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Telomere length, oxidative damage, antioxidants and breast cancer risk

Jing Shen[1,*], **Marilie D. Gammon**[2], **Mary Beth Terry**[3], **Qiao Wang**[1], **Patrick Bradshaw**[2], **Susan L. Teitelbaum**[4], **Alfred I. Neugut**[3], and **Regina M. Santella**[1]

[1]Department of Environmental Health Sciences, Mailman School of Public Health, Columbia University, New York, NY 10032, USA

[2]Department of Epidemiology, School of Public Health, University of North Carolina, Chapel Hill, NC 27599, USA

[3]Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, NY 10032, USA

[4]Department of Community and Preventive Medicine, Mt. Sinai School of Medicine, New York, NY 10029, USA

Abstract

Telomeres play a critical role in maintaining the integrity and stability of the genome, and are susceptible to oxidative damage after telomere shortening to a critical length. In the present study, we explored the role of white blood cell (WBC) DNA telomere length on breast cancer risk, and examined whether urinary 15-F_2-isoprostanes (15-F_{2t}-IsoP) and 8-oxo-7,8-dihydrodeoxyguanosine (8-oxodG), or dietary antioxidant intake modified the relationship between telomere length and breast cancer risk. A population-based case-control study -- the Long Island Breast Cancer Study Project (LIBCSP) was conducted among 1,067 cases and 1,110 controls. Telomere length was assessed by quantitative PCR (Q-PCR). Overall, the mean levels of telomere length (T/S ratio), 15-F_{2t}-IsoP and 8-oxodG were not significantly different between cases and controls. Among pre-menopausal women only, carrying shorter telomeres (Q3 and Q4), as compared with the longest (Q1), was associated with significantly increased breast cancer risk. Age-adjusted OR and 95%CI were 1.71 (1.10–2.67) and 1.61 (1.05–2.45). The 5-F_{2t}-IsoP and 8-oxodG biomarkers did not modify the telomere-breast cancer association. A moderate increase in breast cancer risk was observed among women with the shortest telomeres (Q4) and lower dietary and supplemental intake of β-carotene, vitamin C or E intake (OR (95%CI)=1.48 (1.08–2.03), 1.39 (1.01–1.92) and 1.57 (1.14–2.18), respectively), although the trend test exhibited statistical significance only within the lower vitamin E intake subgroup (P_{trend} =0.01). These results provided the strongest evidence to date that breast cancer risk may be affected by telomere length among pre-menopausal women or women with low dietary intake of antioxidants or antioxidant supplements.

Keywords

Telomeres length; oxidative damage; breast cancer

*Correspondence author: 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: js2182@columbia.edu.

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Telomeres are nucleotide repeats $(TTAGGG)_n$ with associated proteins at the ends of mammalian chromosomes. Telomeres play a critical role in maintaining the integrity and stability of the genome,[1,2] and also participate in the process of cellular DNA damage/repair.[3–5] In most proliferating cells, telomere length is dynamic, and subject to shortening at each cycle of cell division (the so-called end-replication problem).[6] Telomere length measured in surrogate tissues, such as buccal cells, peripheral blood lymphocytes (PBLs), mononuclear cells and white blood cells (WBC) has been considered as one of the applicable susceptibility biomarkers for several kinds of human cancer,[7–13] including breast cancer,[14,15] but not all results are consistent.[16–19]

Levy and colleagues found that telomere length in WBC DNA was significantly shorter in breast cancer patients as compared with healthy individuals.[14] A significantly reduced lymphocyte telomere length in breast cancer cases was noted in another study.[15] However, when the cases and controls were better matched by age, no statistically significant difference in telomere length was detected.[15] Three other studies, including ours, did not observe a significant relationship between shorter telomere length in peripheral blood cell DNA and overall breast cancer risk.[16–18] One study even found a reverse effect, *i.e.* breast cancer patients displayed significantly longer telomere length compared with controls.[19] None of these previous studies has analyzed the effect modification of DNA damage/repair on telomere length and breast cancer risk because of the lack of available data.

DNA damage induced by oxidative stress is another mechanism involved in accelerated telomere shortening,[20] and influences the relationship between telomere length and cancer risk. Telomeres as an initiator of DNA damage responses,[21] play a significant role in distinguishing natural DNA ends from double-strand break ends that trigger DNA damage checkpoints and subsequent repair.[3,22] Due to the high content of guanines, telomeres are especially sensitive to the accumulation of reactive oxygen species (ROS)-induced 8-oxo-7,8-dihydrodeoxyguanosine (8-oxodG) DNA-strand breaks.[23–25] Exposure to oxidative stress can induce a higher level of 8-oxodG formation in the telomere sequences than in other non-telomere sequences.[26,27] Besides the direct effect of oxidative damage on telomeric DNA, it has been proposed that deficient DNA repair capacity may promote telomere erosion. Accumulated single-strand breaks produced by oxidative stress have been found to be less efficiently repaired at telomeres than in the rest of the genome.[28,29] Most of these studies were conducted using *in vitro* or *in vivo* experiments with highly sensitive cell lines or animals. Several studies in humans have indicated the role of DNA oxidative damage in breast tumorigenesis. Plasma malondialdehyde (MDA) was found elevated in breast cancer cases relative to the level in healthy controls[30–32] and the levels of MDA-DNA adducts and 8-oxodG were also significantly higher in breast cancer cases than in controls.[33–36] However, there is a lack of population-based evidence to link both telomere length and oxidative damage to the development of breast cancer.

Using data from the population-based Long Island Breast Cancer Study Project (LIBCSP), we previously reported that dietary antioxidant intake was inversely associated with breast cancer risk,[37] and among a smaller subset of the LIBCSP participants, the urinary oxidative damage biomarker – 15-F_2-isoprostanes (15-F_{2t}-IsoP) appeared to be positively associated with risk, although our estimates were unstable due to the small sample size.[38] In the present study, we now examine whether breast cancer risk is associated with WBC telomere length, and we re-evaluate the association with the urinary oxidative damage biomarkers, but this time among a much larger sample of women from the LIBCSP. We then explore whether oxidative damage markers or dietary antioxidant intake modify the association between telomere length and breast cancer risk.

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Materials and Methods

Study population

The study methods of the LIBCSP have been described in detail previously.[39] The study was conducted with approval from participating institutional review boards, and in accordance with an assurance filed with and approved by the United States Department of Health and Human Services.

In brief, case eligibility included adult female residents of Nassau and Suffolk counties on Long Island, NY, who were of any age or race, who spoke English, and were newly diagnosed with *in situ* or invasive breast cancer between August 1, 1996, and July 31, 1997. Potentially eligible controls were frequency matched to the age distribution of the cases and identified through random digit dialing for women under age 65 years, and through the Center for Medicare and Medicaid Services (CMS) rosters for women 65 and over. Eligible controls were defined as women who spoke English, and resided in the same Long Island counties as the cases, but with no personal history of breast cancer. In-person interviews were completed for 82.1% of cases (n = 1,508) and 62.8% of controls (n = 1,556). Of those who completed the main questionnaire, 73.1% of cases (1,102) and 73.3% of controls (1,141) donated a blood sample. For the current study, sufficient DNA and urine for the biomarker assays was available for 1,061 cases and 1,108 controls. Ethnicity and age distributions were similar between respondents who completed the main questionnaire and the subjects analyzed in the present study (data not shown).

Laboratory methods

Genomic DNA was isolated by standard phenol and chloroform/isoamyl alcohol extraction and RNase treatment as previously described.[40] Telomere length quantification was performed with the quantitative PCR (Q-PCR) method described by Cawthon[41] to determine the relative ratio of telomere (T) repeat copy number to a single-copy gene (S) copy number (T/S ratio) according to a 5-point standard curve (final concentrations from 0.125 to 2 ng/ul using mixed human genomic DNA). This ratio is proportional to the average telomere length. Then, the ratio for each sample was normalized to a reference DNA (a pooled anonymous human WBC DNA sample) in order to standardize between different runs. The gene encoding acidic ribosomal phosphoprotein P0 (*36B4*) was used as the single copy gene. Telomere and *36B4* gene PCRs were always performed in separate 96-wells with each sample run in triplicate. The variations within the triplicate samples were from 16% to 21%. Pearson and Spearman correlation coefficient was higher than 0.88 ($P<0.001$). Details have been described previously.[17] Urinary 15-F_{2t}-IsoP levels were analyzed using immunoassay kits from Oxford Biomedical Research, Inc. (Oxford, MI). Urinary 8-OxodG levels were measured by competitive ELISA as previously described.[38] Both biomarkers were expressed as nmol per mmol/L urinary creatinine. Urinary creatinine was determined by QuantiChrom™ Creatinine Assay Kit (BioAssay System, Hayward, CA). Urinary 15-F_{2t}-IsoP and 8-OxodG data on 801 subjects of the 2,169 total subjects has been reported previously.[38] Tobacco smoke contains several chemical carcinogens, including polycyclic aromatic hydrocarbons (PAHs), that may produce bulky adducts but cause oxidative DNA damage.[42] The PAH-DNA adducts levels were determined by competitive ELISA from the parent study.[40,43] Adduct levels were determined for 873 (79.2%) breast cancer cases and 941 (82.5%) controls who donated sufficient blood for the assay.[43] All assays were performed with the laboratory personnel blinded to the subject's case-control status.

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Data collection

In-person interviews were conducted within a few months of diagnosis by trained personnel. The main questionnaire assessed information on known and suspected risk factors for breast cancer, including family history of breast cancer, reproductive history, menopausal status, exogenous hormone use, physical activity, body mass index (BMI), history of alcohol use and active/passive cigarette smoke (http://epi.grants.cancer.gov/LIBCSP/projects/Questionnaire.html). As part of the in-home interview, a 100-item food frequency questionnaire (FFQ) was self-completed by 98% of participants. The FFQ assessed dietary intake in the year prior to the interview, and supplement intake in the five years prior to the interview. The frequency and portion sizes data were translated to daily intakes of nutrients from both dietary and supplement sources as described previously.[37]

Medical records were abstracted to obtain estrogen receptor and progesterone receptor status of the breast cancer cases.[39]

Statistical methods

Initially, the three biomarkers (telomere length, urinary 15-F_{2t}-IsoP and 8-OxodG) were checked for normality before statistical analyses. All of them were skewed, and were converted into corresponding natural logarithm to achieve a normal distribution. Then, these variables were analyzed as continuous variables. Student’s t test was used to determine the differences for the three biomarkers as continuous variable by case-control status. Pearson's correlation coefficients were used to examine the relationships between telomere length and oxidative damage markers (urinary 15-F_{2t}-IsoP and 8-OxodG) separately in cases and controls.

Telomere length, urinary 15-F_{2t}-IsoP and 8-OxodG were categorized, based on the distribution among controls. For each biomarker, we estimated individual odds ratios (OR) and 95% confidence intervals (95%CI) using unconditional logistic regression. Potential confounding variables were evaluated using a backwards elimination strategy. Covariates were excluded from the full model if they changed the OR of any main effect (telomere length, urinary 15-F_{2t}-IsoP and 8-OxodG) by less than 10%. The factors evaluated and excluded from the final model included age at menarche, parity (nulliparous/parous), lactation (ever/never), history of fertility problems, body mass index (BMI, weight (kg)/ height (m^2)) at reference, BMI at age 20, first degree family history of breast cancer, history of benign breast disease, menopausal status, oral contraceptive use, hormone replacement use, smoking status, alcohol drinking, race, education, religion and marital status (as previously defined).[39,40,43] So, all models used in the current study were only adjusted for the frequency matching factor age at reference (defined as age at diagnosis for cases and age at identification for controls), as well as for the other two biomarkers.

We explored whether the telomere-breast cancer association varied among biologically plausible subgroups, stratifying by suspected breast cancer risk factors that could potentially influence oxidation damage (age at reference; BMI; alcohol drinking per day; active cigarette smoking defined as pack-years; menopausal status; detectable PAH-DNA adducts); oxidative damage biomarkers (urinary 15-F_{2t}-IsoP; 8-OxodG); and antioxidant intake (any fruits, fruit juices, and vegetables; dietary and supplemental β-carotene or vitamin C or vitamin E and dietary α-carotene). The distributions among controls were used to dichotomize at the median the intervals of age group at reference (<50 and ≥50 years old), BMI (<25 and ≥25), smoking pack years (<15, ≥15), alcohol drinking per day (<6.85g/d and ≥6.85g/d), telomere length (≥0.73, <0.73 T/S ratio), urinary 15-F_{2t}-IsoP (<0.71 and ≥0.71) and 8-OxodG (<22.17, ≥22.17); detectable PAH-DNA adduct levels (>15% inhibition in the

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ELISA) *vs.* non-detectable adducts. These potential effect modifications were evaluated on the multiplicative scale by performing likelihood ratio test (LRT) to compare the difference in the log likelihoods from models with or without a cross-product term for the evaluated variables.44 We also considered the association between telomere length and breast cancer risk with the case women categorized by their hormone receptor status, grouped either as estrogen receptor (ER) and progesterone receptor (PR) negative versus ER or PR positive.

All statistical analyses were completed by using Statistical Analysis System 9.0 (SAS Institute, Cary, NC), and two-sided ($P < 0.05$) as the criteria of statistical significance.

Results

Overall, telomere length (T/S ratio) ranged from 0.10–8.70 for cases and 0.10–8.45 for controls, and the mean levels of telomere length, 15-F_{2t}-IsoP and 8-OxodG were not significantly different between cases and controls (1.03 *vs*. 1.05; 1.01 *vs*. 1.04 nmol/mmol creatinine and 25.69 *vs*. 26.05 nmol/mmol creatinine, respectively, Table I). There were no significant correlations between telomere length and urinary 15-F_{2t}-IsoP levels or 8-OxodG levels within cases or controls (data not shown). When biomarkers were categorized by their medians in controls, no significant difference were observed between cases and controls even after adjusting for age at reference alone or age at reference and the other two biomarkers (Table II). Also no significant trends for quartiles of the three biomarkers were noted between breast cancer cases and controls within age-adjusted or multivariable-adjusted models.

The association between telomere length and breast cancer risk was modified by several biologically plausible subgroups (Table III). Among pre-menopausal women, we found that carrying shorter telomeres (Q3 and Q4), as compared with the longest (Q1), were associated with significantly increased breast cancer risks (Q3: OR=1.71, 95%CI=1.10–2.67; Q4: OR=1.61, 95%CI=1.05–2.45). A consistent result was observed in women younger than 50 years with the shortest telomere length (Q3: OR=1.66, 95%CI=1.05–2.65; Q4: OR=1.78, 95%CI=1.15–2.76). Using the likelihood ratio test, significant interaction was found between telomere length and menopausal status and age group (LRT $P_s<0.01$). P_s for interaction were 0.004 and 0.003, respectively. Both P_s for trends were 0.01 indicating that these two variables might be correlated with each other, at least in determining telomere length and breast cancer relationship. No similar increased breast cancer risk was obtained in post-menopausal women or older women (≥50 years). Within women who had no detectable PAH-DNA adducts, increased breast cancer risks were observed for carrying shorter telomeres (Q2: OR=1.90, 95%CI=1.11–3.26; Q4: OR=1.92, 95%CI=1.16–3.18), but the trend test did not show statistical significance (P_{trend} =0.05). No other differences were evident within BMI, alcohol drinking and cigarette smoking subgroups. Analyses conducted with the case women categorized by the ER/PR status of their tumor revealed no significant associations between quartiles of telomere length and breast cancer risk (e.g., ORs for the shortest telomere length Q4 = 0.90 (0.53–1.51) for ER and PR negative breast cancer and 1.12 (0.83–1.50) for ER or PR positive breast cancer).

In table IV, we analyzed the relationships between telomere length by quartile and breast cancer risk according to the status of oxidative damage and antioxidant capacity. Although 15-F_{2t}-IsoP and 8-OxodG biomarkers did not appear to substantially modify the telomere-breast cancer association, a moderate increase in breast cancer risk was observed among women with the shortest telomere length and who also reported lower dietary and supplemental intakes of β-carotene or vitamin C or E (OR (95%CI)=1.48 (1.08–2.03), 1.39 (1.01–1.92) and 1.57 (1.14–2.18), respectively). Statistically significant interactions were observed for telomere length and dietary and supplemental intakes of β-carotene (LRT

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P<0.05) and vitamin E (LRT *P*<0.01). P_s for interaction were 0.035 and 0.004, respectively). Trend tests achieved statistical significance only within the lower vitamin E intake subgroup (P_{trend} =0.01), not for other two subgroups. Other variables indicating antioxidant capacity (any fruits, fruit juices, and vegetables and dietary α-carotene) did not show any modification effect on the relationship between quartile of telomere length and beast cancer risk.

Discussion

Although we did not observe an independent correlation for short telomere length and overall breast cancer risk in the current study, we have provided population-based evidence that breast cancer risk may be modified by telomere length among certain subgroups of women. First, the shortest telomere length was significantly associated with increased breast cancer risk in younger or pre-menopausal women (ORs ranged from 1.61 to 1.78, *P*< 0.03). Second, women with the shortest telomere lengths had moderately increased breast cancer risk when they also had poor antioxidant capacity (lower dietary and supplemental β-carotene, vitamin C or E intake). The ORs ranged from 1.39 to 1.57 (*P*<0.04). However, using urinary 8-oxodG and 15-F_{2t}-IsoP as oxidative damage markers no influence on telomere length and breast cancer risk was observed. Third, among women exposed to lower carcinogen levels (no detectable PAH-DNA adducts), increased breast cancer risks were observed for carrying shorter telomeres (Q2 and Q4), although no significant trend was observed (P_{trend} =0.05).

Previous epidemiological studies to investigate the correlations between telomere lengths in surrogate tissues (including buccal cells, bladder wash samples[45] and peripheral blood cells) and overall cancer risks are quite limited, but still provided some positive evidence. Surrogate tissues frequently serve as a proxy for solid target tissues since they are easily obtained from DNA repositories, and can be conveniently used in large sample epidemiological studies.[46,47] Short telomeres have been observed to increase risks for bladder, esophageal, head and neck, lung, lymphoma, and renal cell cancers that may be influenced by cigarette smoking.[7–13,16,19] Studies on telomere length and breast cancer risk are few and inconclusive. Levy *et al*. first observed an association between shorter telomere length in WBC DNA and increased breast cancer risk.[14] Another study found breast cancer risk significantly increased with the longest telomere, and short telomeres were associated with a better prognostic outcome for younger women.[19] However, not all data reached a significant level [15,16], including our pilot study conducted in sister-sets from the New York site on the Breast Cancer Family Registry (BCFR) in which carrying short telomeres was associated with a non-significant increased breast cancer risk (OR=2.1, 95%CI=0.8–5.5).[17] Also, no statistically significant difference in telomere lengths was detected between breast cancer cases and unaffected controls in another larger cohort study (*P*=0.53).[18] The discrepant results might be due to different methods (terminal restriction fragment (TRF), southern blot, Q-FISH and Q-PCR) used to measure telomere length in dissimilar studies.[15–19] Those approaches with different sensitivity, variability and requirements for DNA quality[48,49] could be the cause of heterogeneity in the previous observations. Even using the same Q-PCR method, the range of T/S ratio also showed wide variation (from 4.7 to 76.3 folds)[7,17,50]. Whether this is due to different reference DNAs used, the influence of the standard curve (covering the potential range of measured DNA concentrations), or variation of studied populations, needs to be further evaluated. These inconsistent results also suggest that telomere length alone might not be sufficiently powerful as an independent biomarker to determine breast cancer risk. Several biologically related pathways (such as hormone and carcinogen metabolism, DNA damage and repair) could interact with telomere length, and would have stronger influences on breast cancer susceptibility. This is consistent with our current observations of significant differences only

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in certain subgroups (pre-menopausal women and women with a poor anti-oxidative capacity).

The potential biological explanation for the more pronounced association found between the shortest telomere length and breast cancer risk among pre-menopausal or younger women have been discussed previously.[17] The first possible reason is the dramatic difference in estrogen level during pre- and post- menopausal periods. Estrogen has anti-inflammatory and antioxidant activities that may contribute to the dynamic of telomere length.[51] Another explanation is that the diversity of genomic instability is relative greater between pre-menopausal cases (with an unstable status) and pre-menopausal controls (usually with a stable status), and can be detected easily. While for post-menopausal women, the genome maybe relatively unstable for both cases and controls because of the influence of either carcinogens or aging itself,[10,52,53] and the smaller variety in genomic stability is not easy observed.

8-OxodG and 15-F_{2t}-IsoP as oxidative damage biomarkers have been associated with several cancers. Our previous study based on a subset of 801 breast cancer cases and controls observed a significantly increased breast cancer risk at higher levels of urinary 15-F_{2t}-IsoP, and this relationship remained after excluding the effect of radiation therapy.[38] It was agreement with the observations that smokers[54] and alcohol drinkers[55] had higher levels of 15-F_{2t}-IsoP, a situation of favorable to carcinogenesis. However, the current analysis of the expanded case-control study (1,061 cases and 1,108 controls) found no statistically significant associations between the two biomarkers (15-F_{2t}-IsoP and 8-OxodG) and breast cancer risk, although the direction of relationship was consistent. Previous studies have indicated that DNA oxidative damage and repair capacity was another important pathway significantly influencing telomere shortening[56] and potentially modulating breast cancer risk. Von Zglinicki reviewed 22 independent experiments, and found an increased telomere shortening rate under conditions of increased oxidative stress in 19 reports from seven different laboratories.[20] The DNA excision repair proteins (XPC, XPE and ERCC2 *etc*.) were found to be up-regulated in cell lines with long telomeres, while integrin, its ligands, and other interacting proteins were down-regulated possibly related to telomere dysfunction.[57] Measuring both telomeric-shortening rate and antioxidant capacity (using DCF fluorescence as an indicator of intracellular peroxide levels), Von Zglinicki observed a significant inverse correlation between telomere-shortening rate and anti-oxidative capacity in more than 20 human fibroblast cell lines.[58] The role of antioxidant defense in affecting telomere length in cell lines was also confirmed for different tissues of the same donor in two independent studies.[56,59] In an *in vitro* study, telomere attrition was accelerated by chronic oxidative stress.[60,61] The rate of telomere shortening in human vascular endothelial cells could be slowed down by Asc2P, an oxidation-resistant derivative of vitamin C.[62] Chondrocytes treated with Asc2P displayed a tendency to protect telomeres from erosion compared with untreated controls.[63] An *in vivo* study also found a relationship between oxidative stress and telomere shortening in male and female rats of the same age. Female rats exhibited longer telomeres and higher expression of the antioxidant enzyme (MnSOD) than male rats.[64] Only one population study confirmed the relationship between higher oxidative stress and telomere shortening.[65] The oxidative stress index used was the ratio of urine 15-F_{2t}-IsoP and vitamin E level. Healthy pre-menopausal women who were the mothers of children with a chronic illness were considered a high oxidative stress group and were found to have a significantly shorter telomeres length (3,110bp) compared with controls (3,660bp) who were age-matched mothers with healthy children.[65] The major mechanisms underlying the link between oxidative damage-telomere shortening and breast cancer risk include 1) the direct damage of ROS on telomeric DNA; 2) the telomeric high content of guanines (G triplet) is particularly susceptible to accumulate oxidative stress-induced 8-OxodG; 3) the repair capacity for oxidative lesions is less efficient in telomeres

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than in the rest of the genome; and 4) a high level estrogen enhanced gene expression of MnSOD, an antioxidant enzyme.[20,23,24,26,27,56,66–69] All these data support the theory that telomere length is determined by the balance between oxidative damage and antioxidant defense capacity.[56] Our current results indicate that women with a poor anti-oxidative capacity and the shortest telomere length are at significantly increased breast cancer risk, and are the first population evidence to endorse this theory.

The advantages of current study include the population-based study design with a relatively large sample size; the quantitative PCR assay with high through-put (96-well plates used) and high sensitivity (ng quantities of DNA can be analyzed) to measure telomere length; the available oxidative status obtained from both urinary biomarkers and the FFQ; and the extensive information on co-variables allowing us to comprehensively control for potential confounders and evaluate effect modifications in the data analysis. A major limitation is samples were collected after breast cancer diagnosis, which restricts our ability to confirm the etiological temporal sequence between telomere length, oxidative biomarkers and breast cancer risk. This limitation provides another possible explanation for the discrepant results obtained with the two urinary oxidative biomarkers (representing current oxidative damage) and the FFQ data (indicating anti-oxidative capacity prior to cancer diagnosis) on telomere-breast cancer relationship. The former data may be influenced by intake of more fruits, vegetables and antioxidants after cancer diagnoses. Our previous data that did not show an association between fruit and vegetable consumption and 15-F2t-IsoP or 8-oxodG levels supports this possibility.[38] Second, we have evaluated available confounders in the analytic models, but we cannot exclude residual confounding caused by unknown factors related to both the dynamics of telomere length and breast cancer risk, including chronic inflammation, telomerase activity, and epigenetic and genetic changes in telomere-related genes.

In summary, our data are the first to suggest that carrying shorter telomeres is associated with a significantly increased breast cancer risk for pre-menopausal women and women with lower dietary and supplemental β-carotene vitamin C or E intake. Additional population data will greatly accelerate our understanding of the role of shortened telomere length, oxidative damage and antioxidants in the development of breast cancer.

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TABLE I

COMPARISON OF MEANS IN THREE BIOMARKERS BETWEEN ACSE AND CONROL, LONG ISLAND BREAST CANCER STUDY PROJECT, 1996–1997

Biomarker	Cases (n=1,061) mean (SD)	Controls (n=1,108) mean (SD)	t	*P*
Telomere length (T/S ratio)	1.03 (1.01)	1.05 (1.03)	0.43	0.67
Range	0.10–8.70	0.10–8.45		
15-F_{2t}-Isoprostane (nmol/mmol creatinine)	1.01 (1.04)	1.04 (1.66)	0.37	0.71
Range	0.02–16.34	0.05–40.59		
8-OxodG (nmol/mmol creatinine)	25.69 (15.76)	26.05 (19.48)	0.47	0.64
Range	3.00–140.09	1.94–304.74		

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TABLE II

ADJUSTED OR AND 95%CI FOR BREAST CANCER ASSOCIATED WITH TELOMERE AND OXIDATIVE BIOMARKERS, LONG ISLAND BREAST CANCER STUDY PROJECT, 1996–1997

Biomarker	Cases (%)	Controls (%)	Age-adjusted OR (95%CI)	Multivariable-adjusted OR (95%CI) *
15-F_{2t}-IsoP (nmol/mmol creatinine)				
< 0.71 (median)	467 (45.21)	541 (50.14)	1.00	1.00
≥ 0.71 (median)	566 (54.79)	538 (49.86)	1.17 (0.98–1.39)	1.15 (0.96–1.37)
< 0.49 (Q1)	227 (21.97)	270 (25.02)	1.00	1.00
0.49–0.70 (Q2)	240 (23.23)	271 (25.12)	1.03 (0.80–1.32)	1.09 (0.84–1.40)
0.71–1.06 (Q3)	288 (27.88)	272 (25.21)	1.20 (0.94–1.53)	1.22 (0.95–1.57)
≥ 1.07 (Q4)	278 (26.92)	266 (24.65)	1.17 (0.91–1.50)	1.17 (0.91–1.50)
			$P_{trend} = 0.12$	$P_{trend} = 0.15$
8-OxodG (nmol/mmol creatinine)				
< 22.17 (median)	510 (49.42)	538 (50.05)	1.00	1.00
≥ 22.17 (median)	522 (50.58)	537 (49.95)	0.97 (0.82–1.15)	0.96 (0.80–1.14)
< 15.27 (Q1)	267 (25.86)	269 (25.02)	1.00	1.00
15.27–22.16 (Q2)	243 (23.55)	269 (25.02)	0.90 (0.70–1.15)	0.95 (0.74–1.21)
22.17–31.67 (Q3)	256 (24.81)	269 (25.02)	0.91 (0.72–1.16)	0.93 (0.73–1.19)
≥ 31.68 (Q4)	266 (25.78)	268 (24.94)	0.93 (0.73–1.19)	0.94 (0.74–1.21)
			$P_{trend} = 0.60$	$P_{trend} = 0.62$
Telomere length (T/S ratio)				
≥ 0.73 (median)	513 (50.00)	534 (49.91)	1.00	1.00
< 0.73 (median)	513 (50.00)	536 (50.09)	0.99 (0.84–1.18)	0.97 (0.82–1.16)
≥ 1.41 (Q1)	252 (24.56)	269 (25.14)	1.00	1.00
0.73–1.40 (Q2)	261 (25.44)	265 (24.77)	1.06 (0.83–1.35)	1.08 (0.85–1.39)
0.35–0.72 (Q3)	226 (22.03)	252 (23.55)	0.95 (0.74–1.22)	0.93 (0.72–1.19)
< 0.35 (Q4)	287 (27.97)	284 (26.54)	1.09 (0.86–1.38)	1.10 (0.86–1.41)
			$P_{trend} = 0.69$	$P_{trend} = 0.73$

* Unconditional logistics regression analysis adjusted by age at reference and the other two biomarkers in the table

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TABLE III

OR AND 95%CI FOR BREAST CANCER ASSOCIATED WITH QUARTILES OF TELOMERE LENGTH STRAFIED BY CO-VARIABLES, LONG ISLAND BREAST CANCER STUDY PROJECT, 1996–1997

Variable	Age-adjusted ORs (95%CIs) of Quartiles of Telomere Length				*P* for trend
	Q1 (longest)	Q2	Q3	Q4 (shortest)	
Age (y)					
< 50	1.00	1.18 (0.75–1.86)	1.66 (1.05–2.65)	1.78 (1.15–2.76) *	0.01
≥ 50	1.00	1.01 (0.75–1.34)	0.74 (0.56–1.00)	0.91 (0.68–1.22)	0.23
Menopausal status					
Pre-menopausal	1.00	1.24 (0.80–1.91)	1.71 (1.10–2.67)	1.61 (1.05–2.45) ‡	0.01
Postmenopausal	1.00	1.05 (0.78–1.43)	0.72 (0.53–0.98)	0.92 (0.67–1.24)	0.19
Ever alcohol drinking					
Nondrinkers	1.00	1.26 (0.85–1.89)	0.79 (0.52–1.19)	1.06 (0.71–1.57)	0.64
Drinkers	1.00	0.96 (0.70–1.30)	1.05 (0.76–1.43)	1.11 (0.82–1.50)	0.28
< 6.85 (gram/day)	1.00	0.91 (0.59–1.41)	1.02 (0.65–1.59)	1.08 (0.70–1.67)	0.52
≥ 6.85 (gram/day)	1.00	0.96 (0.62–1.48)	1.00 (0.64–1.57)	1.10 (0.72–1.67)	0.51
Active cigarette smoking status					
Never	1.00	1.08 (0.75–1.55)	1.03 (0.71–1.49)	0.99 (0.70–1.42)	0.93
Former	1.00	0.99 (0.66–1.49)	0.82 (0.54–1.24)	1.28 (0.86–1.90)	0.28
Current	1.00	1.11 (0.63–1.94)	0.96 (0.54–1.72)	0.98 (0.56–1.73)	0.81
< 15 pack-years	1.00	1.19 (0.60–2.37)	0.93 (0.46–1.88)	1.37 (0.63–2.98)	0.63
≥ 15 pack-years	1.00	0.63 (0.33–1.24)	0.67 (0.32–1.24)	0.94 (0.48–1.82)	0.87
PAH-DNA adducts					
Non detectable	1.00	1.90 (1.11–3.26)	1.28 (0.76–2.18)	1.92 (1.16–3.18)	0.05
Below median	1.00	1.04 (0.68–1.61)	0.87 (0.55–1.38)	1.33 (0.86–2.06)	0.31
Median and above	1.00	0.80 (0.53–1.20)	0.83 (0.53–1.30)	0.80 (0.51–1.25)	0.33
BMI (kg/m2)					
< 25	1.00	0.85 (0.60–1.21)	0.83 (0.57–1.20)	0.94 (0.67–1.31)	0.87
≥25	1.00	1.31 (0.93–1.85)	1.08 (0.76–1.51)	1.27 (0.90–1.78)	0.33

* $P_{\text{interaction}} = 0.003$, Likelihood ratio test $P < 0.01$

† $P_{\text{interaction}} = 0.004$, Likelihood ratio test $P < 0.01$

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TABLE IV

OR AND 95%CI FOR BREAST CANCER ASSOCIATED WITH QUARTILES OF TELOMERE LENGTH STRAFIED BY OXIDATIVE DAMAGE BIOMARKERS, DIETARY AND/OR SUPPLEMENTAL ANTIOXIDANTS INTAKE*, LONG ISLAND BREAST CANCER STUDY PROJECT, 1996–1997

Variable	Age-adjusted ORs (95%CIs) of Quartiles of Telomere Length				*P* for trend
	Q1 (longest)	Q2	Q3	Q4 (shortest)	
15-F_{2t}-IsoP (nmol/mmol creatinine)					
< 0.71 (median)	1.00	1.10 (0.77–1.58)	1.10 (0.76–1.59	1.11 (0.78–1.59)	0.53
≥ 0.71 (median)	1.00	1.10 (0.78–1.55)	0.83 (0.59–1.18)	1.07 (0.76–1.49)	0.96
8-OxodG (nmol/mmol creatinine)					
< 22.17 (median)	1.00	0.95 (0.67–1.36)	0.89 (0.62–1.28)	1.23 (0.88–1.73)	0.18
≥ 22.17 (median)	1.00	1.23 (0.87–1.74)	1.00 (0.70–1.42)	0.92 (0.65–1.31)	0.43
Any Fruits, Fruit Juices, and Vegetables †					
0–34	1.00	1.27 (0.93–1.73)	1.06 (0.77–1.47)	1.16 (0.85–1.58)	0.39
≥ 35	1.00	0.81 (0.55–1.21)	0.83 (0.56–1.24)	0.99 (0.67–1.47)	0.99
Dietary + Supplemental β-carotene (mcg/d)					
0–3817.2	1.00	1.33 (0.97–1.83)	1.10 (0.79–1.52)	1.48 (1.08–2.03) ‡	0.05
≥ 3817.3	1.00	0.82 (0.56–1.21)	0.75 (0.51–1.11)	0.81 (0.55–1.20)	0.25
Dietary + Supplemental Vitamin C (mg/d)					
0–131.0	1.00	1.23 (0.88–1.70)	1.11 (0.80–1.53)	1.39 (1.01–1.92)	0.08
≥ 131.1	1.00	0.93 (0.64–1.35)	0.74 (0.49–1.10)	0.91 (0.62–1.34)	0.43
Dietary + Supplemental Vitamin E (a-te/d)					
0–29.0	1.00	1.28 (0.92–1.77)	1.19 (0.86–1.65)	1.57 (1.14–2.18) §	0.01
≥ 29.1	1.00	0.88 (0.61–1.28)	0.67 (0.45–1.00)	0.78 (0.54–1.14)	0.11
Dietary α -carotene (mcg/d)					
0–267.7	1.00	1.22 (0.89–1.68)	1.06 (0.76–1.46)	1.20 (0.88–1.64)	0.41
≥ 267.8	1.00	0.92 (0.63–1.36)	0.79 (0.53–1.18)	1.12 (0.75–1.66)	0.79

* Variables were derived from a two-step process. Quintiles of the control distribution were used to form fifths. ORs of the last two-fifths and the first three-fifths were similar and therefore collapsed to form these binary variables

† In ½ cup servings per week

‡ P interaction = 0.035, Likelihood ratio test $P < 0.05$

§ P interaction = 0.004, Likelihood ratio test $P < 0.01$

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