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## PREMENOPAUSAL PLASMA CAROTENOIDS, FLUORESCENT OXIDATION PRODUCTS AND SUBSEQUENT BREAST CANCER RISK IN THE NURSES' HEALTH STUDIES

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### Abstract

**Purpose**—High levels of circulating carotenoids are hypothesized to reduce breast cancer risk, potentially due to their antioxidant properties. However, little is known about the relationship between carotenoid exposure earlier in life and risk.

**Methods**—We examined associations of premenopausal plasma carotenoids and markers of oxidative stress and risk of breast cancer among 1,179 case-control pairs in the Nurses' Health Study (NHS) and NHSII. Levels of  $\alpha$ - and  $\beta$ -carotene,  $\beta$ -cryptoxanthin, lycopene and lutein/zeaxanthin were quantified by high-performance liquid chromatography. Three fluorescent oxidation products (FIOP\_360, FIOP\_320, FIOP\_400) were measured in a subset of participants by spectrofluorometry. Multivariate conditional logistic regression was used to estimate odds ratios (OR) and 95% confidence intervals (CI) for breast cancer by quartile, as well as *P*-values for tests of linear trend. We additionally examined whether 45 single nucleotide polymorphisms (SNPs) in five genes involved in oxidative and antioxidative processes or carotenoid availability were associated with risk.

**Results**—Carotenoid measures were not inversely associated with breast cancer risk. No differences by estrogen receptor status were observed, though some inverse associations were

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observed among women postmenopausal at diagnosis. Plasma FLOP levels were not positively associated with risk, and suggestive inverse associations with FLOP\_320 and FLOP\_360 were observed. Several SNPs were associated with carotenoid levels, and a small number were suggestively associated with breast cancer risk. We observed evidence of interactions between some SNPs and carotenoid levels on risk.

**Conclusion**—We did not observe consistent associations between circulating levels of premenopausal carotenoids or FLOP levels and breast cancer risk.

### Keywords

breast cancer; fluorescent oxidation products; carotenoids; oxidative stress

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## INTRODUCTION

The role of dietary factors in breast cancer etiology has attracted substantial interest. Specifically, diets rich in fruits and vegetables have been hypothesized to reduce cancer risk, though results from several large-scale epidemiologic studies of breast cancer have been inconclusive.<sup>1–5</sup> Despite these equivocal findings, it remains biologically plausible that some components of fruits and vegetables have anti-carcinogenic effects. One class of compounds that has attracted considerable interest is carotenoids, fat-soluble nutrients responsible for the coloring of many red, orange, and dark green fruits and vegetables. Some epidemiologic studies of carotenoids and breast cancer risk have suggested a modest protective effect of at least some carotenoids, with stronger results typically seen in studies examining circulating biomarkers than those relying on measures derived from recalled dietary data.<sup>6–16</sup> Most recently, our pooled analysis (n=3,055 cases) reported significant inverse associations between circulating  $\alpha$ -carotene,  $\beta$ -carotene, lycopene, lutein/zeaxanthin and total carotenoids levels and breast cancer risk, with relative risks (RRs) (top vs. bottom quintiles) of 0.78–0.87.<sup>17</sup> The associations with carotenoids may vary by breast cancer subtype, with a stronger protective effect observed for estrogen receptor negative (ER–), compared to estrogen receptor positive (ER+) tumors.<sup>17,18</sup>

The potential mechanisms through which carotenoids may influence breast cancer risk are numerous. Carotenoids may promote cellular differentiation and inhibit tumorigenesis and proliferation, either directly or through the conversion of the provitamin A carotenoids ( $\alpha$ -carotene,  $\beta$ -carotene and  $\beta$ -cryptoxanthin) to retinol. Further, carotenoids may act via their antioxidant properties. At high levels, reactive oxygen species (ROS) produced by endogenous and exogenous processes may induce potentially carcinogenic DNA damage and gene expression modification.<sup>18</sup> Antioxidants, including carotenoids and several endogenous enzymes, may inhibit carcinogenesis by neutralizing these reactive molecules, thereby reducing the burden of oxidative stress. To date, largely null results have been reported from a small number of prospective studies of various oxidative stress biomarkers and breast cancer risk.<sup>19,20</sup> including our analysis in the Nurses' Health Study (NHS),<sup>21</sup> which examined plasma levels of three fluorescent oxidation products (FLOP\_360, FLOP\_320, FLOP\_400). Thus, the roles of oxidative stress and antioxidant activity in breast cancer etiology are not clear.

Presently, little is known about the relationship between carotenoid exposure earlier in life and breast cancer risk. In our pooled analysis,<sup>17</sup> which included data from nearly all published prospective studies of circulating carotenoids and breast cancer, 67% of participants were postmenopausal when biomarkers were measured. Here, we examined the relationship between premenopausal plasma carotenoid and fluorescent oxidation products levels and subsequent breast cancer risk in the NHS and NHSII. To maximize power, we included premenopausal women from previously published data sets in the NHS.<sup>10,21</sup> We also explored interactions between carotenoids and several single-nucleotide polymorphisms (SNPs) in genes hypothesized to predict carotenoid availability or influence ROS formation and neutralization among NHSII participants.

## METHODS

### Study population

The NHS and NHSII are ongoing prospective cohorts, initiated in 1976 and 1989, respectively. The NHS was established among 121,700 registered female nurses, ages 30–55 years; the NHSII was established among 116,430 registered female nurses, ages 25–42 years. At baseline, and on subsequent biennial questionnaires, participants provided updated information about lifestyle factors and medical diagnoses.

### Biospecimen collection

In 1989–1990, 32,826 NHS participants, ages 43–70 years, provided blood samples and responded to a short questionnaire.<sup>22</sup> Participants had their blood drawn and samples were returned to the laboratory, with an icepack, via overnight courier, with 97% arriving within 26 hours of blood draw. Upon arrival, blood samples were centrifuged to isolate plasma, buffy coat, and red blood cell components.

The NHSII blood collection occurred in 1996–99, when 29,611 participants who were cancer-free and ages 32–54 years provided blood samples and answered a short questionnaire.<sup>23</sup> Of these women, 18,521 who were premenopausal and had not been pregnant, breastfed, or used oral contraceptives in the 6 months preceding collection provided samples timed within their menstrual cycle. Participants collected follicular phase blood samples during days 3–5 of their menstrual cycle, and blood and urine samples during the luteal phase, 7–9 days before the anticipated start of their next cycle. Participants were instructed to separate follicular phase via pipette and freeze the plasma component before returning it to the laboratory. Samples were stored with an ice pack and returned to the laboratory via overnight courier; 93% of samples were received within 26 hours of collection. Upon arrival at the laboratory, luteal phase samples were processed similarly to NHS samples. Blood samples from both cohorts were aliquoted into cryotubes and stored in liquid nitrogen freezers.

### NHS case-control selection

Eligible breast cancer cases who were premenopausal at blood draw and diagnosed before June 1, 2010 were selected from a previous analysis of carotenoids and breast cancer risk.<sup>17,24</sup> A total of 498 cases were reported on biennial questionnaires and subsequently

confirmed through medical record review (n=495) or verbal confirmation (n=3). Cases were matched (1:1) to controls age ( $\pm 2$  years), month ( $\pm 1$ ) and time of day of blood draw ( $\pm 2$  hours), fasting status (<2, 2–4, 5–7, 8–11, 12+ hours), and menopausal status at blood draw and diagnosis. A total of 466 pairs were included in carotenoid analyses. FLOP assays were performed for cases diagnosed before June 1, 2006 (383 pairs).

### NHSII case-control selection

Breast cancer cases were participants premenopausal at blood collection and diagnosed before June 1, 2011. Cases were matched to controls on age ( $\pm 2$  years), race, menopausal status at blood draw and diagnosis, luteal day ( $\pm 1$  day for timed samples), month ( $\pm 2$ ), time of day ( $\pm 2$  hours), and fasting status (<2, 2–4, 5–7, 8–11, 12+ hours) of blood draw. Plasma biomarker analyses used one matched case per control. The carotenoids analyses include a total of 677 pairs. FLOP assays were conducted on cases diagnosed prior to June 1, 2007 (401 pairs). For genetic analyses, cases were diagnosed before June 1, 2009, and matched to controls in either a 1:1 or 1:2 frequency (613 cases, 1066 controls). Genetic analyses were restricted to Caucasian participants to reduce the possibility of population stratification.

### Laboratory methods

Case-control pairs were assayed together in random order by technicians blinded to case status. Plasma carotenoids were assayed at the Micronutrient Analysis Laboratory at the Harvard School of Public Health Department of Nutrition using the reversed-phase, high-performance liquid chromatography method previously described by El-Sohemy *et al.*<sup>25</sup> Levels of  $\alpha$ -carotene,  $\beta$ -carotene,  $\beta$ -cryptoxanthin, lutein/zeaxanthin and lycopene were assessed, and summed to calculate total carotenoids. Carotenoids were assayed in 11 batches (9 in NHS; 2 in NHSII); coefficients of variation (CVs) were 15%, with the exception of three batches of  $\alpha$ -carotene, two batches of  $\beta$ -carotene and 1 batch each of  $\beta$ -cryptoxanthin and lycopene, which each had CVs of 20%; 1 batch of  $\beta$ -cryptoxanthin had CV=22%.

FLOP levels were assayed at the University of Cincinnati using a spectrofluorometry method previously described by Wu *et al.*<sup>26</sup> Concentrations of three individual FLOPs were assessed: FLOP\_360 (excitation 360 nm, emission 420 nm), FLOP\_320 (excitation 320 nm, emission 420 nm), and FLOP\_400 (excitation 400 nm, emission 475 nm). FLOPs were assayed in 3 batches (2 in NHS; 1 in NHSII); CVs for all FLOP measures were 20%.

Evidence of batch-to-batch variability in biomarker assays was observed; therefore, NHS carotenoid measurements were recalibrated using replicates of participant samples.<sup>17</sup> FLOP measurements from both cohorts were recalibrated to an average batch using methods previously described by Rosner *et al.*<sup>21,27</sup> The number of participants included in analyses varies by biomarker due to laboratory difficulties that resulted in missing values for some participants.

### SNP selection and genotyping

The Tagger algorithm<sup>28</sup> in the Haploview program<sup>29</sup> and dense genotyping data from the HapMap Release 28 CEU panel was used to select a total of 45 SNPs in five genes (*CAT*, *GPX1*, *SOD2*, *MPO*, *BCMO1*) that were of interest due to their biologic roles in oxidative

and antioxidative processes.<sup>30,31</sup> SNPs were selected to capture variation with a coefficient of determination ( $R^2$ ) >0.8 in a segment spanning 20 kb upstream and 10 kb downstream of each gene. We selected tagging SNPs with a minor allele frequency >5% in the reference panel, including the two candidate SNPs rs1050450 and rs4880, in *GPXI* and *SOD2*, respectively. We included two additional *BCMO1* SNPs, rs1641417 and rs7501331, that were previously genotyped in NHSII.<sup>32</sup>

Genotyping was performed at the Dana Farber/Harvard Cancer Center High Throughput Polymorphism Detection Core, with Taqman OpenArray SNP Genotyping Platform. All case-control pairs were processed in the same batch by technicians blinded to case status, and duplicate samples were included across batches as quality controls. Call rates for all SNPs were >97%.

### Covariates

Covariate information was obtained from biennial questionnaires (height, age at menarche, parity, age at first birth, family history of breast cancer, history of benign breast disease) and blood collection questionnaires (weight, smoking status).

### Statistical analyses

Statistical outliers in plasma biomarkers were identified and removed using the extreme Studentized deviate many-outlier procedure;<sup>33</sup> the number of outliers ranged from 2 ( $\alpha$ -carotene) to 56 (FLOP\_320). Quartile cutpoints were determined using distributions among the controls; results were similar when quintiles were examined. Linear tests for trend were conducted by modeling quartile medians continuously and evaluating the Wald statistic. We additionally cross-classified total carotenoid and individual FLOPs by dichotomizing each biomarker at its median.

We pooled NHS and NHSII data and used conditional logistic regression models adjusted for several breast cancer risk factors to estimate RRs and corresponding 95% confidence intervals (95% CI). To examine variation in associations across subgroups, we evaluated the significance of interaction terms between the ordinal median biomarker variable and binary variables for cohort, BMI, smoking status, and menopausal status at diagnosis using Wald tests. We used polytomous logistic regression<sup>34</sup> to evaluate whether associations varied by ER status, which was determined by medical record review.

Tests for deviation from Hardy-Weinberg equilibrium were conducted among controls for all SNPs using the Pearson's goodness of fit test with a cutoff of 0.01. For all genetic analyses, SNP effects were assumed to be additive, and the number of minor alleles was modeled as an ordinal variable (0, 1, 2).

Associations between SNPs and plasma carotenoid levels were evaluated among controls using age-adjusted generalized linear models. Tests for trend were conducted by modeling number of minor alleles continuously and calculating the Wald statistic. To examine associations between SNPs and breast cancer risk, per-allele RRs were estimated using age-adjusted unconditional logistic regression models. Interactions between SNPs and plasma carotenoid levels on risk were also evaluated using age-adjusted unconditional logistic

regression. Plasma carotenoid levels were dichotomized at their medians, and per-allele RRs were calculated separately by carotenoid level. Likelihood ratio tests were used to calculate *P*-values for interaction terms that were the product of number of minor alleles and continuous carotenoid levels. All *P*-values were two-sided and tests of significance were performed at the  $\alpha=0.05$  level. All analyses were conducted using SAS v. 9.2 (SAS Institute, Cary, NC).

## RESULTS

Overall, 1,143 matched case-control pairs across both cohorts had carotenoid data and 784 pairs had FLOP data; a total of 748 pairs had both carotenoid and FLOP data. Genotype data were available for 597 cases and 1,048 controls, of which 536 cases and 531 controls also had carotenoids measured. Among 1,179 cases and matched controls in the carotenoid or FLOP analyses, average age at blood draw was 46 years (NHS: 48 years; NHSII: 43 years) (Table 1). Cases and controls were similar with regard to most characteristics, though cases were more likely than controls to report a family history of breast cancer and a personal history of benign breast disease. 44% of cases were postmenopausal at diagnosis.

Individual carotenoids were significantly positively correlated with one another; Spearman correlations ranged from  $r=0.28$  ( $\alpha$ -carotene and lycopene) to 0.77 ( $\alpha$ -carotene and  $\beta$ -carotene). Significant correlations also were observed among FLOP measures (FLOP\_360 and FLOP\_320,  $r=0.58$ ; FLOP\_360 and FLOP\_400,  $r=0.73$ ; FLOP\_320 and FLOP\_400,  $r=0.49$ ). Weak, but significant, correlations ranging from  $r=0.10$  ( $\alpha$ -carotene) to 0.23 (lycopene) were observed between FLOP\_360 and carotenoids; FLOP\_320 and FLOP\_360 were not correlated with carotenoids.

Plasma carotenoids were not inversely associated with breast cancer risk overall (Table 2) (e.g., total carotenoids top vs. bottom quartile RR=0.99, 95% CI (0.77–1.28),  $p_{\text{trend}}=0.67$ ). Similarly, FLOP levels were not positively associated with risk (Table 3), though we observed some evidence of inverse associations with FLOP\_360 (top vs. bottom quartile RR=0.68, 95% CI: 0.50, 0.95;  $p_{\text{trend}}=0.07$ ) and FLOP\_320 (RR=0.76, 95% CI: 0.55, 1.06;  $p_{\text{trend}}=0.08$ ).

When we stratified by menopausal status at diagnosis, significant interactions were detected for  $\alpha$ -carotene,  $\beta$ -carotene and total carotenoids ( $p_{\text{interaction}}=0.03$ – $0.05$ ) (Table 4). While no associations were apparent among women premenopausal at diagnosis, suggested or significant inverse associations between plasma carotenoids and breast cancer risk were observed among women postmenopausal at diagnosis (e.g., lycopene top v. bottom quartile RR (95% CI): premenopausal at diagnosis=1.00 (0.70, 1.42),  $p_{\text{trend}}=0.99$ ; postmenopausal at diagnosis=0.66 (0.45, 0.96),  $p_{\text{trend}}=0.02$ ;  $p_{\text{heterogeneity}}=0.03$ ). We further explored these results by examining whether age at blood draw or time between blood draw and diagnosis modified the associations. Associations were similar among women diagnosed <50 and  $\geq 50$  (all  $p_{\text{heterogeneity}} \geq 0.19$ ). The association between lycopene and risk appeared stronger among women with longer intervals between blood draw and diagnosis (top v. bottom quartile RR (95% CI): <8 years between draw and diagnosis=1.03 (0.71, 1.48),  $p_{\text{trend}}=0.58$ ; 8 years between draw and diagnosis=0.63 (0.45, 0.90),  $p_{\text{trend}}=0.01$ ;  $p_{\text{heterogeneity}}=0.02$ ),

though the associations with other carotenoids did not significantly differ by time to diagnosis (all  $p_{\text{heterogeneity}} > 0.19$ ). BMI, smoking, and cohort did not modify the relationship of carotenoids and FIOPs with risk (data not shown).

While associations between carotenoids and breast cancer risk did not vary by ER status (all  $p_{\text{heterogeneity}} > 0.35$ ), FIOp\_400 was more strongly inversely associated with risk of ER-tumors (top v. bottom quartile RR (95% CI): ER- tumors=0.55 (0.29, 1.03),  $p_{\text{trend}}=0.07$ ; ER + tumors=1.15, (0.79, 1.67),  $p_{\text{trend}}=0.75$   $p_{\text{heterogeneity}}=0.04$ ) (Suppl. Table 1). When cross-classifying FIOPs and total carotenoid levels, we observed no evidence that women with low carotenoid and high FIOp levels were at increased risk (data not shown).

Genotype frequencies were in Hardy Weinberg equilibrium among controls in all but one SNP (rs17080528); inspection of the cluster plot indicated no evidence of genotyping error and it was included in analyses. In log-additive models, minor alleles of two *CAT* SNPs were associated with increased breast cancer risk: rs11032686 (per-allele OR=1.31, 95% CI: 1.02, 1.67) and rs7947841 (per-allele OR=1.36, 95% CI: 1.07, 1.74); no other SNPs were associated with risk (Suppl. Table 2). Several SNPs were associated with carotenoids (Suppl. Table 3), including SNPs in the *BCMO1* gene, consistent with our previous report.<sup>35</sup>

Associations between *CAT*, *SOD2*, and *GPX1* SNPs and breast cancer risk varied by plasma carotenoid level (Table 5). For example, an increasing number of A alleles for *CAT* SNP rs11032686 appeared to confer an increased risk among women with high levels of  $\beta$ -cryptoxanthin (per-allele OR (95% CI): low  $\beta$ -cryptoxanthin=0.99 (0.65, 1.52); high  $\beta$ -cryptoxanthin=1.98 (1.26, 3.10);  $p_{\text{heterogeneity}}=0.01$ ).

## DISCUSSION

Overall, we did not find significant associations between premenopausal plasma carotenoid levels and breast cancer risk in this large nested case-control study. However, higher levels of carotenoids during premenopause, particularly lycopene, were suggestively associated with a lower risk of postmenopausal breast cancer. Results did not differ by ER status. While none of the FIOp biomarkers were positively associated with risk, premenopausal levels of FIOp\_360 and FIOp\_320 were suggestively inversely associated with risk. Lastly, we observed that several SNPs in five genes that influence oxidant and antioxidant status were associated with carotenoid levels, and a small number were suggestively associated with breast cancer risk. Our data additionally suggest that the association between some SNPs and risk are modified by carotenoid levels.

The association between circulating carotenoids and breast cancer risk has been examined previously in several epidemiologic studies, with findings generally suggesting weak or modest protective effects. Although null or largely null findings have been reported in some smaller individual studies,<sup>7,9,12</sup> in our pooled analysis, we observed inverse associations between breast cancer risk and  $\alpha$ -carotene,  $\beta$ -carotene, lutein/zeaxanthin and total carotenoids (top v. bottom quintile RRs=0.78–0.87). The same carotenoids were also significantly inversely associated with risk in a previous analysis in the NHS that included both postmenopausal and premenopausal participants (RRs=0.64–0.76).<sup>10</sup>

To date, ours is the first study to specifically investigate premenopausal carotenoids and subsequent breast cancer risk, and our largely null findings may reflect that our study is capturing a different etiologic period than previous analyses, which have assessed exposure later in life. Interestingly, we observed stronger inverse associations among women postmenopausal at diagnosis. This suggests that the effects of carotenoids on risk may differ by hormones, given the stark differences in hormonal milieu by menopausal status. Though we did not observe differences in associations by ER status, stronger inverse associations have been observed with ER- tumors in large pooled studies of dietary<sup>3,16</sup> and circulating<sup>17</sup> carotenoids, suggesting a stronger association of carotenoids with less hormonally-driven tumors. Alternatively, given their role as antioxidants that may reduce oxidative stress-induced DNA damage,<sup>36</sup> the effects of carotenoids may be more pronounced with increasing time since blood collection, reflecting their importance in preventing cancer initiation rather than disease progression. However, the correlation between time since blood collection and postmenopausal status in our data makes it difficult to disentangle these factors.

We found no evidence of a positive association between FIOP levels and overall breast cancer risk; however, we observed suggestive inverse associations with FIOP\_360 and FIOP\_320. FIOP levels are hypothesized to reflect global levels of oxidation, and have been associated with exposures related to oxidative stress, including cigarette smoking, hypertension, and total cholesterol,<sup>37</sup> and with increased risk of coronary heart disease.<sup>38,39</sup> Our prior analysis of FIOP measurements and breast cancer risk in the NHS generally suggested no association, though there was evidence that persistently high levels of FIOP\_320 were associated with increased risk.<sup>21</sup> FIOP measurements quantify interactions between oxidation products with lipids, proteins, carbohydrates, and DNA; it is possible that more specific markers of oxidative stress might have greater relevance to breast cancer etiology, though no associations were observed between prospectively measured urinary markers of lipid peroxidation and breast cancer risk in the Shanghai Women's Health Study.<sup>19,20</sup>

Endogenous enzymatic factors may also be involved in defense against oxidative stress. Proteins encoded by *CAT*, *GPXI* and *SOD2* may reduce the burden of oxidative stress through neutralization of ROS into compounds with less carcinogenic potential, while the MPO enzyme contributes to oxidative stress through formation of oxidants. Previous findings suggest that the effect of *GPXI* SNP rs4880 on breast,<sup>40</sup> cervical<sup>41</sup> and prostate cancer<sup>42</sup> risk may be modified by antioxidant status. We did not observe interactions between rs4880 and carotenoids on breast cancer risk, but found interactions with four other *GPXI* SNPs. We additionally noted interactions between carotenoids, several *CAT* SNPs and breast cancer risk, and an increased risk with two other *CAT* SNPs. At least one functional polymorphism in the promoter region of this gene (rs1001179) has been shown to decrease catalase activity,<sup>43–45</sup> which could hypothetically affect cancer risk. However, results from epidemiologic studies have been inconsistent,<sup>46,47</sup> and our results did not indicate evidence of any significant association with this SNP.

BCMO1 plays a central role in the conversion of provitamin A carotenoids to retinal, and activity of this protein is reduced among women with copies of the rs6564851 minor allele.<sup>48</sup> Consistent with previous findings from the NHS and other studies,<sup>35,48–50</sup> a



positive association between the *G* allele of rs6564851 have been observed with  $\alpha$ -carotene and  $\beta$ -carotene levels, and an inverse association with lutein/zeaxanthin.<sup>35,50</sup> Positive associations between rs12934922 variants and plasma levels of some carotenoids have also been reported,<sup>35,49</sup> though we did not observe significant associations with this SNP. We also observed several significant associations between SNPs in *CAT*, *GPX*, *SOD2* and *MPO* and carotenoids; to our knowledge, these associations have not previously been studied.

There are several limitations to our study. First, carotenoids and FIOPs were assessed at a single time point. However, among postmenopausal women in the NHS, plasma carotenoids had good reproducibility over a three-year period (intraclass correlation (ICC): 0.73–0.80),<sup>51</sup> suggesting that one measurement adequately represents longer-term exposure status. FIOF measurements are fairly representative, with comparable ICCs of 0.44–0.70,<sup>38</sup> although 10-year ICCs were notably lower (0.14–0.30).<sup>21</sup> Additionally, we performed a large number of statistical tests, and at least some of significant associations may be due to chance. Because many of these associations had not been previously evaluated, we opted not to adjust for multiple testing; however, these results should be viewed as exploratory and interpreted with caution. Lastly, we cannot rule out the possibility of unmeasured confounding, despite adjusting our analyses for known and suspected confounders.

Overall, we did not observe significant inverse associations between premenopausal levels of circulating carotenoid and subsequent breast cancer risk in this large nested case-control study. Additionally, we did not observe positive associations with circulating markers of oxidative stress, but instead found suggestive inverse associations with two FIOF measurements. Evidence of inverse associations between some carotenoids and risk appeared limited to postmenopausal breast cancer, suggesting some interaction between these exposures and hormonal factors. Although we conducted many tests, our results suggest that genetic variation in *CAT*, *GPX*, *SOD2* and *MPO* genes may impact circulating carotenoid levels and potentially breast cancer risk. Further work is needed to elucidate the complex roles of oxidant and antioxidant factors in breast carcinogenesis.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## References

1. Van Gils CH, et al. Consumption of vegetables and fruits and risk of breast cancer. *JAMA J Am Med Assoc.* 2005; 293:183–193.

2. Lissowska J, et al. Intake of fruits, and vegetables in relation to breast cancer risk by hormone receptor status. *Breast Cancer Res Treat.* 2008; 107:113–117. [PubMed: 17318377]
3. Jung S, et al. Fruit and vegetable intake and risk of breast cancer by hormone receptor status. *J Natl Cancer Inst.* 2013; 105:219–236. [PubMed: 23349252]
4. Smith-Warner SA, et al. Intake of fruits and vegetables and risk of breast cancer: a pooled analysis of cohort studies. *JAMA J Am Med Assoc.* 2001; 285:769–776.
5. Aune D, et al. Fruits, vegetables and breast cancer risk: a systematic review and meta-analysis of prospective studies. *Breast Cancer Res Treat.* 2012; 134:479–493. [PubMed: 22706630]
6. Toniolo P, et al. Serum carotenoids and breast cancer. *Am J Epidemiol.* 2001; 153:1142–1147. [PubMed: 11415946]
7. Dorjgochoo T, et al. Plasma carotenoids, tocopherols, retinol and breast cancer risk: results from the Shanghai Women Health Study (SWHS). *Breast Cancer Res Treat.* 2009; 117:381–389. [PubMed: 19096929]
8. Kabat GC, et al. Longitudinal study of serum carotenoid, retinol, and tocopherol concentrations in relation to breast cancer risk among postmenopausal women. *Am J Clin Nutr.* 2009; 90:162–169. [PubMed: 19474140]
9. Epplein M, et al. Plasma carotenoids, retinol, and tocopherols and postmenopausal breast cancer risk in the Multiethnic Cohort Study: a nested case-control study. *Breast Cancer Res BCR.* 2009; 11:R49.
10. Tamimi RM, et al. Plasma carotenoids, retinol, and tocopherols and risk of breast cancer. *Am J Epidemiol.* 2005; 161:153–160. [PubMed: 15632265]
11. Sato R, et al. Prospective study of carotenoids, tocopherols, and retinoid concentrations and the risk of breast cancer. *Cancer Epidemiol Biomark Prev Publ Am Assoc Cancer Res Cosponsored Am Soc Prev Oncol.* 2002; 11:451–457.
12. Maillard V, et al. Serum carotenoid, tocopherol and retinol concentrations and breast cancer risk in the E3N-EPIC study. *Int J Cancer J Int Cancer.* 2010; 127:1188–1196.
13. Aune D, et al. Dietary compared with blood concentrations of carotenoids and breast cancer risk: a systematic review and meta-analysis of prospective studies. *Am J Clin Nutr.* 2012; 96:356–373. [PubMed: 22760559]
14. Mignone LI, et al. Dietary carotenoids and the risk of invasive breast cancer. *Int J Cancer J Int Cancer.* 2009; 124:2929–2937.
15. Nagel G, et al. Dietary beta-carotene, vitamin C and E intake and breast cancer risk in the European Prospective Investigation into Cancer and Nutrition (EPIC). *Breast Cancer Res Treat.* 2010; 119:753–765. [PubMed: 19565333]
16. Zhang X, et al. Carotenoid intakes and risk of breast cancer defined by estrogen receptor and progesterone receptor status: a pooled analysis of 18 prospective cohort studies. *Am J Clin Nutr.* 2012; 95:713–725. [PubMed: 22277553]
17. Eliassen AH, et al. Circulating carotenoids and risk of breast cancer: pooled analysis of eight prospective studies. *J Natl Cancer Inst.* 2012; 104:1905–1916. [PubMed: 23221879]
18. Klaunig JE, Kamendulis LM. The role of oxidative stress in carcinogenesis. *Annu Rev Pharmacol Toxicol.* 2004; 44:239–267. [PubMed: 14744246]
19. Dai Q, et al. Oxidative stress, obesity, and breast cancer risk: results from the Shanghai Women's Health Study. *J Clin Oncol Off J Am Soc Clin Oncol.* 2009; 27:2482–2488.
20. Lee KH, et al. Breast cancer and urinary biomarkers of polycyclic aromatic hydrocarbon and oxidative stress in the Shanghai Women's Health Study. *Cancer Epidemiol Biomark Prev Publ Am Assoc Cancer Res Cosponsored Am Soc Prev Oncol.* 2010; 19:877–883.
21. Fortner RT, Tworoger SS, Wu T, Eliassen AH. Plasma fluorescent oxidation products and breast cancer risk: repeated measures in the Nurses' Health Study. *Breast Cancer Res Treat.* 2013; 141:307–316. [PubMed: 24046001]
22. Hankinson SE, et al. Alcohol, height, and adiposity in relation to estrogen and prolactin levels in postmenopausal women. *J Natl Cancer Inst.* 1995; 87:1297–1302. [PubMed: 7658481]
23. Tworoger SS, Sluss P, Hankinson SE. Association between plasma prolactin concentrations and risk of breast cancer among predominately premenopausal women. *Cancer Res.* 2006; 66:2476–2482. [PubMed: 16489055]

24. Eliassen AH, et al. Plasma carotenoids and risk of breast cancer over 20 years of follow-up. *Am J Clin Nutr*. 2002; 76:172–179. [PubMed: 12081831]
25. El-Soheemy A, et al. Individual carotenoid concentrations in adipose tissue and plasma as biomarkers of dietary intake. *Am J Clin Nutr*. 2002; 76:172–179. [PubMed: 12081831]
26. Wu T, Rifai N, Roberts LJ 2nd, Willett WC, Rimm EB. Stability of measurements of biomarkers of oxidative stress in blood over 36 hours. *Cancer Epidemiol Biomark Prev Publ Am Assoc Cancer Res Cosponsored Am Soc Prev Oncol*. 2004; 13:1399–1402.
27. Rosner B, Cook N, Portman R, Daniels S, Falkner B. Determination of blood pressure percentiles in normal-weight children: some methodological issues. *Am J Epidemiol*. 2008; 167:653–666. [PubMed: 18230679]
28. De Bakker PIW, et al. Efficiency and power in genetic association studies. *Nat Genet*. 2005; 37:1217–1223. [PubMed: 16244653]
29. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinforma Oxf Engl*. 2005; 21:263–265.
30. Ambrosone CB. Oxidants and antioxidants in breast cancer. *Antioxid Redox Signal*. 2000; 2:903–917. [PubMed: 11213491]
31. Lietz G, Lange J, Rimbach G. Molecular and dietary regulation of beta,beta-carotene 15,15'-monooxygenase 1 (BCMO1). *Arch Biochem Biophys*. 2010; 502:8–16. [PubMed: 20599666]
32. Hendrickson SJ, et al. Plasma carotenoid- and retinol-weighted multi-SNP scores and risk of breast cancer in the National Cancer Institute Breast and Prostate Cancer Cohort Consortium. *Cancer Epidemiol Biomark Prev Publ Am Assoc Cancer Res Cosponsored Am Soc Prev Oncol*. 2013; 22:927–936.
33. Rosner B. Percentage Points for a Generalized ESD Many-Outlier Procedure. *Technometrics*. 1983; 25:165–172.
34. Marshall RJ, Chisholm EM. Hypothesis testing in the polychotomous logistic model with an application to detecting gastrointestinal cancer. *Stat Med*. 1985; 4:337–344. [PubMed: 4059720]
35. Hendrickson SJ, et al.  $\beta$ -Carotene 15,15'-monooxygenase 1 single nucleotide polymorphisms in relation to plasma carotenoid and retinol concentrations in women of European descent. *Am J Clin Nutr*. 2012; 96:1379–1389. [PubMed: 23134893]
36. Frei B. Reactive oxygen species and antioxidant vitamins: mechanisms of action. *Am J Med*. 1994; 97:5S–13S. discussion 22S–28S. [PubMed: 8085584]
37. Wu T, Willett WC, Rifai N, Rimm EB. Plasma fluorescent oxidation products as potential markers of oxidative stress for epidemiologic studies. *Am J Epidemiol*. 2007; 166:552–560. [PubMed: 17615091]
38. Jensen MK, et al. Fluorescent oxidation products and risk of coronary heart disease: a prospective study in women. *J Am Heart Assoc*. 2013; 2:e000195. [PubMed: 24103570]
39. Wu T, Rifai N, Willett WC, Rimm EB. Plasma fluorescent oxidation products: independent predictors of coronary heart disease in men. *Am J Epidemiol*. 2007; 166:544–551. [PubMed: 17615090]
40. Wang S, et al. Association between manganese superoxide dismutase (MnSOD) Val-9Ala polymorphism and cancer risk - A meta-analysis. *Eur J Cancer Oxf Engl* 1990. 2009; 45:2874–2881.
41. Tong SY, et al. Functional polymorphism in manganese superoxide dismutase and antioxidant status: their interactions on the risk of cervical intraepithelial neoplasia and cervical cancer. *Gynecol Oncol*. 2009; 115:272–276. [PubMed: 19706356]
42. Mikhak B, et al. Manganese superoxide dismutase (MnSOD) gene polymorphism, interactions with carotenoid levels and prostate cancer risk. *Carcinogenesis*. 2008; 29:2335–2340. [PubMed: 18784358]
43. Ahn J, et al. Associations between catalase phenotype and genotype: modification by epidemiologic factors. *Cancer Epidemiol Biomark Prev Publ Am Assoc Cancer Res Cosponsored Am Soc Prev Oncol*. 2006; 15:1217–1222.
44. Nadif R, et al. Association of CAT polymorphisms with catalase activity and exposure to environmental oxidative stimuli. *Free Radic Res*. 2005; 39:1345–1350. [PubMed: 16298864]

45. Bastaki M, et al. Genotype-activity relationship for Mn-superoxide dismutase, glutathione peroxidase 1 and catalase in humans. *Pharmacogenet Genomics*. 2006; 16:279–286. [PubMed: 16538174]
46. Ahn J, et al. Associations between breast cancer risk and the catalase genotype, fruit and vegetable consumption, and supplement use. *Am J Epidemiol*. 2005; 162:943–952. [PubMed: 16192345]
47. Quick SK, et al. Effect modification by catalase genotype suggests a role for oxidative stress in the association of hormone replacement therapy with postmenopausal breast cancer risk. *Cancer Epidemiol Biomark Prev Publ Am Assoc Cancer Res Cosponsored Am Soc Prev Oncol*. 2008; 17:1082–1087.
48. Lietz G, Oxley A, Leung W, Hesketh J. Single nucleotide polymorphisms upstream from the  $\beta$ -carotene 15,15'-monooxygenase gene influence provitamin A conversion efficiency in female volunteers. *J Nutr*. 2012; 142:161S–5S. [PubMed: 22113863]
49. Leung WC, et al. Two common single nucleotide polymorphisms in the gene encoding beta-carotene 15,15'-monooxygenase alter beta-carotene metabolism in female volunteers. *FASEB J Off Publ Fed Am Soc Exp Biol*. 2009; 23:1041–1053.
50. Ferrucci L, et al. Common variation in the beta-carotene 15,15'-monooxygenase 1 gene affects circulating levels of carotenoids: a genome-wide association study. *Am J Hum Genet*. 2009; 84:123–133. [PubMed: 19185284]
51. Kotsopoulos J, et al. Reproducibility of plasma and urine biomarkers among premenopausal and postmenopausal women from the Nurses' Health Studies. *Cancer Epidemiol Biomark Prev Publ Am Assoc Cancer Res Cosponsored Am Soc Prev Oncol*. 2010; 19:938–946.

**Table 1**

Characteristics of the premenopausal study population at blood draw, Nurses Health Study and Nurses Health Study II<sup>a</sup>

	NHS		NHSII	
	Case (n=498)	Control (n=498)	Case (n=681)	Control (n=681)
Age at blood draw, years	48.3 (3.2)	48.3 (3.2)	43.4 (4.0)	43.6 (3.9)
Age at menarche, years	12.3 (1.3)	12.5 (1.3)	12.4 (1.3)	12.5 (1.4)
BMI at age 18, kg/m <sup>2</sup>	21.1 (2.7)	21.3 (2.8)	20.8 (3.0)	21.0 (2.8)
Height, inches	64.7 (2.4)	64.6 (2.3)	65.3 (2.5)	64.8 (2.6)
BMI at blood draw, kg/m <sup>2</sup>	25.1 (4.3)	25.2 (4.9)	24.8 (4.8)	25.6 (5.4)
Weight change since age 18	10.6 (10.5)	10.5 (11.0)	10.9 (11.0)	12.2 (11.7)
Parity, children	2.6 (1.1)	2.6 (1.2)	1.7 (1.2)	2.0 (1.3)
Age at first birth, years	24.9 (2.9)	24.9 (2.8)	27.3 (4.6)	26.4 (4.5)
Physical activity, MET-hrs/wk	13.7 (22.3)	14.1 (16.8)	17.2 (19.7)	18.2 (23.1)
Alcohol consumption, g/d	4.3 (7.7)	4.5 (7.2)	3.9 (6.7)	3.2 (6.0)
History of benign breast disease, %	58	48	20	13
Family history of breast cancer, %	12	6	18	10
	Median (IQR)		Median (IQR)	
Alpha-carotene, ug/dL	7.1 (4.5–11)	7.2 (4.6–12)	7.0 (4.5–12)	7.2 (4.6–11)
Beta-carotene, ug/dL	23 (15–36)	23 (15–35)	24 (16–38)	23 (15–37)
Beta-cryptoxanthin, ug/dL	10 (7.3–15)	10 (7.6–14)	10 (7.0–14)	10 (7.1–14)
Lycopene, ug/dL	43 (32–53)	43 (33–55)	42 (32–53)	43 (34–55)
Lutein-zeaxanthin, ug/dL	22 (16–28)	21 (16–27)	17 (13–22)	17 (13–22)
Total carotenoids, ug/dL	108 (86–142)	110 (87–139)	107 (83–136)	107 (83–134)
FIOP_360, FI/mL	205 (170–247)	209 (178–252)	210 (173–250)	211 (175–252)
FIOP_320, FI/mL	313 (254–426)	335 (261–451)	338 (278–441)	345 (277–455)
FIOP_400, FI/mL	65 (55–78)	66 (56–79)	64 (53–78)	64 (52–78)

<sup>a</sup>Values are means (SD) or percentages and are standardized to the age distribution of the study population; biomarker measures are not age-adjusted.

Table 2

Relative risks (RR) of breast cancer and 95% confidence intervals (CI) according to quartile of premenopausal carotenoid levels (ug/dL)

	RR	RR 95% CI	RR 95% CI	RR 95% CI	P <sub>trend</sub>
<b>α-Carotene</b>					
Quartile Cutpoints	<4.6	4.6 to <7.2	7.2 to <12	12	
Case/Control No.	298/285	286/284	263/283	286/281	
Unadjusted RR (95% CI)	1.0 (reference)	0.96 (0.76, 1.21)	0.89 (0.70, 1.12)	0.97 (0.77, 1.23)	0.85
Multivariate RR <sup>a</sup> (95% CI)	1.0 (reference)	0.93 (0.73, 1.19)	0.85 (0.67, 1.09)	0.93 (0.73, 1.19)	0.62
<b>β-Carotene</b>					
Quartile Cutpoints	<15	15 to <23	23 to <36	36	
Case/Control No.	282/284	285/284	259/282	306/282	
Unadjusted RR (95% CI)	1.0 (reference)	1.01 (0.80, 1.28)	0.92 (0.73, 1.17)	1.10 (0.87, 1.39)	0.44
Multivariate RR <sup>a</sup> (95% CI)	1.0 (reference)	0.97 (0.76, 1.23)	0.87 (0.68, 1.13)	0.99 (0.77, 1.28)	0.98
<b>β-Cryptoxanthin</b>					
Quartile Cutpoints	<7.3	7.3 to <10	10 to <14	14	
Case/Control No.	302/285	262/282	290/281	275/281	
Unadjusted RR (95% CI)	1.0 (reference)	0.87 (0.69, 1.11)	0.97 (0.77, 1.23)	0.92 (0.73, 1.17)	0.72
Multivariate RR <sup>a</sup> (95% CI)	1.0 (reference)	0.82 (0.64, 1.05)	0.93 (0.72, 1.19)	0.83 (0.64, 1.07)	0.27
<b>Lutein/Zeaxanthin</b>					
Quartile Cutpoints	<14	14 to <19	19 to <24	24	
Case/Control No.	263/282	288/282	278/279	298/284	
Unadjusted RR (95% CI)	1.0 (reference)	1.10 (0.87, 1.39)	1.07 (0.85, 1.36)	1.14 (0.89, 1.47)	0.36
Multivariate RR <sup>a</sup> (95% CI)	1.0 (reference)	1.06 (0.83, 1.35)	1.03 (0.80, 1.31)	1.02 (0.78, 1.34)	0.96
<b>Lycopene</b>					
Quartile Cutpoints	<33	33 to <43	43 to <55	55	
Case/Control No.	324/282	253/282	299/284	254/282	
Unadjusted RR (95% CI)	1.0 (reference)	0.78 (0.62, 0.99)	0.91 (0.73, 1.15)	0.78 (0.61, 0.99)	0.10
Multivariate RR <sup>a</sup> (95% CI)	1.0 (reference)	0.81 (0.64, 1.04)	0.92 (0.72, 1.17)	0.80 (0.62, 1.02)	0.14
<b>Total Carotenoids</b>					
Quartile Cutpoints	<85	85 to <109	109 to <136	136	
Case/Control No.	279/283	297/276	247/278	288/274	

	<b>RR</b>	<b>RR 95% CI</b>	<b>RR 95% CI</b>	<b>RR 95% CI</b>	<b>P<sub>trend</sub></b>
Unadjusted RR (95% CI)	1.0 (reference)	1.09 (0.86, 1.39)	0.90 (0.70, 1.15)	1.07 (0.84, 1.36)	0.81
Multivariate RR <sup>a</sup> (95% CI)	1.0 (reference)	1.09 (0.85, 1.40)	0.86 (0.66, 1.11)	0.99 (0.77, 1.28)	0.67

<sup>a</sup> Adjusted for BMI at blood draw (<25, 25-<25.9, 30 kg/m<sup>2</sup>), age at menarche ( 11, 12, 13, 14 years old), alcohol intake (non-drinker, 5, 5.01-10, 10.01-15, >15 grams/day), parity/age at first birth (nulliparous, 1 child/<25 years old, 1 child/ 25 years old, 2 children/<25 years old, 2 children/ 25 years old), family history of breast cancer (yes/no), history of benign breast disease (yes/no)

**Table 3**  
Relative risks (RR) of breast cancer and 95% confidence intervals (CI) according to quartile of premenopausal FIOP levels (FI/mL)

	RR	RR 95% CI	RR 95% CI	RR 95% CI	$P_{\text{trend}}$
<b>FIOP_360</b>					
Quartile Cutpoints	<176	176 to <209	209 to <252	252	
Case/Control No.	216/187	172/192	200/193	176/192	
Unadjusted RR (95% CI)	1.0 (reference)	0.77 (0.57, 1.02)	0.90 (0.67, 1.20)	0.77 (0.57, 1.04)	0.21
Multivariate RR <sup>a</sup> (95% CI)	1.0 (reference)	0.71 (0.52, 0.96)	0.85 (0.63, 1.16)	0.68 (0.50, 0.95)	0.07
<b>FIOP_320</b>					
Quartile Cutpoints	<271	271 to <339	339 to <453	453	
Case/Control No.	193/179	200/184	172/180	161/183	
Unadjusted RR (95% CI)	1.0 (reference)	0.99 (0.74, 1.33)	0.86 (0.62, 1.19)	0.80 (0.58, 1.09)	0.11
Multivariate RR <sup>a</sup> (95% CI)	1.0 (reference)	0.97 (0.71, 1.32)	0.83 (0.59, 1.16)	0.76 (0.55, 1.06)	0.08
<b>FIOP_400</b>					
Quartile Cutpoints	<53	53 to <64	64 to <79	79	
Case/Control No.	177/190	200/194	208/192	186/195	
Unadjusted RR (95% CI)	1.0 (reference)	1.12 (0.83, 1.51)	1.17 (0.87, 1.58)	1.04 (0.77, 1.42)	0.96
Multivariate RR <sup>a</sup> (95% CI)	1.0 (reference)	1.14 (0.83, 1.56)	1.14 (0.83, 1.57)	1.03 (0.74, 1.44)	0.92

<sup>a</sup> Adjusted for BMI at blood draw (<25, 25-<25.9, 30 kg/m<sup>2</sup>), age at menarche ( 11, 12, 13, 14 years old), alcohol intake (non-drinker, 5, 5.01-10, 10.01-15, >15 grams/day), parity/age at first birth (nulliparous, 1 child/<25 years old, 1 child/ 25 years old, 2 children/<25 years old, 2 children/ 25 years old), family history of breast cancer (yes/no), history of BBD (yes/no)



Table 4

Relative risks (RR) of breast cancer and 95% confidence intervals (CI) according to quartiles of premenopausal carotenoid and FIOP levels, by menopausal status at diagnosis<sup>a</sup>

	N, cases	Quartile 1		Quartile 2		Quartile 3		Quartile 4		<i>P</i> <sub>trend</sub>	<i>P</i> <sub>heterogeneity</sub> <sup>b</sup>
		RR	(95% CI)	RR	(95% CI)	RR	(95% CI)	RR	(95% CI)		
<b>α-Carotene</b>											
Premenopausal at diagnosis	535	1.0	(reference)	1.10 (0.77, 1.58)	0.85 (0.59, 1.23)	1.17 (0.81, 1.68)	0.49	0.05			
Postmenopausal at diagnosis	491	1.0	(reference)	0.92 (0.63, 1.35)	0.98 (0.68, 1.42)	0.73 (0.49, 1.06)	0.10				
<b>β-Carotene</b>											
Premenopausal at diagnosis	535	1.0	(reference)	0.89 (0.62, 1.28)	1.09 (0.75, 1.58)	0.98 (0.68, 1.41)	0.88	0.41			
Postmenopausal at diagnosis	492	1.0	(reference)	0.92 (0.63, 1.35)	0.77 (0.52, 1.14)	0.95 (0.63, 1.42)	0.82				
<b>β-Cryptoxanthin</b>											
Premenopausal at diagnosis	531	1.0	(reference)	0.92 (0.64, 1.33)	1.22 (0.85, 1.76)	0.87 (0.59, 1.27)	0.61	0.40			
Postmenopausal at diagnosis	491	1.0	(reference)	0.80 (0.55, 1.15)	0.72 (0.49, 1.05)	0.85 (0.57, 1.26)	0.48				
<b>Lutein/Zeaxanthin</b>											
Premenopausal at diagnosis	531	1.0	(reference)	1.28 (0.89, 1.83)	1.37 (0.96, 1.97)	1.19 (0.81, 1.75)	0.45	0.13			
Postmenopausal at diagnosis	488	1.0	(reference)	0.82 (0.55, 1.22)	0.79 (0.53, 1.17)	0.89 (0.60, 1.33)	0.76				
<b>Lycopene</b>											
Premenopausal at diagnosis	535	1.0	(reference)	0.82 (0.58, 1.18)	1.13 (0.80, 1.60)	1.00 (0.70, 1.42)	0.65	0.03			
Postmenopausal at diagnosis	489	1.0	(reference)	0.92 (0.63, 1.34)	0.73 (0.50, 1.06)	0.66 (0.45, 0.96)	0.02				
<b>Total Carotenoids</b>											
Premenopausal at diagnosis	526	1.0	(reference)	1.07 (0.75, 1.54)	0.89 (0.62, 1.28)	1.13 (0.78, 1.62)	0.61	0.04			
Postmenopausal at diagnosis	482	1.0	(reference)	1.17 (0.81, 1.70)	0.78 (0.52, 1.16)	0.79 (0.53, 1.19)	0.12				

<sup>a</sup> Adjusted for BMI at blood draw (<25, 25-<25.9, 30 kg/m<sup>2</sup>), age at menarche ( 11, 12, 13, 14 years old), alcohol intake (non-drinker, 5, 5.01-10, 10.01-15, >15 grams/day), parity/age at first birth (nulliparous, 1 child/<25 years old, 1 child/ 25 years old, 2 children/<25 years old, 2 children/ 25 years old), family history of breast cancer (yes/no), history of benign breast disease (yes/no);

<sup>b</sup> *P*-heterogeneity calculated by evaluating significance of cross-product of ordinal median carotenoid variables and menopausal status using Wald tests

Table 5

Summary of significant interactions between carotenoid levels (>/< median)<sup>a</sup> and SNPs in *CAT*, *SOD2*, *GPXI*, *MPO* and *BCMO1* on breast cancer risk in NHSII

Carotenoid	Gene	rs Number	Genotype	Minor allele frequency, cases/controls (%)	Mean carotenoid level, ug/dL <sup>b</sup>	P-value <sup>c</sup>	Low carotenoid		High carotenoid		P <sub>interaction</sub>
							Per-minor allele RR (95% CI) <sup>d</sup>	Per-minor allele RR (95% CI) <sup>d</sup>			
β-carotene	<i>CAT</i>	208679	AA	88/87	24	0.15	0.76 (0.45, 1.27)	1.07 (0.65, 1.77)	0.04		
			AG	12/12	21						
			GG	0.0/0.6	20						
	<i>GPXI</i>	17080528	CC	44/47	25	0.22	0.79 (0.61, 1.03)	1.10 (0.85, 1.41)			
			CT	47/41	23						
			TT	9/12	23						
β-cryptoxanthin	<i>CAT</i>	11032686	GG	80/84	10	0.02	0.99 (0.65, 1.52)	1.98 (1.26, 3.10)	0.01		
			AG	19/16	8.7						
			AA	0.7/0.5	9.2						
	<i>CAT</i>	1535721	GG	62/63	10	0.01	0.88 (0.65, 1.19)	1.26 (0.92, 1.71)			
			AG	35/33	9.4						
			AA	3/5	8.4						
<i>CAT</i>	7947841	GG	79/84	10	0.03	1.12 (0.74, 1.70)	2.00 (1.28, 3.12)				
		AG	20/16	8.8							
		AA	1/0.6	8.5							
Lutein/zeaxanthin	<i>SOD2</i>	5746151	CC	88/89	17	0.58	1.69 (1.01, 2.83)	0.92 (0.56, 1.52)	0.02		
			CT	10/10	17						
			TT	1/0.5	23						
Lycopene	<i>SOD2</i>	2758352	GG	64/62	41	0.004	1.10 (0.82, 1.49)	0.74 (0.55, 1.00)	0.02		
			AG	32/33	44						
			AA	3/5	48						
<i>SOD2</i>		2077560	TT	25/26	46	0.01	1.06 (0.83, 1.35)	1.20 (0.94, 1.54)	0.02		

Carotenoid	Gene	rs Number	Genotype	Minor allele frequency, cases/controls (%)	Mean carotenoid level, ug/dL <sup>b</sup>	P-value <sup>c</sup>	Low carotenoid		High carotenoid		P <sub>interaction</sub>
							Per-minor allele RR (95% CI) <sup>d</sup>	Per-minor allele RR (95% CI) <sup>d</sup>			
			AT	49/52	42						
			AA	26/22	41						
<i>SOD2</i>		2758329	TT	24/26	45	0.03	1.14 (0.89, 1.45)	1.10 (0.85, 1.41)		0.04	
			CT	51/52	42						
			CC	25/22	41						
<i>SOD2</i>		5746151	CC	88/89	42	0.22	1.51 (0.91, 2.49)	1.01 (0.61, 1.69)		0.02	
			CT	10/10	44						
			TT	1/0.5	57						
Total carotenoids	<i>CAT</i>	1535721	GG	62/63	109	0.06	0.96 (0.70, 1.30)	1.14 (0.85, 1.54)		0.02	
			AG	35/33	106						
			AA	3/5	91						
<i>CAT</i>		208679	AA	88/87	108	0.07	0.66 (0.40, 1.11)	1.27 (0.75, 2.13)		0.02	
			AG	12/12	101						
			GG	0.0/0.6	82						
<i>GPX1</i>		17080528	CC	44/47	107	0.60	0.88 (0.67, 1.14)	1.00 (0.78, 1.29)		0.02	
			CT	47/41	106						
			TT	9/12	105						

<sup>a</sup> Carotenoid values dichotomized at median;

<sup>b</sup> Carotenoid levels from age-adjusted generalized linear models among controls only;

<sup>c</sup> Type III Wald P-value from modeling number of minor alleles continuously;

<sup>d</sup> OR (95% CI) calculated from unconditional age-adjusted logistic regression models;

<sup>e</sup> P-heterogeneity from likelihood ratio tests with 1 degree of freedom. NOTE: Frequencies may not add up to 100% due to rounding.