.76
Regular Alcohol Consumption	No	157	2.59		201	2.55	
	Yes	128	2.59	0.98	156	2.56	0.70
Age at Menarche	≤11	57	2.65		84	2.60	
	12	72	2.60		82	2.61	
	13	79	2.56		107	2.53	
	14	55	2.57		41	2.47	
	>14	24	2.53	0.49	44	2.48	0.01
Oral Contraceptive Use[†]	Nonusers	30	2.62		80	2.56	
	Users	75	2.61	0.90	158	2.57	0.98
Parity	0	64	2.51		73	2.59	

		Cases			Controls		
Risk Factor		**N**	**Log-Carbonyl**	**P**	**N**	**Log-Carbonyl**	**P**
	1	37	2.66		50	2.59	
	2	128	2.61		133	2.53	
	3	44	2.55		68	2.52	
	≥4	14	2.58	0.13	34	2.56	0.40
Age at First Birth	<22	29	2.65		47	2.44	
	22–23	27	2.54		44	2.51	
	24–26	45	2.62		65	2.54	
	27–30	64	2.60		69	2.60	
	≥31	58	2.63	0.68	57	2.58	0.03
Menopausal Status	Pre	103	2.61		236	2.57	
	Post	174	2.58	0.49	104	2.53	0.22
HRT[‡]	Never	136	2.58		58	2.59	
	Ever	38	2.58	0.92	43	2.43	0.01
Race	Caucasian	181	2.64		211	2.60	
	Hispanic	69	2.49		103	2.47	
	Other	37	2.53	0.001	44	2.54	0.0003

[†]Premenopausal women

[‡]Postmenopausal women

Table 2

Univariate and multivariate estimates of the association among age, BMI, cigarette smoking, alcohol consumption, age at menarche, oral contraceptive use, parity, menopausal status, HRT use and race and the log of plasma protein carbonyl levels among unaffected sisters.

Risk Factor		β (95% CI)*	β (95% CI)** n=326	β (95% CI)*** n=344
Age (years)	18–39	Reference	Reference	
	40–49	−0.02 (−0.09, 0.05)	−0.03 (−0.10,0.05)	
	50–59	−0.09 (−0.17, −0.01)	−0.07 (−0.04, 0.17)	
	≥60	0.03 (−0.07, 0.13)	−0.01 (−0.16, 0.14)	
BMI (kg/m2)	< 22.0	Reference	Reference	
	22.0–24.3	−0.07 (−0.15, 0.01)	0.06 (−0.14, 0.03)	
	24.4–27.9	−0.04 (−0.12, 0.04)	−0.03 (−0.11, 0.05)	
	≥28.0	−0.05 (−0.13, 0.03)	−0.01 (−0.10, 0.07)	
Cigarette Smoking Status	Never	Reference	Reference	
	Ever	−0.004(−0.06,0.05)	−0.002 (−0.07, 0.06)	
Regular Alcohol Consumption	No	Reference	Reference	
	Yes	0.01 (−0.05,0.07)	−0.01 (−0.07,0.06)	
Age at Menarche	<13	Reference	Reference	Reference
	≥13	−0.10(−0.15, −0.04) ‡	−0.07 (−0.13, −0.01) †	−0.08 (−0.14, −0.03) †
Oral Contraceptive Use	Nonusers	Reference	Reference	
	Users	−0.03 (−0.09,0.03)	−0.02 (−0.08, 0.05)	
Parity	Nulliparous	Reference	Reference	
	Parous	−0.04 (−0.11,0.03)	−0.02(−0.09, 0.06)	
Menopausal Status	Pre	Reference	Reference	
	Post	−0.04 (−0.10, 0.02)	0.04 (−0.07, 0.15)	
HRT	Nonusers	Reference	Reference	Reference
	Users	−0.16 (−0.23, −0.08)‡	−0.15 (−0.25, −0.05)‡	−0.14 (−0.21, −0.06)‡
Race	Caucasian	Reference	Reference	Reference
	Hispanic	−0.13 (−0.19, −0.07)‡	−0.15 (−0.22, −0.07)	−0.14 (−0.20, −0.07) †
	Other	−0.06 (−0.14, 0.03)	−0.09 (−0.19,0.01)	−0.06 (−0.14, 0.03)

* Univariate, Age n=358, BMI n=353, Smoking status n= 358, Alcohol consumption n=357, Median age at menarche n=358, Oral contraceptive use n=356, Parity n=358, Menopausal status n=340, HRT use n=345, Race n=358.

** Saturated Model

*** Best Model

† p<0.01

‡ p<0.001

Table 3

Multivariate estimates of the association among BMI, cigarette smoking status, alcohol consumption, age at menarche, oral contraceptive use, parity, HRT use and race and the log of plasma protein carbonyls in unaffected siblings stratified by menopausal status.

Risk Factor		Premenopausal			Postmenopausal		
		β(95% CI)*	β (95% CI)**	P	β (95% CI)*	β (95% CI)**	P
BMI (kg/m^2)	< 22.0	Reference	Reference		Reference	Reference	
	22.0–24.3	−0.10 (−0.19, −0.01)	−0.08 (−0.17,0.01)	0.09	−0.02 (−0.79, 0.16)	0.004 (−0.16, 0.17)	0.96
	24.4–27.9	−0.08 (−0.17, 0.02)	−0.05 (−0.14, 0.03)	0.25	0.05 (−0.12, 0.22)	0.04 (−0.11,0.20)	0.58
	≥28.0	−0.11 (−0.21, −0.02)	−0.10 (−0.20, −0.01)	0.03	0.08 (−0.08, 0.24)	0.19 (0.04, 0.35)	0.02
Cigarette Smoking Status	Never	Reference			Reference		
	Ever	0.01 (−0.06, 0.08)			0.01 (−0.11, 0.12)		
Regular Alcohol	No	Reference			Reference		
Consumption	Yes	0.03 (−0.04, 0.10)			−0.03 (−0.16, 0.10)		
Age at Menarche	<13	Reference	Reference		Reference	Reference	
	≥13	−0.10 (−0.17, −0.03)	−0.10 (−0.17, −0.03)	0.005	−0.10 (−0.22, 0.01)	−0.02(−0.13, 0.10)	0.76
Oral Contraceptive Use	Nonusers	Reference			Reference		
	Users	−0.01 (−0.06, 0.08)			−0.09 (−0.20, 0.03)		
Parity	Nulliparous	Reference	Reference		Reference	Reference	
	Parous	−0.002 (−0.08, 0.08)	0.04 (−0.05,0.12)	0.39	−0.15 (−0.30, 0.001)	−0.19(−0.34, −0.04)	0.01
HRT	Nonusers	Reference	Reference		Reference	Reference	
	Users	−0.24 (−0.46, −0.03)	−0.21 (−0.43, −0.004)	0.05	−0.16 (−0.27, −0.05)	−0.17(−0.28, −0.06)	0.004
Race	Caucasian	Reference	Reference		Reference	Reference	
	Hispanic	−0.10 (−0.18, −0.03)	−0.10 (−0.18, −0.02)	0.01	−0.23 (−0.36, −0.11)	−0.27(−0.40, −0.14)	<0.0001
	Other	−0.07 (−0.18, 0.03)	−0.07 (−0.18, 0.04)	0.19	−0.03 (−0.19, 0.14)	−0.13(−0.30,0.03)	0.12

*univariate

**Best Model, premenopausal n=227; postmenopausal n = 100

Table 4

Adjusted odds ratios and 95% confidence intervals for breast cancer associated with plasma protein carbonyls

Protein Carbonyl	Cases n (%)	Controls n (%)	OR * (95% CI)	OR ** (95% CI)
≤ 11.0	52 (20)	80 (25)	Reference	Reference
11.1–13.0	54 (20)	78 (24)	1.18 (0.67–2.06)	1.43 (0.77–2.66)
13.1–15.3	84 (32)	83 (25)	2.04 (1.08–3.88)	2.36 (1.14–4.89)
>15.3	76 (28)	85 (26)	1.95 (0.96–3.94)	1.87 (0.83–4.18)
Continuous	266	326	1.80 (0.75–4.32) β= 0.58	1.99 (0.74–5.36) β= 0.69

* Adjusted for age, $p_{trend} = 0.03$

** Adjusted for age at menarche, menopausal status, $p_{trend} = 0.11$