TEA CONSUMPTION, OXIDATIVE STRESS, AND BREAST CANCER RISK

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ABSTRACT

Dongyu Zhang: Tea consumption, oxidative stress, and breast cancer risk
(Under the direction of Hazel B. Nichols)

Purpose: Oxidative stress, which features the imbalance between reactive oxygen species and antioxidant defenses, is associated with carcinogenesis. Tea contains natural antioxidants and has anti-tumor properties. However, limited epidemiologic studies investigated the association between tea and oxidative stress. Previous epidemiologic studies investigating tea and breast cancer risk have not addressed the potential for associations to vary by breast cancer subtype. **Methods:** The Sister Study is a nationwide cohort study that enrolled women across the US and Puerto Rico from 2003-2009. In aim 1, we included 889 premenopausal women from a nested case-control study within the Sister Study and investigated associations between black or green tea and oxidative stress which was measured by urinary F_2-isoprostane (F_2-IsoP) and a primary metabolite, 2,3-dinor-5,6-dihydro-15-F_{2t}-isoprostane (15-F_{2t}-IsoP-M). In aim 2, we investigated the association between black or green tea and breast cancer risk in the full cohort of 49,214 women. The analysis addressed variation by estrogen receptor (ER) status and other biologically important factors. **Results:** (Aim 1) We observed green tea was not associated with urinary F_2-IsoP and 15-F_{2t}-IsoP-M. Black tea was not associated with urinary F_2-IsoP. However, drinking at least 5 cups of black tea per week (compared to 0 cup/week) was associated with a higher level of urinary 15-F_{2t}-IsoP-M (geometric mean difference (GMD)=0.09, 95% CI 0.01, 0.17). Additionally adjusting for

caffeine intake attenuated the association towards null (GMD=0.07, 95% CI -0.02, 0.16). (Aim 2) A total of 3,044 breast cancer patients were diagnosed from 49,214 participants. High-level black (hazard ratio (HR)=0.86, 95% CI 0.76, 0.98, ≥5 compared to 0 cups/week) and green tea (HR=0.84, 95% CI 0.73, 0.97, ≥5 compared to 0 cups/week) consumption were associated with a lower breast cancer risk. Both black and green tea consumption were inversely associated with ER+ breast cancer risk. Associations with ER- breast cancer risk were similarly inverse but non-significant. **Conclusions:** Contrary to previous experimental and clinical studies, we did not find an inverse association between black or green tea and oxidative stress. However, drinking at least 5 cups of black or green tea per week (compared to 0) was associated with a lower breast cancer risk.

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15-F_{2t}-IsoP-M	2,3-dinor-5,6-dihydro-15-F_{2t}-isoprostane
8-OHdG	8-Oxo-2'-deoxyguanosine
AA	Arachidonic acid
BMI	Body mass index
BOSS	Biomarkers of Oxidative Stress Study
CI	Confidence interval
DAG	Directed acyclic graph
DNA	Deoxyribonucleic acid
EC	Epicatechin
ECG	Epicatechin gallate
EGC	Epigallocatechin
EGCG	Epigallocatechin-3-gallate
EGFR	Epidermal growth factor receptor
EPIC	European Prospective Investigation into Cancer and Nutrition
ER	Estrogen receptor
F_2-IsoP	F_2-isoprostanes
FFQ	Food Frequency Questionnaire
HNE	Hydroxynonenal
GC	Gas chromatography
GM	Geometric mean
GMD	Geometric mean difference
GSH	Glutathione

GSSG	Glutathione disulfide
HER2	Human epidermal growth factor receptor 2
HR	Hazard ratio
MCF-7	Michigan Cancer Foundation-7
MDA	Malondialdehyde
MET	Metabolic equivalent
MS	Mass spectrometry
NF-κB	Nuclear factor-kappaB
NIEHS	The National Institute of Environmental Health Sciences
OR	Odds ratio
PG	Prostaglandin
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
RR	Risk ratio
SEER	Surveillance, Epidemiology, and End Results Program
SD	Standard deviation
VEGF	Vascular endothelial growth factor
WHO	World Health Organization
WHI	Women's Health Initiative

CHAPTER 1. BACKGROUND

1.1 Overview

Breast cancer is the most commonly diagnosed cancer among women worldwide, and it accounts for about 25% of all cancers in women [1]. In the United States, recent statistics in Surveillance, Epidemiology, and End Results (SEER) Program demonstrate that breast cancer incidence was 125 per 100,000 women in 2013 (the most recent year of data available in SEER), and the lifetime risk is approximately 12% [2]. The 5-year relative survival of female breast cancer patients was 89.7% during 2006-2012 [2].

Tea is one of the most popular beverages around the world because of its good taste, low cost, and potential beneficial health effects [3]. Some studies suggest a chemopreventive effect of tea drinking on cancer development due to its suppressive effect to oxidative stress, which is defined as the imbalance between the production of reactive species and antioxidant defenses [1]. However, epidemiologic studies have yielded heterogeneous results. This heterogeneity may be due, in part, to differences in study design, exposure measurement, or study populations. For instance, some studies treat green tea, black tea, and oolong tea as a single category of "non-herbal" tea without considering their differing chemical composition. Other studies have been unable to evaluate potential dose-response relations, or heterogeneity by breast cancer subtype. Tea can be classified as non-herbal and herbal tea. The former type originates from leaves of tea trees (e.g., green tea, black tea, and oolong tea) and the latter refers to infusions of fruit or herbs made without the leaves of tea trees [4]. Non-herbal tea and herbal tea contain different chemical compounds; particularly, non-herbal tea contains flavonoids and caffeine, whereas herbal tea

usually does not contain these compounds [5]. Flavonoids are a class of antioxidants found in some plants, and over 5,000 natural subtypes of flavonoids have been identified such as anthoxanthin, anthocyanidin, and catechin [5]. Non-herbal tea (e.g., green black, and oolong tea) is rich in flavonoids, and flavonoids can account for about 30% of the dry weight of fresh tea leaves [6]. Flavonoids can scavenge oxygen- derived free radicals in human body. In vitro studies showed that flavonoids has anti-inflammatory, anti-oxidant, and anti-tumor properties [7]. Previous epidemiologic studies indicated that caffeine consumption was associated with a reduced risk of malignant tumors [8, 9], and laboratory research also showed that low dose of caffeine could induce p53-associated apoptosis [10, 11].

Although these beneficial compounds have been identified in non-herbal tea, their concentrations can vary across different types, for example green tea has a higher level of (-)-epigallocatechin-3-gallate (EGCG) than black tea [12]. Henning et al. [13] reported that green tea extract (697.1 mg from 3 tea bags) had a higher concentration of flavonoids than black tea (546.5 mg from 4 tea bags), while black tea extract (268.9 mg from 4 tea bags) had higher concentration of caffeine than green tea (110.9 mg from 3 tea bags). Flavonoids and caffeine may differentially influence oxidative stress or cancer risk; therefore, pooling green tea and black tea in analysis may mask effects with one constituent or the other.

Catechins are bioactive polyphenols that comprise a

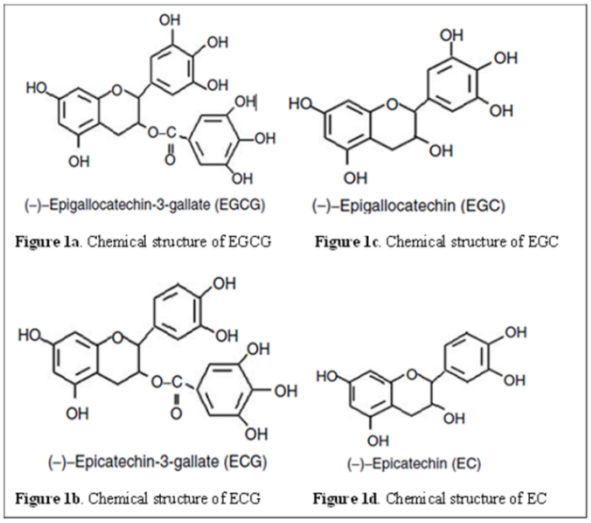


Figure 1. Chemical structure of catechin in non-herbal tea.

subgroup of flavonoids (flavan-3-ols) (**Figure 1**). Four major types of catechins are found in green and black tea: (-)-epicatechin (EC),3 (-)-epigallocatechin (EGC), (-)-epicatechin-3-gallate (ECG), and (-)-epigallocatechin-3-gallate (EGCG) [14-16]. Among these chemical compounds, EGCG was shown to inhibit the growth and metastasis of tumor cells in some laboratory studies. However, a high dosage of tea consumption may be required to inhibit cancer development due the relatively low bioavailability of EGCG [17]. EGCG is the main catechin in tea from *Camellia sinensis*, including black and green tea; depending on water temperature and time of brewing, a cup of green tea may contain 100-200 mg EGCG [18]. Previous in vitro studies suggest that EGCG may inhibit the growth of human cancer cell lines and induce apoptosis [19]. In addition, in mouse models, Ju et al. [20] reported that EGCG solution inhibited the development of small intestinal tumors in a dose-dependent manner.

Breast cancer is commonly classified according to the estrogen receptor (ER) status of the tumor; the majority of breast cancers are ER positive [2]. By binding to the ER, estrogen can activate downstream signal pathway and induce cell proliferation [21]. Some evidence indicates that EGCG may downregulate the function of estrogen receptors [22]. Therefore, green tea or black tea consumption may be differentially associated with ER positive compared to ER negative breast cancers by inhibiting ER binding to reduce the proliferation rate and prevent tumor promotion.

My proposal aims to investigate the association between tea consumption, oxidative stress, and breast cancer. The proposal will also address the limitations found in previous epidemiologic studies in exposure measurement, analytic approaches, and potential heterogeneity by evaluating the type, frequency and duration of tea consumption and examining potential variation according to tumor subtype.

1.2 Oxidative stress and its measurement

Oxidative stress is defined as a disturbance in the balance between the production of reactive species and antioxidant defenses [23]. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are the main two groups of reactive species which are essential in oxidative stress process. ROS and RNS can be produced when human body is under sustained environmental stress, including but not limited to malnutrition, inflammation, and infection [24]. Increased levels of ROS or RNS can result in damage to cell structure such as random damage to proteins, lipids and DNA. and may induce somatic mutations or neoplastic transformation [25] and increase the risk of cancer initiation.

Lipid peroxidation, which can be caused by highly reactive free radical species, is a typical feature of oxidative stress [26]. There are diverse kinds of product in lipid peroxidation, and a group of prostaglandin (PG) F_2-like products are found in these metabolic markers, which are termed F_2-isoprostanes (F_2-IsoPs) [27]. Urinary F2-isoprostanes (F_2-IsoPs) and 2,3-dinor-5,6-dihydro-15-F2t-isoprostane (15-F_{2t}-IsoP-M) will be used as the biomarker of oxidative stress in our analysis. **Figure 2** shows the synthesis pathway

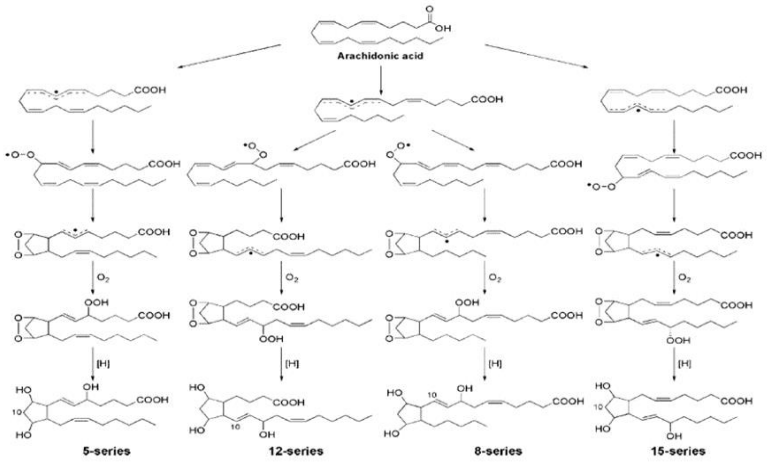


Figure 2. Biochemical synthesis of isoprostane

of F_2-IsoPs, particularly, F_2-IsoPs are formed as a result of peroxidation of arachidonic acid (AA) by free radicals. In vivo studies provide evidence that measurement of F_2-IsoPs in plasma or other body fluids can be used as an index to reflect levels of oxidative stress [28]. The primary metabolite of 15-F2t-isoprostane (the most common F2-IsoP), 15-F_{2t}-IsoP-M, is produced via β-oxidation and is less affected by renal production [29]. Another classical marker of lipid peroxidation, malondialdehyde (MDA), may vary in response to oxidized lipids present in the diet [30]. Isoprostanes, our chosen biomarker, are less affected by lipids in the diet [30].

1.3 Evidence for an association between tea consumption and oxidative stress

Clinical and epidemiologic studies, as well as trial results, support a potential inverse association between tea consumption and oxidative stress. In a clinical study of 34 men and women in Portugal, Coimbra et al. [31] found that regular green tea drinking (1L/d) for 4 weeks could reduce the serum levels of the lipid peroxidation products, malondialdehyde (MDA) and malonyldialdehyde+4-hydroxy-2(E)-nonenal (MDA+4-HNE) . In a randomized double-blind, placebo-controlled trial of 56 subjects, Bogdanski et al. [32] observed that the total antioxidant status was improved for people in the tea-extract treated arm. Klaunig et al. [33] reported that green tea consumption reduced oxidative DNA damage, lipid peroxidation, and radical generation. Particularly, they observed that green tea reduced 8-hydroxy-2' -deoxyguanosine (8-OHdG) in white blood cells and MDA in urine. Panza et al. [34] found that green tea reduced the post-exercise concentration of lipid hydroperoxide and increased the levels of total antioxidants such as polyphenols and glutathione (GSH) in a sample of 14 men. Hodgson et al. [35] conducted a cross-over study investigating the association between urinary F_2-isoprostanes and tea drinking (green and black tea) among a group of people (N=35) with borderline hypertension

or mild elevated serum lipid, but they reported a null association. Dorjgochoo et al. [29] investigated 845 Chinese women in a cross-sectional study and observed a non-significant association between any tea drinking and urinary levels of F_2-IsoPs and 15-F_{2t}-IsoP-M after multivariable adjustment. Limitations of these previous studies require further investigation of the effect of tea drinking and oxidative stress. Most studies investigating this association had small sample sizes, and this could introduce imprecision for statistical analysis yielding a wide confidence interval. Many of these studies used clinical populations, which may compromise generalizability if participants differ from the general population in aspects of disease, diet pattern, and health related behaviors. Previous studies reported the effect of green tea, but few of them investigated that of black tea and other types of herbal tea. Therefore, it is necessary to use a larger, population-based sample to disentangle the underlying association between tea consumption and oxidative stress levels.

1.4 Evidence for an association between tea consumption and breast cancer

Population-based studies have yielded heterogeneous results regarding the association between tea consumption and breast cancer risk. To our knowledge, 15 cohort studies have reported 17 effect estimates (1 study reported effect measures from 2 sub-cohorts, and 1 study reported effect measures for green tea and black tea separately) for the association between tea consumption and breast cancer risk. Of these, 1 obtained a statistically significant and positive association (1 cup/d: RR=1.12, 95% CI 1.01-1.24 [36]) and 2 reported statistically significant and inverse associations between tea consumption and breast cancer risk. An inverse association for tea drinking (>3 cups/d) and breast cancer risk (RR=0.79, 95% CI 0.62-1.01) was reported by Fagherazzi et al. [37] in a cohort study in France that identified 2,868 breast cancer cases during

a median 11-year follow-up. In a second French study, Hirvonen et al. [38] reported a strong inverse association between tea consumption and breast cancer risk (RR=0.43, 95% CI 0.20-0.93); however, estimates were based on only 95 breast cancers diagnosed among 4,396 women during a median 6.6 year follow-up. The remaining 14 effect measures were not statistically significant (**Figure 3**); however, based on the direction of the effect estimate, 2 appeared null, 5 reported positive associations, and 7 reported inverse associations.

By synthesizing these effect measures by a random-effects model, the pooled RR was 1.00 (95% CI 0.94-1.07), suggesting that there is a no association between tea consumption and breast cancer risk. However, some methodological limitations make this conclusion less robust. Six studies treated any tea drinking as the exposure of interest, ignoring the biochemical heterogeneity between herbal and non-herbal tea, since only the latter could contain potential anti-tumor agent (EGCG and caffeine). There is a biochemical heterogeneity among non-herbal teas; for example, black tea extract had higher concentration of caffeine than green tea [13]; however, 3 studies treated green tea and black tea as the same in analysis, which could obscure the true effect of the one containing higher anti-tumor agents. Four studies conducted in Asia reported the effect measures of green tea and black tea separately, but they may be less generalizable to the US population based on potential modifying factors. For example, Bernstein et al. [39] reported that the estrogen levels were significantly higher among women in the US compared to China. In addition, the prevalence of overweight or obesity among the US women older than 40 is higher than 65% [40], whereas this number is less than 35% in China [41]. Serum estrogen level and obesity can directly and indirectly affect the bioactivity of estrogen receptor and induce the proliferation effect [42], and these hormonal effects can be stronger than nutritional chemopreventive effects from tea consumption; thus, this indicates a potential that the

association with tea should be much stronger among Asian women. Additionally, interpretation of the prior literature is limited by the diversity of previous study populations and differing levels of baseline risk. For example, 75% of the study population in Key et al. [43] were present in Hiroshima or Nagasaki bombing, suggesting that the modest associations with dietary factors could be obscured due to a relatively larger effects of ionizing radiation exposure. To address these limitations, our study will analyze the association between different types of tea and breast cancer risk within a well-characterized population of U.S. women.

The individual cohort studies reported the following results: In Japan, Suzuki et al. [44] synthesized the data of 2 cohort studies, which yield a total of 42,122 female participants. The authors observed a suggested positive association between green tea consumption and breast cancer risk (222 breast cancer cases, OR=1.32, 95% CI 0.83–2.10) for people consuming 3-4 cups of green tea per day, but the association was not apparent for women consuming 5 or more cups per day(OR=0.90, 95% CI 0.56–1.45). Also in Japan, Key et al. [43] investigated the association between green and black tea and breast cancer risk in a prospective study of 34,759 women, including 427 cases. Effect estimates for black (≥5 cups/wk: RR=1.10, 95% CI 0.82–1.48, 55 cases) and green (≥5 cups/wk: RR=0.86, 95% CI 0.62–1.21, 100 cases) tea were in different directions and not statistically significant. Boggs et al. [45] identified 1,268 breast cancer cases in a cohort of 52,062 at the US, and they reported a null association (2-3 times/d: IRR=1.02, 95% CI 0.78-1.32) between non-herbal tea consumption and breast cancer risk after an average follow-up of 10 years. Adebamowo et al. [46] followed a cohort (n=90,638) of participants for 8 years and identified 710 breast cancer cases in the US, and they reported that any tea drinking was not associated with the risk of breast cancer (RR=1.18, 95% CI 0.88-1.58). Bhoo-Pathy et al. [47] identified 681 breast cancer patients in a prospective cohort in the

Netherland with a sample size of 27,323, and they reported an inverse but non-significant association between any tea consumption and breast cancer risk (HR=0.90, 95% CI 0.68-1.19) after an average follow-up period of 9.6 years. Additionally, by using data from European Prospective Investigation into Cancer and Nutrition (EPIC) cohort, Bhoo-Pathy et al. [48] identified 10,198 breast cancer cases from 335,060 participants after 11 years follow-up and found that any tea drinking was not associated with breast cancer risk regardless of menopausal status (Premenopausal: moderately low: HR=0.98, 95% CI 0.80-1.21 moderately high: HR=0.97, 95% CI 0.79-1.20 Postmenopausal: moderately low: HR=1.00, 95% CI 0.93-1.08 moderately high: HR=0.98, 95% CI 0.91-1.06). By following 72,861 participants for 7.3 years in China, Dai et al. [49] identified 614 breast cancer cases and reported that regular green tea drinking was not associated with breast cancer risk (HR=1.04, 95% CI 0.88-1.26). Ganmaa et al.[50] investigated a cohort of 85,987 for a long period (22 years) and obtained 5,272 breast cancer cases using Nurses' Health Study (NHS), and they found that any tea drinking was not associated with breast cancer risk (2-3.9 cups/d RR=0.96, 95% CI 0.86-1.07). Iwasaki et al. [51] identified 931 breast cancer patients from a cohort of 97,432 after 11 years follow-up in Japan, and they reported that green tea consumption was associated with a positive but non-significant risk (1-2 cups/wk HR=1.19, 95% CI 0.80-1.76) of breast cancer. Ishitani et al.[52] identified 1,171 breast cancer cases from 39,310 participants in Women's Health Initiative (WHI), and they found that any tea drinking was not associated with breast cancer risk (≥2 cups/d: RR=1.03, 95% CI 0.85-1.25). Zheng et al. [53] identified 1,015 breast cancer patients from a cohort of 35,369 after 8 years observation in the US, and they reported that non-herbal tea consumption was associated with an increased but non-significant risk of breast cancer (≥2 cups/d RR=1.14, 95% CI 0.92-1.41). Goldbohm et al. [54] observed 605 breast cancer patients from a sub-cohort of 1,598 from a

prospective cohort study in the Netherland, and they found black tea was positively and non-significantly associated with the risk of breast cancer risk (3 cups/d RR=1.18, 95% CI 0.76-1.83).

Nine case-control studies, involving 13 effect estimates, have reported on the association between tea consumption and breast cancer risk (**Figure 3**). Overall, 3 significant and inverse effect estimates were obtained. Zhang et al. [55] found that regular green tea consumption was associated with a 40% relative decrease in the risk of breast cancer in southern China (1,009 cases, 1,009 controls; OR[once per day]=0.57, 95% CI: 0.38-0.85). Also in China, Shrubsole et al. [56] conducted a population-based case-control study (case:3,371, control: 3,380) and found that regular green tea drinking was associated with a reduced risk of breast cancer (OR=0.88, 95% CI 0.79-0.88). Wu et al. [57] also reported a significant inverse association of green tea consumption on breast cancer in the US (501 cases, 594 controls; OR=0.58, 95% CI 0.36-0.93), but not black tea consumption (OR=0.96, 95% CI 0.67-1.37). Of the rest of the non-significant effect measures, the direction of association was positive in 3 studies, inverse in 4 studies, and null in 3 studies.

The synthesized effect measures in random-effects model indicated a slightly inverse association between tea consumption and breast cancer risk (RR=0.91, 95% CI 0.80-1.03). However, considerable methodological limitations also exist in these studies. Four case-control studies reported effect of any tea consumption, which obscured the potential chemopreventive effect of non-herbal tea. Four studies were conducted among Asian population, compromising generalizability to the US population. Most importantly, cancer development is a long-term process and it can take a long time period from tumor initiation to clinical diagnosis [58], thus, past exposure is etiologically more important than recent exposure. However, only 1 case-control study measured tea consumption 5 years before index date. Some preclinical symptoms may

appear within a short period prior to cancer diagnosis, and this may make patients change diet habits; therefore, measuring tea consumption in this recent period may not capture etiologically significant exposure.

The main outcomes of these case-control studies include: Baker et al.[59] studied whether black tea could reduce the risk of breast cancer in a hospital-based study in the US, and reported a null association for premenopausal breast cancer (398 cases, 480 controls; 1 cup/d: OR=0.94, 95% CI 0.60-1.46) and possible positive association for postmenopausal breast cancer (1534 cases, 1415 controls; 1 cup/d: OR=1.17, 95% CI 0.92–1.49); however, neither of these estimates are statistically significant. Ewertz et al.[60] identified 1,486 cases and 1336 controls in a case-control study conducted in Denmark, and they reported a null association for any tea drinking (3-4 cups/d: HR=0.98, 95% CI 0.76-1.25). McLaughlin et al. [61] conducted a case-control study of 3,234 women and found that any tea drinking was not associated with breast cancer risk (ever used vs. never used: OR=0.97, 95% CI 0.81-1.16). Rabstein et al. [62] conducted a population-based case-control study (case: 1,020, control: 1,047) in German, and they found that any tea drinking was associated with a reduced but non-significant risk (≥4 cups/d: OR=0.79, 95% CI 0.50-1.25) of breast cancer. Also in China, Li et al. [63] conducted a hospital-based case-control study (case: 756, control: 1,545); overall, they found that regular tea drinking (any type) was associated with an increased but non-significant risk of breast cancer (>3 cups/d OR=1.25, 95% CI 0.85-1.84). Inoue et al. [64] identified 380 breast cancer cases in a nested case-control study conducted in Singapore after 12 years observation, and they found that regular green tea drinking (daily OR=1.00, 95% CI 0.82-1.22) and black tea drinking (weekly OR=0.85, 95% CI 0.62-1.17) were not associated with breast cancer risk.

Biologic plausibility

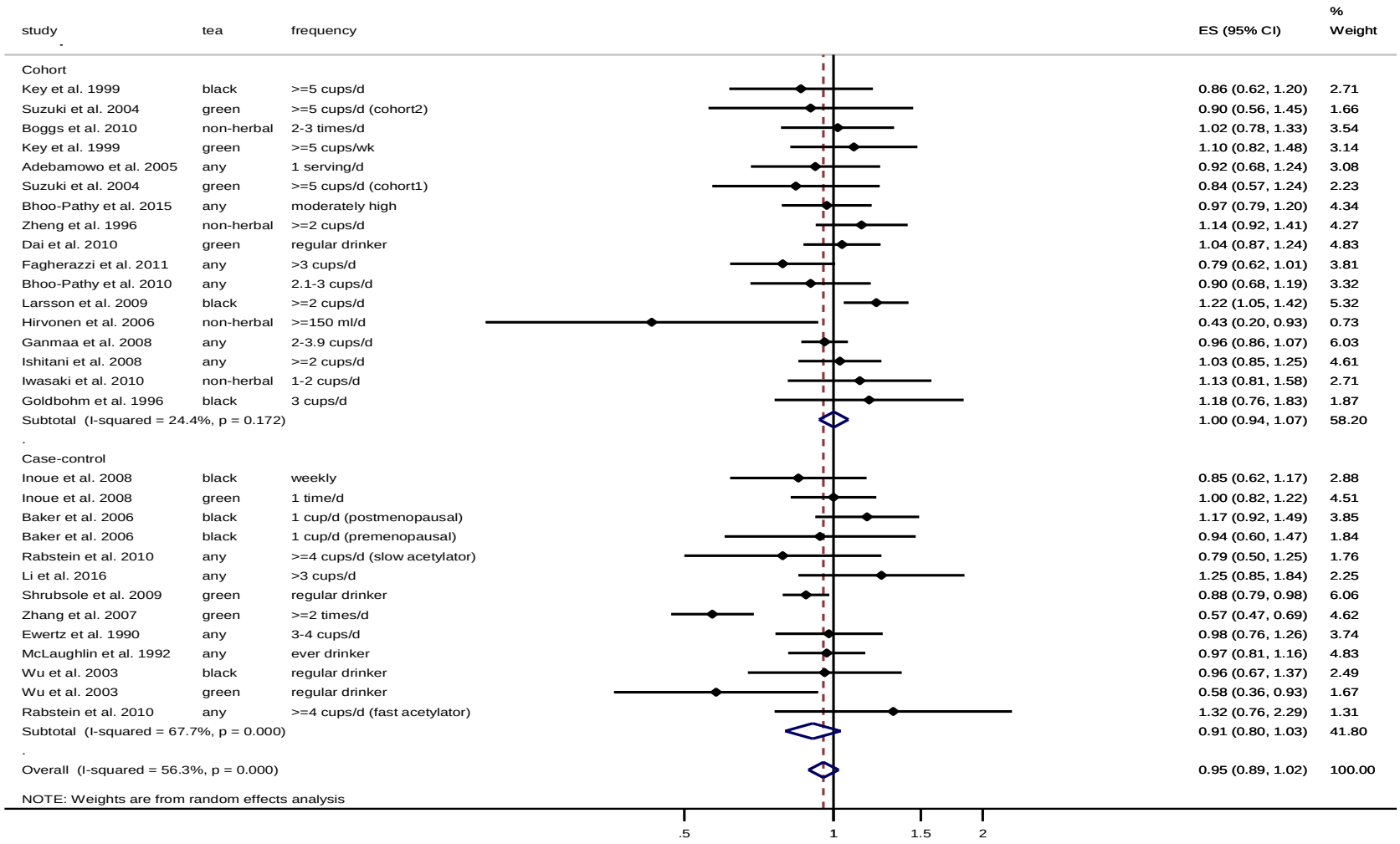
Several mechanisms may be involved in the effect of tea consumption to breast cancer risk. Particularly, previous studies reported that tea can lower oxidative stress levels by inhibiting lipid peroxidation, and this may play an important role in pathogenesis of breast cancer. A recent systematic review of 10 epidemiological studies [65] reported evidence that higher oxidative stress was associated with increased postmenopausal breast cancer risk. Among postmenopausal women, lower oxidative stress level among tea drinkers may thus translate to lower breast cancer risk.

Laboratory research [66] found that green tea extract, which contained EGCG, could reduce the levels of MDA and 4-HNE in male rats in their blood, liver, and brain tissue. In a randomized controlled trial of 35 people, Basu et al. [67] reported that green tea drinking (4 cups/day) significantly decreased levels of MDA in treatment group (-0.39±0.06 μM, $p<0.0001$) versus controls after 8 weeks of follow-up. *In vitro* studies reported that caffeine-derived oxygen-centered radical could be formed in chemical reaction between caffeine and hydroxyl radical [68], suggesting the anti-oxidative stress properties of black and green tea which contain substantial amount of caffeine. Laboratory science also supports a role of oxidative stress in tumor development and growth through somatic mutations [25]. Oxidative stress can cause damage to fundamental biological molecules in cells, including molecules regulating cell proliferation and repair, such as DNA and relevant proteins [25]. ROS can promote the formation of adducts of pyrimidines and alkyl radical [69]. This may cause DNA double-strand breaks, which can lead to mutations and chromosomal rearrangements [70]. The control of DNA transcription and maintenance of DNA synthesis by NF-κB protein complexes and *P*53 gene are also sensitive to oxidative stress [24, 71]. Hursting et al. [72] reported that oxidative stress could

lead to adverse genetic change and DNA damage by triggering the P13K/Akt signaling, which could increase the risk of tumor formation. DNA mutation plays an important role in process of carcinogenesis and higher levels of oxidative DNA lesions (8-OH-G) were observed in many malignant tumors, which strongly suggests that such damage is associated with the etiology of cancer [73]. Additionally, oxidative stress can influence the activity of cellular receptors that are associated with cell growth or differentiation, which can affect the risk of tumor development [74]. For example, Leon-Buitimea et al.[75] found that the phosphorylation levels of EGFR were increased with the elevation of oxidative stress levels in MCF-10A cells which belong to a non-tumorigenic epithelial cell line. This supports the hypothesis that increased human mammary cell activation could be associated with oxidative stress via affecting EGFR-dependent signaling pathway.

Several studies reported associations between oxidative stress and breast cancer. Dai et al. [76] reported differential effect measures between oxidative stress and breast cancer risk by menopausal status in a case-control study (case: 436, control: 852); although most of their effect measures were not statistically significant, higher levels of urinary isoprostane were positively associated with breast cancer risk among postmenopausal women (15-F_{2t}-IsoPM [3rd tertile]: OR=1.47, 95% CI 0.86-2.53) but were inversely associated with the risk among premenopausal women (15-F_{2t}-IsoPM [3rd tertile]: OR=0.68, 95% CI 0.41-1.14). In a case-control study, Rossner et al. [77] reported that urinary 15-F_{2t}-IsoP levels were significantly associated with an increased risk of breast cancer among postmenopausal women (≥0.99 vs. <0.45 nmol/mmol creatinine, OR=2.06, 95% CI 1.15-3.69) but such association was not observed among premenopausal women (≥0.99 vs. <0.45 nmol/mmol creatinine, OR=1.85, 95% CI 0.91-3.76). Similar patterns were also reported by Nichols et al. [78] using a nested case-control study

Figure 3. Forest plot of available epidemiological data reporting association between tea and breast cancer risk



(N=453 cases, 901 controls) within the Sister Study. Nichols et al. [78] reported that oxidative stress was associated with a reduced risk of breast cancer among premenopausal women (350 cases, OR=0.55, CI: 0.31-0.96) after adjustment in a multivariable model.

Among premenopausal women, oxidative stress does not appear to increase breast cancer risk, and higher levels may in fact be associated with lower breast cancer risk [65]. Although oxidative stress can cause damage to important cellular molecules such as DNA and RNA, a low-to-moderate level of oxidative stress can stimulate immune system and induce leukocytosis [79, 80], which can enhance immune surveillance and inhibit tumor growth. However, the physiological function of immune system exhibits an age-related change, and the development of immunosenescence is associated with aging process [81, 82], suggesting that oxidative stress-induced cellular immune enhancement is less likely to be as strong in older women. Among women with relatively younger age, the ability to repair damaged DNA is much stronger compared to old women [83], and this can result in a lower probability of cancer development for younger women when other factors are similar. Given this evidence, oxidative stress-induced DNA damage can be weaker and oxidative stress-induced cellular immune enhancement can be stronger among premenopausal women, and this can partly explain a potential inverse association between oxidative stress and premenopausal breast cancer. Conversely, among postmenopausal women, with the decline in ability to repair damaged DNA and immunosenescence, oxidative stress is associated with an increased risk of breast cancer.

In addition to impacting oxidative stress, tea consumption may influence the risk of breast cancer via other pathways or mechanisms. The catechin EGCG, found in non-herbal tea, [11], has been found to have antitumor effects in pre-clinical research. Mittal et al. [84] reported that EGCG could reduce the viability and lead to apoptosis in breast carcinoma MCF-7 cells

without causing adverse effect on the growths of normal mammary cells. In addition, it was reported that EGCG could be cytotoxic to breast tumor cells regardless of estrogen receptor (ER) status [85]. Liang et al. [86] found that, after treatment with EGCG, breast tumor cells were substantially reduced in both ER positive and ER negative cell lines. ECG and EGC were also observed to have an inhibition effect to cancer growth. Vergote et al. [87] observed that EGC substantially inhibited the growth of breast tumor cell lines (MCF-7 and MDA-MB-231), but it did not affect the growths of normal breast epithelial cells.

As an alternative or additional pathway to EGCG exposure, beneficial effects of tea consumption may occur through its caffeine content. Non-herbal teas, even decaffeinated, contain some caffeine. Caffeine is a central nervous system stimulant [88], and some of its biological effects are inversely associated with the tumor initiation and promotion. Al-Ansari et al. [89] found that caffeine could up-regulate the expression of some tumor suppressor proteins such as p16, p21, p53, and caveolin-1 in active breast stromal fibroblasts, suggesting that caffeine may reduce the risk of breast cancer. Moreover, angiogenesis plays an essential role in breast cancer development, since solid tumors usually needs new blood vessels to transport nutrients before growing beyond 1-2mm [90], and caffeine may have the potential to prevent tumor promotion by inhibiting the angiogenesis. For example, by observing human umbilical vein endothelial cells, Li et al. [91] found that caffeine was associated with an inhibiting effect to angiogenesis and could induce endothelial cell death.

1.5 The NIEHS Sister Study

The proposed research will be conducted with data from the National Institute of Environmental Health Sciences (NIEHS) Sister Study. The Sister Study is a nationwide cohort

study that enrolled 50,884 women between the ages of 35 and 74 in the United States and Puerto Rico during 2003-2009. At enrollment, participants should have at least one sister who had been diagnosed with breast cancer but no personal breast cancer history [18]. During an enrollment home visit, study participants had height, weight, waist and hip circumference and blood pressure measured and contributed blood, urine, hair, toenail, and home dust samples using standard protocols [92].

As part of the enrollment interview, participants reported frequency of black and green tea consumption as well as cups consumed per serving. The clear definition and categorization of tea consumption allows us to investigate the association between tea and oxidative stress and breast cancer risk by different tea types in a dose-response manner. Moreover, as compared to treating any tea as the exposure of interest, investigation by tea types (black tea vs. green tea) also allows us to compare if association differs by tea types and discuss the underlying biochemical mechanism related to the heterogeneity.

1.6 Potential public health impact

In the United States, female breast cancer exerts tremendous burden on women's health care and health system. Mariotto et al. [93] estimated that the United States national expenditure of cancer care in 2010 was $124.57 billion, and medication cost associated with female breast cancer was ranked top ($16.50 billion). In addition, the authors predicted that there will be a 32% relative increase in the cost by the year of 2020 [93]. Thus, a potential chemopreventive effect of tea consumption on breast cancer development may identify a low cost strategy for breast cancer prevention. Moreover, tea is one of the most popular drinks in the world, second only to water

[94], and is a very accessible product. These factors make it an ideal chemopreventive agent for breast cancer prevention

CHAPTER 2. AIMS

Tea originated from Southwest China, and was used as a medicinal drink in ancient periods. To date, tea remains one of the most popular beverages in the world, and contains many chemicals that can be beneficial to health. For example, green tea and black tea contain epigallocatechin gallate (EGCG) and caffeine which have been found to have some preventive effect for cardiovascular disease, diabetes, and cancer [95-97]. EGCG is the main catechin in tea from *Camellia sinensis,* and *in vitro* studies suggest that EGCG may inhibit the growth of human cancer cell lines and induce apoptosis [19]. Caffeine is a central nervous system stimulant, and may have the potential to prevent tumor promotion by inhibiting the angiogenesis [91].

Breast cancer is the most commonly malignant tumor among women worldwide with 1.5 million new breast cancer cases diagnosed annually worldwide [98, 99]. The pathogenesis of breast cancer involves several biological mechanisms, including oxidative stress [76, 100, 101]. Oxidative stress describes an imbalance between production of free radicals and anti-oxidant mechanisms, which can disrupt cellular proliferation and repair [23]. Previous studies support a positive association between oxidative stress levels and postmenopausal breast cancer risk [76, 77].

Some human studies have suggested an inverse association between tea consumption and oxidative stress. However, these studies have been limited by small sample sizes (N<50) and a

singular focus on green tea consumption, despite potentially beneficial constituents such as EGCG in other types of tea including black tea [12, 13].

Epidemiologic studies of tea consumption and breast cancer risk have rarely examined potential differences between non-herbal tea and herbal tea, despite potential biologic heterogeneity due to EGCG and caffeine content. We propose to use the National Institute of Environmental Health Sciences (NIEHS) Sister Study to address these limitations. The Sister Study is a nation-wide cohort of 50,884 women ages 35 to 74 years with a family history of breast cancer enrolled during 2003-2009 and actively followed for breast cancer outcomes [85]. In a sample of ~900 Sister Study participants, baseline urinary oxidative stress has been measured by gas chromatography/negative ion chemical ionization mass spectrometry [86]. Participants also reported their consumption frequency of black and green tea using food frequency questionnaires [87] allowing for a more detailed analysis of tea consumption patterns. The aims of this proposal are:

1. To investigate the cross-sectional association between tea consumption (including type and frequency) and oxidative stress, as measured by urinary isoprostane concentrations. We hypothesized that tea consumption was inversely associated with oxidative stress levels in a sample of 889 premenopausal women without breast cancer enrolled in the Sister Study.

2. To investigate the association between tea consumption (type and cups/week) and breast cancer risk in the NIEHS Sister Study prospective cohort of 49,214 women. We hypothesized that tea consumption was associated with lower breast cancer risk. The association

would be more substantial for breast cancer with certain characteristics (e.g. ER+ or postmenopausal breast cancer).

Tea is a non-toxic and accessible agent in daily life, and may have beneficial health effects for breast cancer risk and other chronic diseases. These characteristics make it an ideal breast cancer prevention strategy.

CHAPTER 3. METHODS

3.1 Study design

To examine the association between tea and oxidative stress in Aim 1, we proposed to analyze a cross-sectional sample of ~900 premenopausal women enrolled in the Sister Study. These women were previously selected as control participants for a nested case-control of oxidative stress and premenopausal breast cancer. To be eligible for selection, women had to meet the following criteria at study enrollment: 1) aged 35 to 54 years, 2) premenopausal (defined as having at least one menstrual cycle in the previous 12 months), 3) at least one intact ovary, and 4) a blood and urine sample collected at baseline. Women aged 54 or younger were considered premenopausal if the only reason for not experiencing menses was hysterectomy (without bilateral oophorectomy). Two controls were matched to each identified breast cancer case on the basis of age and enrollment year, and these controls were free of

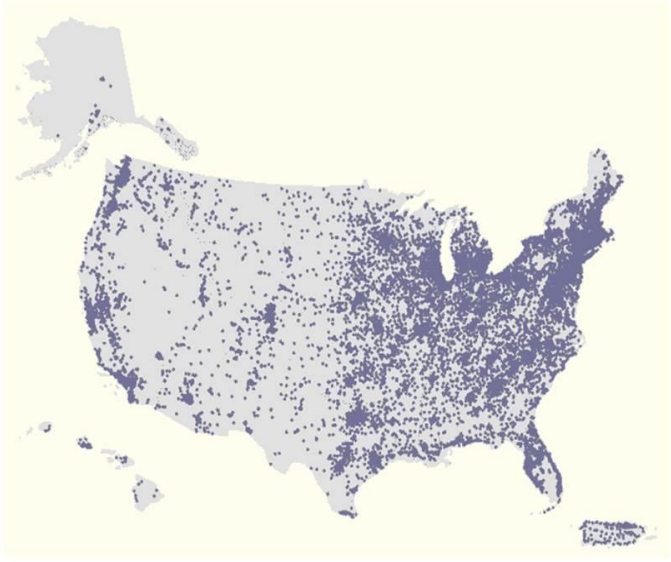


Figure 4 . Source of study population

breast cancer at the time of their matched case's diagnosis.

To examine tea consumption and breast cancer risk in Aim 2, we proposed to use the full NIEHS Sister Study cohort (N=50,884) with prospective follow-up for incident breast cancer diagnoses. Briefly, Sister Study eligibility criteria included: 1) a sister (living or deceased) who was diagnosed with breast cancer; 2) aged 35-74 years at study entry; 3) no personal breast cancer history; 4) United States or Puerto Rico residents (**Figure 4**).

3.2 Data collection in the NIEHS Sister Study

Enrolled Sister Study participants completed telephone interviews that sought information on reproductive and medical history, environmental and occupational exposures, and lifestyle factors. Blood and other biological and environmental specimens were collected during scheduled home visits. Anthropometric data, such as height, weight, waist circumference, and blood pressure were measured at the home visit as well. Participants have been followed annually to update contact information and report diagnosis of selected medical conditions including breast cancer. Participants complete comprehensive questionnaires about changes in exposures and health status every 2–3 years. Study retention has been high, with 92% of participants completing their most recent scheduled follow-up activity (annual update or comprehensive questionnaire) [102].

3.3 Exposure, outcome, and covariate measurement

3.3.1 Measurement of tea consumption, the primary exposure in Aims 1 and 2

Tea consumption was measured at baseline using the self-administered Block 98 food frequency questionnaire (FFQ) [103], which included black and green tea consumption during the previous 12 months. Within the FFQ, participants reported their frequency of tea

consumption and the cups consumed each time. Frequency was reported at 9 levels, ranging from "never" to "everyday". Participants reported how many cups of tea they consumed each time as "1 cup, 2 cups, 3-4 cups, and 5 or more cups."

3.3.2 Oxidative stress measurement, the outcome for Aim 1

As part of study enrollment activities in 2003-2009, study participants collected 60 ml of first-morning urine in a study-provided collection cup which was pre-tested for concentration of phthalates before being used. During the urine collection, no preservative was added into the sample. Participants recorded times of last urination before the collection and cigarette or alcohol consumption during the past 24 hours. Urinary specimens did not include preservative, but study subjects were asked to refrigerate the sample until it was picked up by the examiner that day [104]. Examiners prepared the samples for shipment on ice. On receipt by the study biorepository laboratory, urine samples were aliquotted and stored at -80℃. In 2012, as part of a nested case-control study of oxidative stress and premenopausal breast cancer risk, urinary levels of F2-IsoP and 15-F2t-IsoP-M were measured utilizing gas chromatography/negative ion chemical ionisation mass spectrometry (GC/NICI-MS) at the Eicosanoid Core Laboratory at Vanderbilt University Medical Center. Protocols for these chemical analysis approach and procedures have been described [105-108]. Before chemical analysis, laboratory investigators first thawed the sample and diluted 0.250 ml urine in 10 ml pH 3 water, and they acidified the sample to pH 3 with 1N hydrogen chloride (HCL). The Sister Study stored the urinary samples in several straws (0.5 ml), and one straw is used for each chemical analysis. Thus, there is no potential for previous freeze/thaw cycles. The storage time of the urinary sample will not affect the isoprostane measures in it. Urine is a better matrix than blood/plasma in measuring

biomarkers related to oxidative stress, for it has a lower organic/inorganic metal content that can be oxidized [109]. Morales et al. [107] compared the concentration of urinary 15-F2t-isoprostane from 20 participants and found that storage of urine samples at -70℃ for 18 months did not significantly affect the concentration. The GC/NICI-MS was carried out on an Agilent 5973 Inert Mass Selective Detector which was accompanied with an Agilent 6890n Network GC system (Agilent Labs, Torrance, CA) interfaced with an Agilent computer. The lower limit of detection of F2-isoprostane was within the range of 4 pg/mL. In biological fluids, the precision of this analytical method was +6% and the accuracy was 94% [106].The lower limit of sensitivity for the metabolite was about 8 pg/mL, and the precision and accuracy of this assay was +7% and 95%, respectively [107]. A total of 77 batches were run, and each batch contained 18 study participant samples and 2 quality control (QC) samples. There were 6 trios among the 18 subjects' samples, each trio consisted of 1 case and 2 controls. During chemical analysis, cases and controls were analyzed together within each batch to remove the potential for batch effects to bias case-control analyses. All laboratory investigators were masked to case-control or QC status. The coefficient of variation for QC duplicates was 16.0% for F2-IsoP and 12.5% for 15-F2t-IsoP-M, respectively [110] . Urinary concentrations of F2-IsoP and 15-F2t-IsoP-M were adjusted for creatinine (ng/mg of creatinine) to correct for urine diluteness, but data of unadjusted F2-IsoP and 15-F2t-IsoP-M and urinary creatinine levels were also recorded.

3.3.3 Breast cancer identification, the outcome for Aim 2

In the Sister Study, incident breast cancers were initially self-reported, followed by medical record validation by trained abstractors. Breast cancer diagnoses could be reported via annual health updates, biennial or triennial detailed questionnaires, or reported anytime using the

study website or phone line. Approximately 6 months after breast cancer diagnosis, participants were asked to complete a breast cancer follow-up questionnaire, including permission to obtain relevant medical records. Medical records were reviewed by trained abstractors to verify the breast cancer diagnosis, tumor characteristics, and treatment details. The National Death Index and the Social Security Death Index were used to obtain dates and causes of death.

Estrogen receptor status of the tumor was collected both by self-report and by medical record review, with high agreement between the two (99% for ER positive invasive breast cancers and 83% for ER negative) [111]. Menopausal status at breast cancer diagnosis was defined according to whether participants were within 12 months of their last menses (premenopausal) or ≥ 12 months of last menses based on questionnaire responses before and after diagnosis. In addition, patients who had the following characteristics were also categorized as postmenopausal: 1) bilateral oophorectomy, 2) hysterectomy with ovary tissue, uterine artery embolization, endometrial ablation, or miscellaneous procedures at an age older than 55, 3) chemotherapy, radiation, or other treatment permanently stopping their period prior to spontaneous menopause, or 4) use of ovary suppressing medications that suppress menstrual periods at an age older than 55.

3.3.4 Covariate measurement

Age

Date of birth was collected at study enrollment and age will be included as a covariate in cross-sectional analyses and as the time scale in time-to-event analyses. Among Japanese women, Inoue et al. [112] reported that the distribution of age among tea non-drinkers and drinkers was different; specifically, 58.4% of non-drinkers compared to 78.1% of heavy tea

drinkers (≥7 cups/d) were older than age 50. Aging is an inherent process within human molecular, organ, and system levels, and some evidence proposes that ROS increases with age [113]. Breast cancer is more prevalent among older women, and SEER reported that 10.7% of breast cancer patients were diagnosed before 45 years, and 25.7% of cases were diagnosed between 55 and 64 years based on data from 2009 to 2013 [2].

Race/ethnicity

Race and ethnicity were asked at enrollment and categorized as American Indian or Alaska, Asian, Black or African American, Native Hawaiian or other Pacific Islander, and White. Ethnicity was classified by the question, "Do you consider yourself to be Hispanic or Latina?"

Racial or ethnic groups are associated with different patterns of tea consumption, for example, Grigg et al. [114] reported people in the United Kingdom (UK) consumed more tea (2.5 kg per capita per year) than people in the US (0.4 kg per capita per year) and South Africa (0.6 kg per capita per year). In the U.S., Morris et al. [115] found African Americans had higher levels of oxidative stress than whites, although Nichols et al. [78] observed no significant association between race and oxidative stress in the Sister Study. Yost et al. [116] reported there was a discrepancy in breast cancer incidence rate across different race/ethnicity groups based on samples collected in California, they found that White (192.7 per 10,000) and Black (155.4 per 10,000) women had higher incidence rate and Asian (96.4 per 10,000) women had the lowest.

Income and education level

Income and education level were self-reported at enrollment and included the total income from all household members before taxes in the past year in the following categories:

less than $20,000, $20,000 to $49,999, $50,000 to $99,999, $100,000 to $200,000, and more than $200,000. Study subjects also reported the number of adult and minor household members and the highest level of school they had completed categorized into 10 levels, ranging from no formal schooling to doctoral degree.

By investigating 6,928 women in Shanghai, Shrubsole et al. [56] found people who never drank green tea had relatively lower education level and income. In addition, Nichols et al. [78] found that education and income levels were inversely associated with the urinary concentration of 15-F_{2t}-IsoP-M in the Sister Study. Income and education level can reflect people's socioeconomic status, which can influence their chance of obtaining preventive intervention and or being diagnosed with breast cancer. Therefore, income and education level were treated as confounders in our analysis of tea consumption and oxidative stress or breast cancer.

Coffee consumption

Coffee consumption was measured using the exact same scenario in tea consumption. Regular coffee (non-decaf) contains substantial amount of caffeine and will be included in analysis. Specifically, we measured the frequency of consumption at 9 levels from never drink to drink every day; we also measured the cups of coffee consumed each time. Participants reported how many cups of tea they consumed each time as "1 cup, 2 cups, 3-4 cups, and 5 or more cups." Coffee consumption was treated as an effect modifier since both tea and coffee can contain caffeine which was potentially associated with breast cancer development.

Red meat consumption

Red meat consumption includes beef, pork, and lamb and included frequency and portion

sizes in 4 levels (1/8 lb, 1/4 lb, 1/2 lb, and 3/4 lb). Schwarz et al. [117] interviewed 932 women in a cross-sectional study conducted in Austria, and found that women who were regular tea consumers ate less meat, which supported that tea consumption could be inversely associated with red meat intake. Guo et al. [118] reported that high levels of red meat consumption was associated with an increased risk of breast cancer by synthesizing evidence from 14 cohort studies.

Total energy intake

Total energy intake was calculated from the Block 98 FFQ [103]. People who consume higher energy tend to eat more food, and this elevates their chance of tea drinking with meals. A clinical research study of 10 adults (8 females 2 males) found that an 8-week alternate day calorie restriction significantly reduced serum levels of 8-isoprostanes, a biomarker of oxidative stress [119]. By investigating 53,793 Japanese women, Iwasaki et al. [51] reported that women consuming more than 10 cups of green tea per day had higher daily energy intake as compared to those who consumed less than 1 cup each week. In a multicenter cohort study, Zhang et al. [120] reported that high levels of energy intake might increase breast cancer risk independent of physical activity and body size. Decarli et al. [121] reported energy intake was associated with an increased breast cancer risk after adjusting for other macronutrients and alcohol consumption, particularly, the risk was increased for 1% when energy intake was increased for 100 kcal.

Caffeine

Both black and green tea contain caffeine which was found to have anti-oxidative stress and anti-tumor effects in experimental and clinical research [50, 52, 68, 88, 89, 91]. In our

analysis, caffeine in black tea was calculated from the FFQ by NutritionQuest [34]. The USDA Food Composition Databases indicated that one cup of black tea contains 47.2 mg caffeine [122]. Caffeine of coffee (regular and decaffeinated) and green tea was assigned based on the USDA Food Composition Databases [122] as 95.2 mg caffeine per cup of regular coffee, 2 mg caffeine per cup of decaffeinated coffee, and 24.8 mg caffeine per cup of green tea.

Physical activity

At baseline, study participants were asked to report the number of hours per week they spent engaging in specific activities, and weekly energy expenditures were calculated using the metabolic equivalent (MET) values for each activity as listed in established guidelines [123]. Total physical activity was estimated by summing the MET-h/week of sports or exercise sessions and daily activities.

Physical activity may be a confounder for the association between tea consumption and oxidative stress or breast cancer risk. By investigating 15,367 Japanese women, Inoue et al. [112] reported that 15.4% of black tea non-drinker had at least 2 times of physical exercise per week, 16.1% of moderate (1-2 cups/d) black tea drinker had at least 2 times of physical exercise per week, and 18.8% of frequent (3+ cups/d) black tea drinker had at least 2 times of physical exercise per week. These numbers suggest a potential connection between tea consumption and physical activity level. In addition, by investigating 888 participants in the Sister Study, Anderson et al. [110] found that physical activity was inversely associated with F_2-IsoPs (P_{trend}=0.003). By synthesizing 48 observational study in a systematic review, Monninkhof et al. [124] reported that physical activity was associated with a 15-20% reduction in the risk of breast cancer.

Smoking

At baseline, participants were asked, "Have you smoked at least one cigarette per day, on average, over the past 12 months?" Study participants were subsequently classified as current, former and never smokers. Participants also reported the years that they had smoked at least one cigarette per month across the life course, and the number of cigarettes per day, to create a measure of cumulative pack-years, and also an age grid of age-specific smoking patterns.

Smoking may be a confounder for the association between tea consumption and oxidative stress or breast cancer risk. By investigating 15,367 Japanese women, Inoue et al. [112] reported that frequency of tea drinking was inversely associated with cigarette smoking. Within the Sister Study, Nichols et al. [78] reported that F_2-IsoPs and 15-F_{2t}-IsoP-M were associated with smoking status; current smokers had higher urinary concentrations of these biomarkers. By analyzing 73,388 women in the American Cancer Society's Cancer Prevention Study II (CPS-II) Nutrition Cohort, Gaudet et al. [125] reported that active smoking was significantly associated with breast cancer risk (HR=1.24, 95% CI 1.07-1.42, 3,721 cases). These epidemiological studies support our hypothesis that smoking may be a confounder in our analyses.

Alcohol consumption

At enrollment, participants completed a self-administered Block 98 FFQ [103]. The FFQ included alcohol consumption in the past 12 months, including beer, non-alcoholic beer, wine or wine coolers, and liquor or mixed drinks. Consumption frequency and glasses consumed at each time were recorded.

Alcohol consumption may be a confounder of associations with breast cancer. Wu et al. [31] reported a positive correlation between alcohol consumption and tea drinking; particularly, 15.0% of light tea drinkers consumed alcohol, 30.9% of moderate tea drinkers consumed alcohol, and 46.0% of heavy drinkers consumed alcohol among 1,037 study participants in Taiwan. Inoue et al. [112] investigated 15,367 Japanese women and found that women consuming higher dosage of tea drank less alcohol. Further, a recent systematic review and meta-analysis [126] included 98 epidemiological studies and reported alcohol drinking was associated with a 22% (95% CI: 9–37%) excess risk of breast cancer. These studies suggest that alcohol consumption may be associated with tea consumption and is an independent predictor of breast cancer risk, making it a potential confounder in our analyses.

Alcohol consumption can also be an effect modifier for association with breast cancer. Alcohol consumption may affect the risk of breast cancer via impacting the estrogen level of the blood, and it has been reported that alcohol usage was positively associated with postmenopausal estrogen level in blood [127]. A cross-sectional study involving 130 Chinese women found that blood level of estrone was significantly heterogeneous ($p=0.03$) across green tea drinker (25.8 pg/ml), black tea drinkers (35.0 pg/ml), and tea non-drinkers (29.5 pg/ml), and estradiol was marginally heterogeneous across these participants ($p=0.08$) [128], suggesting that tea drinking was associated with estrogen level. Since breast tumor initiation can be a downstream event of elevated estrogen level [129], alcohol consumption may modify the association by impacting estrogen.

Epidemiological studies support this connection. Wu et al. [57] found inverse associations between green tea consumption and breast cancer risk among both alcohol drinkers and non-drinkers; however, the effect measure of drinkers (OR=0.20, 95% CI 0.06-0.66) was

more substantial than that of non-drinkers (OR=0.74, 95% CI 0.52-1.05). This suggests a potential interaction between tea consumption and alcohol usage in carcinogenesis of breast cancer.

Body mass index (BMI)

Height and weight were measured at baseline during the home visit by trained examiners using digital self-calibrating scales and metal measuring tapes. Measurements were taken three times without shoes, and the outcomes were rounded to the nearest whole pound for weight and quarter of an inch for height. BMI was calculated as height/(weight)^2 in kg/m^2. When examiner measurements are not available, self-reported values are used.

Obesity may be a key confounder of association between tea consumption and oxidative stress or breast cancer. Inoue et al. [112] reported that green tea drinkers had a relatively high level of physical activity and fruit consumption than non-drinkers; these factors indicated a relatively healthy lifestyle and were shown to be inversely associated with obesity [130, 131]. Fruit consumption and physical activity can be indicator for health lifestyle, and obesity can be the outcome of unhealthy lifestyle. Thus, we hypothesized that tea consumption could be indirectly and inversely associated with obesity. Evidence for a positive association between obesity and of oxidative stress levels was reported by the parent nested case-control study in the Sister Study [78]. Obesity is also an established risk factor for postmenopausal breast cancer [132]. In postmenopausal women, androgens can be converted to estrogen in peripheral fat tissue by aromatase and increase breast cancer risk [133, 134].

BMI can also be conceptualized as a potential effect modifier of the association between tea consumption and oxidative stress, as well as of the association between tea consumption and

breast cancer risk among postmenopausal women. Tea was reported to prevent plasma lipid peroxidation whereas obesity was shown to be an independent risk factor for plasma lipid peroxidation [66, 135]. Isoprostanes are the downstream biomarker of lipid peroxidation, thus, obesity can modify the association between tea and urinary isoprostane levels. EGCG derived from tea can downregulate the function of estrogen receptor on breast tumor cell, which may inhibit breast cancer cell proliferation before it moves into the stage of tumor promotion [22], but obesity-induced estrogen expression can activate estrogen receptor and result in downstream mitogenesis [136]. This suggests that obesity can modify the association via impacting signaling pathway of estrogen receptor. The association between tea consumption and oxidative stress is expected to be modest and may be more apparent among women in the normal range (18.5-24.9 kg/m^2) than overweight or obese ranges (>25.0 kg/m^2) of BMI. Similarly, the association between tea consumption and breast cancer risk may also be more readily detected among women with BMI 18.5-24.9 kg/m^2.

Menopausal status

Menopausal status at breast cancer diagnosis was defined according to whether participants were within 12 months of their last menses (premenopausal) or ≥ 12 months of last menses based on questionnaire responses before and after diagnosis. In addition, patients who had the following characteristics were also categorized as postmenopausal: 1) bilateral oophorectomy, 2) hysterectomy with ovary tissue, uterine artery embolization, endometrial ablation, or miscellaneous procedures at an age older than 55, 3) chemotherapy, radiation, or other treatment permanently stopping their period prior to spontaneous menopause, and 4) use of ovary suppressing medications that suppress menstrual periods at ages older than 55.

Menopausal status can be an effect modifier between tea consumption and breast cancer risk. Epidemiologic studies suggested that associations between tea consumption and breast cancer risk were in different directions when results were stratified by menopausal status. Li et al. [63] reported that tea consumption was inversely associated with the risk of breast cancer among premenopausal women (OR=0.62, 95% CI 0.40-0.97) but positively associated with the risk among postmenopausal women (OR=1.40, 95% CI 1.00-1.96). Although these findings contradict our primary hypothesis, they illustrate the potential for differential association between tea consumption and breast cancer risk.

Sleep duration

Although the association between sleep duration and breast cancer risk remains controversial, epidemiologic evidence suggests night work shift and exposure to light during night is associated with a higher breast cancer risk [92]. In addition, a good sleep pattern is associated with a healthier lifestyle pattern, which is indirectly associated with tea drinking. Therefore, we treated sleep duration as a confounder. Sleep duration was measured by a self-report approach at baseline.

3.4. Analysis plan for Aim 1: Tea consumption and oxidative stress

3.4.1 Hypothesis

For aim 1, the outcome of interest was oxidative stress as measured by urinary concentrations of F2-IsoP and 15-F2t-IsoP-M. The exposure of interest was type and frequency of tea consumption categorized as green and black tea. We hypothesized that tea consumption was inversely associated with oxidative stress.

3.4.2 Statistical analysis

Black and green tea consumption was categorized into 4 levels (0, <1, 1-<5, and ≥ 5 cups/week). Cups consumed each week was calculated by multiplying frequency of consumption (times per week) and serving size (cups consumed each time) together. Tea consumers with missing serving size information were assigned a serving size of 1 cup per serving. We first checked the normality of F2-IsoP and 15-F2t-IsoP-M via histograms, and a log-transformation may was conducted since the distributions of these variables were not normally distributed. Descriptive statistics included means and standard deviations of continuous covariates and proportions of categorical covariates and tea consumption.

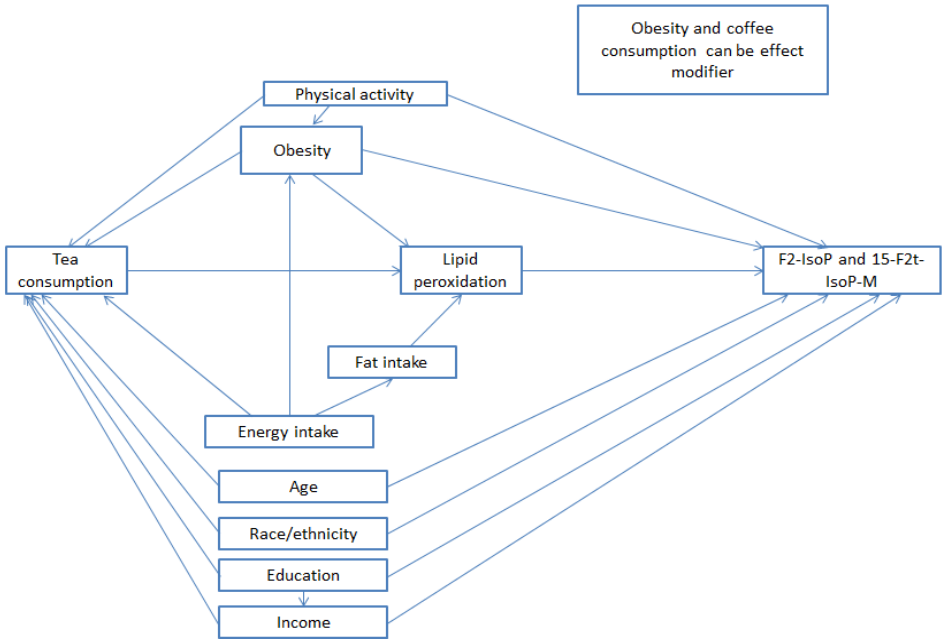
A linear regression was applied to investigate the association between tea consumption and oxidative stress. In the model, tea consumption was the independent variable and log-transformed F2-IsoP and/or 15-F2t-IsoP-M were the dependent variable. The parent and metabolite biomarkers were treated as two separate biomarkers in the model. The metabolite, 15-F2t-IsoP-M, was produced from F2-IsoP under β-oxidation which was a normal process in human body. The role of β-oxidation was considered.

The assumptions of the linear regression (independence, linearity, normality, and equal variance) were examined by residual plot. The effect estimates (average difference in geometric mean of urinary F2-IsoPs and 15-F2t-IsoP-M) obtained in unadjusted linear regression were used to compare with the estimates obtained in multivariable model, and the change in the estimate could provide information about the sensitivity to statistical adjustment.

A multivariable model was built with adjustment for the following covariates: age, race, education, income, smoking, physical activity, BMI, and total energy intake. Confounder selection was based on use of a directed acyclic graph (DAG) and the prior literature (**Figure 5).**

$E[biomarker]= \beta_0+\beta_1*tea\ consumption+ \beta_2*age + \beta_3*BMI\ (or\ obesity)+ \beta_4*race/ethnicity+ \beta_5*physical\ activity+ \beta_6*smoking+ \beta_7*income+ \beta_8*education+ \beta_9*energy\ intake.$

Figure 5. DAG for aim 1



Subgroup analyses were conducted to assess potential effect modification by obesity and NSAID use. In order to investigate if the association differed between subgroups, we compared the point estimates and 95% CI of the geometric means obtained in each subgroup. In stratified analyses based on BMI, participants were classified as overweight or obese ($BMI \geq 25\ kg/m^2$) and normal or underweight ($BMI < 25\ kg/m^2$). In subgroup analyses based on coffee consumption, participants were classified as ever drinkers

and non-drinkers. In addition to subgroup comparison, we used log-likelihood ratio test to assess if the interaction product term was statistically significant in the multivariable model.

3.4.3 Power calculation

The null hypothesis for this analysis was that oxidative stress was not associated with tea consumption. The reference group in analysis was women never drinking any tea, and comparison groups include women who drank herbal, green, or black tea. We assumed that 900 women without breast cancer who had urinary concentrations of F2-IsoP and 15-F_{2t}-IsoP-M measured were eligible for inclusion in analyses. The prevalence of tea drinking in the general population varied, and we set the proportion of any tea drinker as 20%, 30%, and 40%. The proportion of herbal tea and non-herbal tea drinkers were assumed to be similar in a certain population, and proportions of green tea drinkers and black tea drinkers were assumed to be similar as well. Anderson et al. [110] reported that the geometric mean of F_2-IsoP and 15-F_{2t}-IsoP-M was 1.44 (SD 0.75) ng/mgCr and 0.71 (SD 0.32) ng/mgCr, respectively, and we treated this as the average levels of biomarkers among premenopausal women. We hypothesized that tea consumption is associated with at least 15% reduction in these biomarkers. We used a two-sample independent comparison approach for power calculation, the powers were all above 0.80. Moreover, we assumed that the power equals 0.80, and we obtained detectable effect measures as follows (**Table 1a and b**).

Table 1a. Power calculation for aim 1, assumption: a 15% reduction in biomarkers

Hypothesized geometric mean (SD)	**Proportion of herbal tea drinker**	**Power**	**Proportion of green/black tea drinker**	**Power**
F_2-IsoP				
1.22 (0.75)	10%	0.79	5%	0.54
1.22 (0.75)	15%	0.92	7.5%	0.74
1.22 (0.75)	20%	0.97	10%	0.87
15-F_{2t}-IsoP-M				
0.60 (0.32)	10%	0.90	5%	0.67
0.60 (0.32)	15%	0.97	7.5%	0.86
0.60 (0.32)	20%	0.99	10%	0.95

Table 1b. Power calculation for aim 1, assumption: power equals 0.80

Power	**Proportion of herbal tea drinker**	**Geometric mean**	**Proportion of green/black tea drinker**	**Geometric mean**
F_2-IsoP				
0.80	10%	1.22	5%	1.14
0.80	15%	1.26	7.5%	1.20
0.80	20%	1.28	10%	1.24
15-F_{2t}-IsoP-M				
0.80	10%	0.62	5%	0.58
0.80	15%	0.63	7.5%	0.61
0.80	20%	0.64	10%	0.62

3.5 Analysis plan for Aim 2: Tea consumption and breast cancer risk

3.5.1 Hypothesis

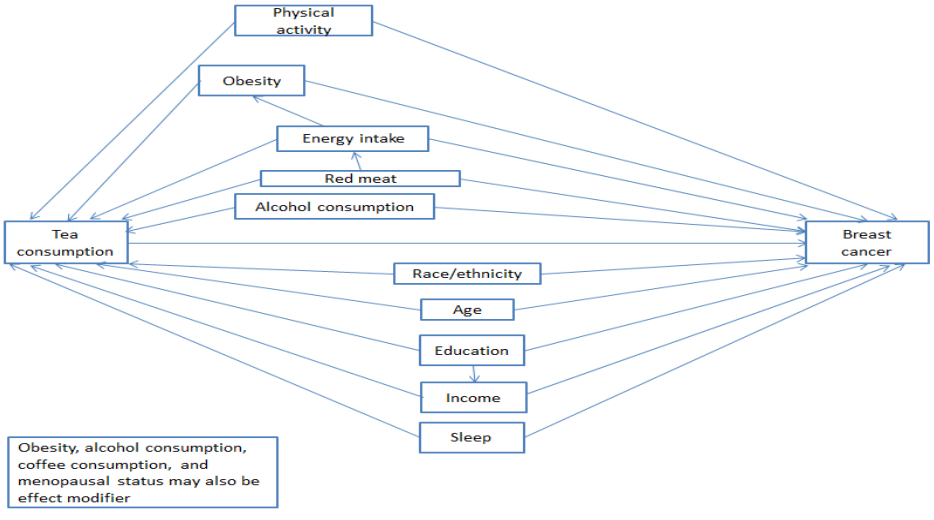
For aim 2, we hypothesized that tea consumption was inversely associated with postmenopausal breast cancer risk but might not be associated with premenopausal breast cancer risk.

3.5.2 Statistical analysis

Tea consumption was coded using the same scenario in aim 1, and breast cancer was coded as a binary variable. Descriptive statistics included means and standard deviations of continuous covariates and proportions of categorical covariates and tea consumption.

In time-to-event analysis, age was the time scale and all participants entered the risk set at the age of enrollment. The endpoint was the age of breast cancer diagnosis and women was censored follow-up at their age at last contact date, age at bilateral prophylactic mastectomy, or death. Age-adjusted hazard ratios (HRs) and confidence intervals (CIs) for breast cancer risk according to categories of included covariates were calculated using univariate Cox proportional hazards models. Multivariable Cox proportional hazards regression models were used to estimate the HR and 95% CI for breast cancer risk according to tea consumption [137]. The assumption of proportionality will be examined by the test of a non-zero slope developed by Therneau and Grambsch in which Schoenfeld residual was calculated [138]. Left truncation was considered as late entry in our analysis, and women entered the risk set at the age of the enrollment.[139]. The following variables were adjusted for in the multivariable Cox proportional hazards model: BMI, alcohol consumption, race/ethnicity, education level, income, education level, red meat, smoking, physical activity, caffeine intake, total energy intake, and sleep. Confounder selection was based on use of a directed acyclic graph (DAG) and the prior literature (**Figure 6).**

Figure 6. DAG for aim 2



$\log(ht)=\log(h0)+\beta_1*\text{tea consumption}+\beta_2*\text{alcohol consumption}+\beta_3*\text{BMI (or obesity)}+\beta_4*\text{race/ethnicity}$

+β_5*education level+ β_6*income+ β_7*education level+β_8*red meat+ β_9*smoking+ β_{10}*energy intake+ β_{11}*sleep+ β_{12}*caffeine intake

Potential effect measure modification according to alcohol consumption and obesity was evaluated using stratified analyses. In subgroup analysis based on obesity, participants were classified as obese (BMI≥30 kg/m^2) and non-obese (BMI<30 kg/m^2). In subgroup analysis based on alcohol consumption, participants were classified as ever drinkers and non-drinkers. In addition to subgroup comparison based on alcohol use and obesity, we used log-likelihood ratio test to assess if the interaction product term between tea consumption and obesity or alcohol consumption was significant in the multivariable model.

To assess potential heterogeneity according to menopausal status at diagnosis, we constructed separate regression models for pre- and postmenopausal breast cancer. Premenopausal breast cancer models included women who were classified as premenopausal at breast cancer diagnosis, and censored women at their reported age of menopause during follow-up.

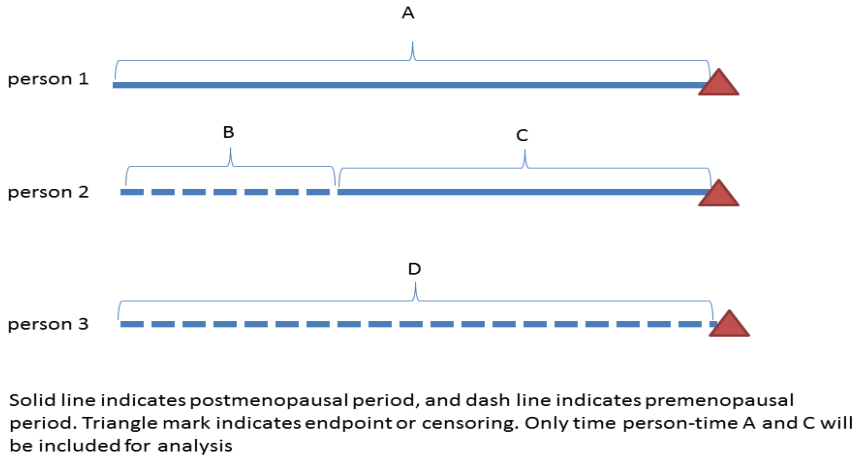


Figure 7. Analysis rationale for postmenopausal breast cancer

Postmenopausal breast cancer models included women who were postmenopausal at study enrollment, and allowed

women to enter the risk set at their age of menopause if they became menopausal during follow-up (**Figure 7**).We still generated an interaction term between menopausal status and tea consumption and used a log-likelihood ratio test to assess if there was an interaction between tea and menopausal status.

To assess potential heterogeneity according to ER status, we used a joint Cox model for analysis in which each person contributed to 2 event (ER+ and ER- breast cancer) times. The joint Cox model fitted a cause-specific hazard model which considered the compering risk framework between ER+ and ER- cancer and allowed for the other covariates in the model to have the same effects across the different subtypes. In the joint Cox model, the parameters of ER+ cancer (β_1) and ER- cancer (β_2) were estimated simultaneously by stratifying on event type and using a robust variance estimator to account for the correlation between the survival times of the two subtypes. A Wald test was used to compare if $\beta_1=\beta_2$ [140], and P values derived from the tests indicated if association between tea and breast cancer differed by subtypes.

On average, the annual risk of bilateral breast cancer is about 0.5% [141], but it can be 3% in carriers of a *BRCA1* or *BRCA2* mutation [142]. Bilateral breast cancers were excluded in analysis if they had different ER status.

A sensitivity analysis was conducted by excluding the first 6 months of person-time to reduce the potential for undiagnosed breast cancer to influence dietary behaviors.

3.5.3 Power calculation

For our power calculations, the null hypothesis was that breast cancer risk in tea consumption group was not statistically different from that in non-drinker group. The reference group in analysis was women who never drank a specific type of tea, and comparison groups

included women who drank green or black tea. The Sister Study cohort included 50,884 women and approximately 3,000 incident breast cancers. The prevalence of tea drinking in the general population varies, and we set the proportion of any tea drinker as 20%, 30%, and 40%. The proportion of herbal tea and non-herbal tea drinkers were assumed to be similar in a certain population, and proportions of green tea drinkers and black tea drinkers were assumed to be similar as well. We assumed that tea consumption was associated with a moderate reduction (15%) in breast cancer risk. The hypothesized risk reduction was made based on estimates reported in a large cohort study conducted in France in which similar number of breast cancer cases (2,868) and cohort size (67,703) were identified [37]; in that study, researchers reported that people consuming more than 3 cups of tea per day were associated with a marginally significant risk reduction (HR=0.79, 95% CI 0.62-1.01). By treating alpha level at 0.05, we yield the power as follows (**Table 4**).

Table 2. Power calculation for aim 2

Hypothesized RR	**Proportion of any tea drinker**	**Proportion of herbal tea drinker**	**Proportion of green/black tea drinker**	**Power**
0.85	20%	10%	5%	0.59
0.85	30%	15%	7.5%	0.73
0.85	40%	20%	10%	0.80
0.81	20%	10%	5%	0.80
0.84	30%	15%	7.5%	0.80
0.85	40%	20%	10%	0.80

3.6 Strengths and limitations

There are several strengths of our proposed research. Our research has a sufficient number of breast cancer cases identified during the study follow-up for our proposed analyses, since women in the Sister Study, on average, have a two-fold increase in breast cancer risk. This can enable us to identify more cases, and larger sample size and case number ensure more favorable precision and less random error. In addition, since the cohort consists of women across

the whole country, our study can have a good representativeness of the US population. The Sister Study sent follow-up questionnaires to ascertain the time-varying menopausal status. Menopausal status is biologically important, as we have mentioned, tea consumption can have differential effect to women with different menopausal status. Thus, recording the age at menopausal can make it possible for us to analyze the association with premenopausal and postmenopausal breast cancer separately and avoid misclassification. Different types of tea contain differential levels of (-)-epigallocatechin-3-gallate (EGCG), and the Sister Study measured the consumption of each tea type separately, making it possible to analyze the effect of each type and avoid obscuring the chemopreventive effect of certain type (e.g. green tea).

Our study also has some limitations. Tea consumption can be a time-varying variable, but it was only measured once at baseline by retrospective recall approach. This might introduce misclassification and bias the effect measure towards null in our analysis. In addition, urinary oxidative biomarkers do not only reflect the oxidative stress level of breast epithelial cells, since lipid peroxidation can occur in tissues other than breast. For example, urinary F2-IsoPs and 15-F2t-IsoP-M [143] was found to be associated with the level of neurodegenerative diseases, suggesting that non-case women with such disease may also have a higher levels of urinary F2-IsoPs and 15-F2t-IsoP-M and this may turn the association towards null.

Given our strengths, we are more likely be able to identify the effect of each type of tea separately and make our outcome more generalizable to the US women.

CHAPTER 4. TEA AND OXIDATIVE STRESS

4.1 Background

Oxidative stress describes the imbalance between reactive oxygen species (ROS) and antioxidant defenses [1, 2]. In humans, persistent oxidative stress can disturb homeostasis, induce inflammation, and damage deoxyribonucleic acid (DNA) [25, 144-146]. This disruption may contribute to the pathogenesis of cancer, diabetes, and neurodegenerative disease [74, 147, 148]. Lipid peroxidation caused by ROS is a marker of oxidative stress [149]. Urinary F_2-isoprostanes (F_2-IsoPs) and a primary metabolite, 2,3-dinor-5,6-dihydro-15-F2t-isoprostane (15-F_{2t}-IsoP-M), are stable biomarkers of lipid peroxidation [29, 150]. Some epidemiologic studies report that higher levels of urinary F_2-isoprostanes are associated with an increased risk of cancer and cardiovascular diseases [77, 151].

Tea is a popular and accessible beverage worldwide [3] and may have beneficial health effects for diabetes, cardiovascular disease, and cancer [152-154]. Green tea and black tea contain polyphenols that have anti-oxidative properties [155]. For example, the polyphenol epigallocatechin-3-gallate (EGCG) is a natural anti-oxidant found in green and black tea [156, 157]. Experimental studies of the association between tea consumption and oxidative stress [32, 33, 67] assigned participants to high levels of tea consumption for a short period of time (e.g. 4 cups of green tea/day for 8 weeks or placebo beverage). Study outcomes showed that participants in the intervention group had lower levels of blood malondialdehyde (MDA), an oxidative stress biomarker, or higher total antioxidant status as compared to the placebo group. These findings support a potential inverse association between tea consumption and oxidative stress.

However, in the general population, tea consumption patterns may reflect longer durations of lower consumption levels. It is unknown whether these patterns also translate to benefits for oxidative stress. Here we conducted a cross-sectional analysis of 889 premenopausal women to examine the association between tea consumption and oxidative stress.

4.2 Methods

Data for this analysis come from the National Institute of Environmental Health Sciences (NIEHS) Sister Study. Study participants (N=50,884) aged 35 to 74 years were enrolled between 2003 and 2009 across the United States and Puerto Rico [158]. To be eligible, participants had at least one sister who had been diagnosed with breast cancer, but no personal history of breast cancer [102]. All participants provided written consent at enrollment. Study protocols were approved by the Institutional Review Board of the NIEHS, the National Institutes of Health, and the Copernicus Group.

4.2.1 Population for analysis

Within the Sister Study, 1,367 women were identified (456 cases, 911 controls) for a nested case-control study investigating oxidative stress and breast cancer risk among premenopausal women. To be eligible for the nested case-control study, participants had to meet the following criteria: age <54 years, premenopausal status (at least one menstrual cycle in the previous 12 months or hysterectomy with≥1 ovary conserved), and have an available blood and urine sample from enrollment. Controls were matched to cases with a ratio of 2:1 on the basis of age and enrollment year and were free of breast cancer at the time of their matched case's diagnosis. For this analysis, information from the 911 controls was used. We further excluded

women missing data of black and green tea consumption levels (N=22), which yielded 889 participants for this study.

4.2.2 Oxidative stress measurement

At Sister Study enrollment, participants self-collected approximately 60 ml of first-morning void urine in a study-provided collection cup [158]. Participants refrigerated samples without preservative until they were picked up by study examiners who shipped the samples on ice to the study repository [159]. On receipt, urine samples were aliquotted and stored at -80℃. In 2012, samples were retrieved and urinary concentrations of F_2-IsoP and 15-F_{2t}-IsoP-M were measured using gas chromatography/negative ion chemical ionization mass spectrometry at the Eicosanoid Core Laboratory at Vanderbilt University Medical Center. The mean storage time of urinary samples was 8.3 years. Protocols for chemical analysis and procedures have been described in detail [106-108, 160]. A total of 77 batches were run; each batch contained 18 samples from study subjects (12 controls and 6 cases) and two quality control (QC) samples. The coefficient of variation for QC duplicates was 16.0% and 12.5% for F_2-IsoP and 15-F_{2t}-IsoP-M, respectively [161]. Urinary levels of F_2-IsoP and 15-F_{2t}-IsoP-M were adjusted for creatinine (ng/mg Cr) to correct for urine diluteness.

4.2.3 Exposure and covariate measurement

During an enrollment home visit, trained examiners measured height and weight without shoes. These measurements were taken three times and values were rounded to the nearest quarter inch for height and whole pound for weight [162]. Body mass index (BMI) was calculated as weight (kg)/ height $(m)^2$. Black and green tea consumption during the past 12

months was measured by the self-administered Block 98 food frequency questionnaire (FFQ) at study enrollment [163]. Within the FFQ, participants reported their frequency of tea consumption and the cups consumed each time [158]. Frequency was reported at 9 levels, ranging from "never" to "everyday". Participants reported how many cups of tea they consumed each time as "1 cup, 2 cups, 3-4 cups, or 5 or more cups." Regular (non-decaffeinated) coffee consumption was measured using the same methods described above. Total energy intake and caffeine from soda and black tea was calculated from the FFQ by NutritionQuest [34, 75]. We assigned caffeine levels to coffee (regular and decaffeinated) and green tea on the basis of data from USDA Food Composition Databases [164]. Each cup of regular coffee was assigned 95.2 mg caffeine, each cup of decaffeinated coffee 2 mg caffeine, and each cup of green tea 24.8 mg caffeine. On average, one cup of black tea contains 47.2 mg caffeine [164].

Weekly energy expenditures at enrollment were calculated as metabolic equivalents (METs) and total physical activity was calculated by summing the MET-h/week of all sports, physical exercise, and daily activity self-reported at enrollment. Participants were also asked for information about the total annual income from all household members and the highest level of school they had completed as well as their age and race/ethnicity.

4.2.4 Statistical analysis

Tea consumption was categorized into 4 levels (0, <1, 1-<5, and ≥ 5 cups/week). The consumption value was obtained by multiplying frequency of consumption (times per week) and serving size (cups consumed each time) together. Tea consumers with missing serving size information (22 for black tea, 24 for green tea) were assigned a serving size of 1 cup per serving (the most common serving size for black (56.7%) and green (76.0%) tea consumption). For

coffee consumption, 1 and 2 cups per serving were about equally common (37.0% and 38.5%, respectively), and coffee consumers with missing serving size information were also assigned as drinking 1 cup per serving. Coffee consumption was categorized into 4 levels: 0, <10, 10-<15, and ≥15 cups/week. Caffeine intake was categorized based on approximate quartiles (<33.9, 33.9 -<111.2, 111.2 -<205.2, and ≥205.2 mg/day). BMI categories were defined based on WHO guidelines as underweight/normal weight (<24.9 kg/m^2), pre-obesity (25.0-29.9 kg/m^2), obesity class I (30.0-34.9 kg/m^2), obesity class II (35.0-39.9 kg/m^2), and obesity class III (≥40 kg/m^2) [21] . Total energy intake and physical activity were categorized to approximate quartiles. Annual household income was categorized reflect low, moderate, and high level values in the general population [35].

The distribution of urinary F_2-IsoP and 15-F_{2t}-IsoP-M concentrations was right-skewed, thus, a natural log-transformation was applied for these biomarkers to approximate normality. Geometric means (GMs) and 95% confidence intervals (95% CIs) of urinary F_2-IsoP and 15-F_{2t}-IsoP-M were calculated for each level of tea consumption and by other covariates. Univariate geometric mean differences (uGMD) and 95% CIs of urinary F_2-IsoP and 15-F_{2t}-IsoP-M were calculated for each covariate using linear regression, and adjusted geometric mean difference (aGMD) was calculated in multivariable models adjusting for all these covariates. uGMD of urinary F_2-IsoP and 15-F_{2t}-IsoP-M (ln ng/mg Cr) across different levels of tea consumption were calculated using unadjusted linear regression. To calculate aGMD of isoprostanes across tea consumption levels, the linear regression model adjusted for age (35-<40, 40-<45, 45-<50, and ≥50 years), race (non-Hispanic white, non-Hispanic black, and other), body mass index (BMI, <25, 25-<30, 30-<35, 35-<40, and ≥40 kg/m^2), education (high school or less, some college or undergraduate, and graduate school), annual income (0-<$50,000, $50,000-<$100,000, and

≥$100,000), smoking status (never, former, current), total energy intake (<1,230.40, 1,230.40-<1,528.70, 1528.70-<1,974.90, and ≥1,974.90 kcal/day), and physical activity (<28.11, 28.11-<44.16, 44.16-<65.99, ≥65.99MET-hours/week) as potential confounders [165]. We also evaluated the impact of additional adjustment for caffeine (<33.9, 33.9 -<111.2, 111.2 -<205.2, and ≥205.2 mg/day) [68, 166]. The assumptions of the linear regression (linearity, independence, multivariate normality, homoscedasticity) were examined by scatterplots of urinary isoprostanes vs. tea consumption and plots of the residuals vs. fitted values of the regression model; results did not suggest that assumptions were violated.

Subgroup analyses were conducted to address potential effect modification of associations between tea and oxidative stress according to overweight (BMI<25 kg/m^2 vs. BMI≥25 kg/m^2) and regular coffee consumption (drinker vs. non-drinker). Interaction terms between tea consumption and these covariates were included in multivariable linear regressions, and log-likelihood ratio tests were used to assess if the interaction terms were statistically significant. Two-sided P values <0.05 were considered to be statistically significant. All statistical analyses were conducted with Sister Study Data Release 6.0 using Stata 13.0 (College Station, TX: StataCorp, LLP).

4.3 Results

Of the 889 participants in our analysis, the average age at baseline was 47.28 (SD 4.45) and the majority were non-Hispanic white (87.29%). The geometric means of urinary F_2-IsoP and 15-F2t-IsoP-M were 1.44 (95% CI 1.39-1.49) and 0.71 (95% CI 0.69-0.73) ng/mg Cr, respectively. Table A.1 presents geometric means and mean differences of urinary isoprostanes according to participant characteristics. Both F2-IsoP and 15-F2t-IsoP-M decreased slightly as age increased, but differences were not statistically significant. Average F2-IsoP and 15-F2t-

IsoP-M levels among non-Hispanic black women were lower compared to white women. Levels of both isoprostanes were positively associated with BMI and inversely associated with income and physical activity. Current smokers had higher levels of both isoprostanes compared to never smokers, but associations were statistically significant only for 15-F2t-IsoP-M. Inverse associations with education were also observed for 15-F_{2t}-IsoP-M, but not F_2-IsoP. High-level coffee consumption (≥15 cups/week) was not associated with F_2-IsoP or 15-F_{2t}-IsoP-M. The relationship between total energy intake and F_2-IsoP or 15-F_{2t}-IsoP-M appeared to be non-significant.

Associations between black and green tea consumption and caffeine intake with urinary oxidative stress measures are shown in Table A.2. Black tea consumption was more common than green tea consumption; 18.6% of women reported never drinking black tea, while 45.9% of women reported never drinking green tea. The highest level of consumption, ≥5 cups per week, was reported by 24.9% and 7.6% of women for black and green tea consumption, respectively. Overall, black and green tea consumption were not associated with F_2-IsoP levels (Table A.2). However, mean concentrations of 15-F_{2t}-IsoP-M were higher for black tea consumption of 5 cups per week or more compared to 0 cups per week (aGMD=0.09, 95% CI 0.01-0.17). High-level green tea consumption (≥5 cups/week compared to 0) was not significantly associated with 15-F_{2t}-IsoP-M (aGMD=0.05, 95% CI -0.06-0.16).

Caffeine intake was not associated with F_2-IsoP. As compared to the lowest quartile (<33.9 mg/day), higher levels of caffeine intake were positively associated with 15-F_{2t}-IsoP-M, but there was no consistent increase across quartiles. Additional adjustment for caffeine intake attenuated the association between black tea and 15-F_{2t}-IsoP-M towards the null (Table A.2).

Associations between tea consumption and urinary F_2-IsoP or 15-F_{2t}-IsoP-M were not modified by overweight (Table A.3) or regular coffee consumption (Table A.4).

4.4 Discussion

Our analysis did not provide support for an inverse association between tea consumption and urinary F_2-IsoP or 15-F_{2t}-IsoP-M, high-quality biomarkers of oxidative stress. Green tea consumption was not associated with either F_2-IsoP or 15-F_{2t}-IsoP-M. Black tea consumption was not associated with F_2-IsoP; however, drinking at least 5 cups of black tea per week (compared to none) was associated with higher 15-F_{2t}-IsoP-M concentrations before adjustment for caffeine.

Clinical studies have found an inverse association between tea consumption and oxidative stress [32, 33, 67, 167]. For example, by observing 19 people in a 5-day experimental study, Stote et al. [167] found that green tea consumption could lower plasma levels of isoprostanes. However, subjects in this study consumed a higher level of tea (e.g. 2 servings of green tea/day for 5 days) than was commonly consumed in our population-based sample of the U.S. women. In addition, this study only enrolled 19 obese people at high risk of insulin resistance, which may have compromised the generalizability of their outcomes.

A cross-sectional epidemiologic study [29] of 845 Chinese women observed an almost null association between any tea drinking and urinary levels of F_2-IsoPs (geometric mean: never drinker: 1.62, ever drinker: 1.65, p=0.72) and 15-F_{2t}-IsoP-M (geometric mean: never drinker: 0.56, ever drinker: 0.61, p=0.06) after adjustment for age, education, occupation, smoking, BMI, multivitamin supplement use, fruit and vegetable intakes, plasma total carotenoids, tocopherols, and retinol, assay batch, and urinary tea polyphenols. However, the ever/never analysis did not consider level of consumption or potential heterogeneity between black and green tea [29].

Green and black tea differ in concentrations of polyphenols (e.g. EGCG) and caffeine [13, 168]. For example, green tea has a higher level of EGCG compared to black tea [169], while black tea contains more caffeine [13]. Such heterogeneity could obscure associations between different types of tea and oxidative stress.

15-F_{2t}-IsoP-M is the metabolite of F_2-IsoP under beta-oxidation [29]. Both black and Both black and green tea contain EGCG and caffeine which have been found to facilitate beta-oxidation on the basis of laboratory evidence [170, 171]. The suggested positive associations between black tea and caffeine with 15-F_{2t}-IsoP-M, but not F_2-IsoP, may be due, in part, to related increases in beta-oxidation pathways. We did not observe an association between green tea and 15-F_{2t}-IsoP-M; however, there were few high-level green tea consumers in our analysis.

Our results regarding the association between caffeine and oxidative stress are similar to an experimental study [172] that assigned 20 participants caffeine (5mg/kg) or placebo before physical exercise that observed a positive association between caffeine and plasma MDA using blood samples collected immediately after exercise. However, other experimental studies have reported inverse associations between caffeine intake and oxidative stress using other caffeine dosages or different biomarkers of antioxidant activity or oxidative stress [173, 174]. For example, Metro et al. [173] assigned 2.5 mg/kg caffeine to 15 subjects twice per day for 1 week and observed that plasma glutathione, a marker of antioxidant activity, increased 106% after ingestion. Moreover, Zeraatpishe et al. [174] assigned 20 male participants 5 mg/kg caffeine or placebo and observed a substantial decrease in plasma 8-hydroxydeoxyguanosine (8-OHdG), a marker of oxidative stress, associated with caffeine ingestion. Taken together, the association between caffeine and oxidative stress deserves further investigation.

Strengths of our study included the use of a general population sample, the clear categorization of tea type, and detailed information on sociodemographic and lifestyle factors for statistical adjustment. The use of urinary isoprostanes provided a stable biomarker of oxidative stress. A previous biochemical study suggested that plasma isoprostanes stored appropriately are stable for at least 10 years [150]. Given that urinary isoprostanes are less prone to auto-oxidation than isoprostanes in plasma [29], our samples were likely to be equally or more stable. In some previous studies, MDA was used as the biomarker of oxidative stress [33, 67]; however, MDA is more affected by dietary lipid consumption [30] and can be generated from non-lipid sources such as bile pigments [175], which may cause measurement error. 8-OHdG is another biomarker of oxidative stress which is an end product of non-enzymatic DNA oxidation [109]. However, levels of 8-OHdG can be influenced by DNA repair capacity which makes 8-OHdG an indicator of the combined effects of oxidative stress-associated damage and DNA repair capability. Furthermore, a previous study of 10 healthy volunteer suggests that there is not a significant diurnal variation of urinary isoprostanes [176], whereas many studies suggest that diurnal variation of urinary 8-OHdG is substantial [109, 177, 178]. These characteristics make urinary isoprostanes a more desirable biomarker of oxidative stress.

Our study also has some limitations. First, our sample included only premenopausal women, which may compromise the external validity for men or older women. Second, tea consumption was measured by retrospective self-report and measurement error could be introduced. Moreover, our study is a cross-sectional analysis with one-time urinary sampling, which impedes cause and effect interpretation.

Our study contributes real-world data regarding associations between tea and oxidative stress to inform use of antioxidant products. We did not observe an inverse association between

green or black tea consumption and urinary isoprostanes, which does not support the hypothesis that tea consumption reduces oxidative stress.

CHAPTER 5. TEA AND BREAST CANCER RISK

5.1 Background

Breast cancer is the most commonly diagnosed malignant tumor among women in the United States [2, 3, 179]. In the United States, the estimated number of new invasive breast cancer and carcinoma *in situ* diagnoses in 2017 was 252,710 and 63,410, respectively [2]. In addition to its high incidence, side effects and high cost associated with breast cancer treatment emphasize the importance of primary prevention [180-182].

Tea is one of the most popular beverages worldwide [3]. Green tea and black tea are produced from the leaves of the plant *Camellia sinensis* [57]. Many types of organic chemical compounds are found in black and green tea, including polyphenols and caffeine [153]. These chemicals may have anti-tumorigenic properties [183]. For instance, *in vitro*, clinical, and epidemiologic studies suggest that (-)-epigallocatechin-3-gallate (EGCG), the main polyphenol in green and black tea [12, 17], may be beneficial for cancer prevention [17, 183-186]. On the basis of laboratory evidence, Mittal et al. [187] reported that EGCG increased apoptosis in breast carcinoma MCF-7 cells, which represent estrogen receptor (ER) positive tumors, without causing adverse effects on the growth of normal mammary cells.

However, beneficial effects of tea consumption for breast cancer have not been consistently observed in previous epidemiologic studies. Inconsistent results may be due, in part, to chemical heterogeneity between tea types or etiological heterogeneity between breast cancer subtypes. For example, green tea has a higher level of EGCG than black tea [12], whereas black tea contains higher concentrations of caffeine [188]. Thus, studies ignoring differences in

chemical constitution may obscure potential chemopreventive effects. Additionally, EGCG may inhibit estrogen receptor (ER) activity and result in differential effects according to breast tumor ER expression [22, 189]. However, few previous epidemiologic studies have considered these potential sources of heterogeneity.

To examine the association between different types of tea and ER-defined breast cancer risk, we used data from the National Institute of Environmental Health Sciences (NIEHS) Sister Study.

5.2 Methods

Data from the NIEHS Sister Study was used for analysis [158]. The Sister Study is a prospective cohort study that enrolled 50,884 women between the ages of 35 and 74 across the United States and Puerto Rico from 2003 to 2009. Participants were free of breast cancer but had a sister who was diagnosed with breast cancer at enrollment [18]. All participants provided written informed consent. Study protocols were approved by the Institutional Review Board of the NIEHS, the National Institutes of Health, and the Copernicus Group.

5.2.1 Data collection

At enrollment, green and black tea consumption during the past 12 months was measured by the self-administered Block 98 food frequency questionnaire (FFQ) [103]. Within the FFQ, participants reported their frequency of tea consumption and the cups consumed each time. Frequency was reported at 9 levels, ranging from “never” to “everyday”. Participants reported how many cups of tea they consumed each time as “1 cup, 2 cups, 3-4 cups, or 5 or more cups.” Alcohol consumption, red meat intake (e.g. pork, beef, lamb), and coffee consumption were also

measured by the FFQ [103]. Caffeine from soda and black tea, as well as total energy intake, was calculated from the FFQ by NutritionQuest [34]. Caffeine levels of regular and decaffeinated coffee, as well as caffeine in green tea, were assigned based on the USDA Food Composition Databases [122] as 95.2 mg caffeine per cup of regular coffee, 2 mg caffeine per cup of decaffeinated coffee, and 24.8 mg caffeine per cup of green tea. On average, one cup of black tea contains 47.2 mg caffeine [122].

Breast cancer diagnosis was self-reported via annual health updates, detailed follow-up questionnaires, and through telephone calls, e-mails, or correspondence with the Sister Study helpdesk. Participants who reported a new breast cancer diagnosis were contacted 6 months after diagnosis for additional information about the diagnosis and treatment, and to request permission to access their medical records [18, 60]. Medical records were reviewed by trained abstractors to verify the breast cancer diagnosis, tumor characteristics, and treatment details. Estrogen receptor status of the tumor was abstracted from pathology reports when available and by self-report if not. Agreement between self-reports and medical records was high (99.3% for ER+ and 83.1% for ER- breast cancer) [60].

Menopausal status was measured at enrollment and during follow-up. Women were considered postmenopausal if they were ≥12 months from last menses or reported having bilateral oophorectomy; hysterectomy at an age older than 55; chemotherapy, radiation, or other treatment that permanently stopped their period prior to spontaneous menopause; or use of ovarian suppressing medications at an age older than 55.

At enrollment, weekly energy expenditures were calculated in metabolic equivalents (MET) and total physical activity was obtained by summing the MET-h/week of all sports, physical exercise, and daily activity reported. Sleep duration was self-reported at enrollment as

the average hours of sleep per day. Participants were also asked for information about the total income from all household members and the highest level of school completed. Height and weight were measured at home visits by trained examiners. Measurements were taken three times and obtained numbers were rounded to the nearest quarter inch for height and whole pound for weight [162], and body mass index (BMI) was calculated as weight(kg)/height(m^2). Age and race/ethnicity were self-reported.

Women with the following characteristics were excluded from analysis: 1) had missing data on breast cancer status (n=118); 2) cancer diagnosis or end of follow-up occurred prior to completion of study enrollment activities (n=10); 3) age at cancer diagnosis or end of follow-up was missing (n=30); or 4) had missing data for black and green tea consumption (n=1,512).

5.2.2 Statistical analysis

Black tea consumption was categorized to approximate quartiles as follows: never drinker, <1, 1-<5, and ≥5 cups/week. Green tea consumption was categorized with the same cut points for consistency. Tea consumption levels (cups/week) were calculated by multiplying the frequency of consumption (times per week) by the serving size (cups consumed each time). Tea consumers with missing serving size information (N=1,223, 2.5% of black tea consumers; N=1,038, 2.1% of green tea consumers) were assigned a serving size of 1 cup as it was the most common serving size reported by black (57.5%) and green (73.0%) tea consumers. Regular coffee consumers with missing serving size information were also assigned a serving size of 1 cup as the most common serving size; 38.7% of participants reported drinking 1 cup each time and 38.5% reported drinking 2 cups. Regular coffee consumption was categorized into 4 levels (0, <10, 10-<15, and ≥15 cups/week) to align with previous studies of coffee consumption

among US women [75, 190]. Alcohol consumption was measured by calculating cups consumed per week and were categorized as 0, <1, 1-<7, and ≥7 drinks/week to align with previous reports from the Behavioral Risk Factor Surveillance System [67]. Red and cured meat consumption during the past 12 months was measured by FFQ as ounce-equivalent per day. BMI categories were defined on the basis of guidelines used in WHO as underweight/normal weight (<24.9 kg/m^2), pre-obesity (25.0-29.9 kg/m^2), obesity class I (30.0-34.9 kg/m^2), obesity class II (35.0-39.9 kg/m^2), and obesity class III (≥40 kg/m^2) [21]. Meat consumption, total energy intake, and physical activity were categorized to approximate quartiles. Average daily sleep hours was categorized into 3 levels (<7, 7-<8, and ≥8 hours/day) based on thresholds published by the National Sleep Foundation [70, 191, 192].

Log-rank tests were used to investigate if breast cancer risk was different across tea consumption levels. Univariate and multivariable Cox proportional hazards models, which used age as the time scale, were used to calculate the hazard ratios (HRs) between black or green tea consumption and breast cancer risk. Late entries of the participants were treated as left truncation, and women entered the risk sets at the age of enrollment. The ending time for women diagnosed of breast cancer was age at cancer diagnosis. For women without breast cancer, the ending time was the age at the last contact. In multivariable Cox regression models, we also conducted an overall test to investigate whether estimates of different tea consumption levels, taken as a whole, were simultaneously non-significant. The trend test was conducted by treating the median value in each category of tea consumption as a continuous variable in the model, and $p<0.05$ indicated a significant linear trend. The proportionality assumption of the Cox model was examined with the non-zero slope test using scaled Schoenfeld residuals [138, 193]; there was no evidence of violation.

Covariates included in the multivariable model were chosen based on *a priori* knowledge regarding their relation with tea consumption and/or breast cancer and included: race (non-Hispanic white, non-Hispanic black, and other), body mass index (BMI, <25, 25-<30, 30-<35, 35-<40, and ≥40 kg/m^2), education (high school or less, some college or undergraduate, and graduate school), annual household income (<$50,000, $50,000-<$100,000, and ≥$100,000), smoking status (never, former, current), alcohol consumption (0, <1, 1-<7, and ≥7 drinks/week), total energy intake (<1,197.2, 1,197.2-<1,545.6, 1545.6-<1,961.2, and ≥1,961.2 kcal/day), physical activity (<27.1, 27.1-<44.4, 44.4-<67.2, and ≥67.2 MET-hours/week), meat consumption (<0.70, 0.70-<1.23, 1.23-<2.01, and ≥2.01 ounce-equivalent/day), total caffeine intake (<31.6, 31.6-<105.8, 105.8-<205, and ≥205 mg/day), and average daily sleep duration (<7, 7-<8, ≥8 hours/day).

Subgroup analyses were conducted to examine potential effect modification of associations between tea and breast cancer risk according to obesity (BMI ≥30 kg/m^2 vs. BMI <30 kg/m^2) and alcohol or coffee consumption (drinker vs. non-drinker). Log-likelihood ratio tests were used to investigate if interaction terms between tea consumption and each these covariates were statistically significant.

To investigate the association between tea consumption and premenopausal breast cancer, we included premenopausal person-time during the follow-up in the model; women who became postmenopausal without breast cancer diagnosis were treated as censoring events at the time of postmenopause for premenopausal breast cancer analysis. For postmenopausal breast cancer risk analysis, women who were premenopausal at enrollment entered the risk set at the age they became postmenopausal during follow-up. Log-likelihood ratio tests were also used to examine the significance of interaction between tea and menopausal status.

We used joint Cox regression models to examine whether associations between tea consumption and breast cancer risk differed by ER status [140]. In joint Cox model, each person contributed to 2 event (ER+ and ER- breast cancer) times, and parameters of ER+ cancer (β_1) and ER- cancer (β_2) were estimated simultaneously by stratifying on event type and using a robust variance estimator to account for the correlation between survival times of the two subtypes. The joint Cox model fitted a cause-specific hazard model which considered the compering risk framework between ER+ and ER- cancer and allowed for the other covariates in the model to have the same effects across the different subtypes.

We further investigated associations between black or green tea consumption and triple negative (ER-/PR-/HER-) breast cancer by treating other subtypes as censoring events.

A sensitivity analysis was conducted by excluding the first 6 months of person-time to reduce the potential for undiagnosed breast cancer to influence dietary behaviors.

Two-sided P values <0.05 were considered to be statistically significant. All statistical analyses were conducted with Stata 13.0 (College Station, TX: StataCorp, LLP) and SAS v9.4 (SAS Institute Inc., Cary, NC).

5.3 Results

A total of 49,214 women contributed to analysis. The mean age at enrollment was 55.7 (SD 9.0) years. The median follow-up time was 8.4 years and 3,044 women were diagnosed with breast cancer during follow-up. Table B.1 presents participants' sociodemographic and health-related characteristics at enrollment and age-adjusted HRs for each covariate. The majority of participants were between 45 and 65 years (69.6%), non-Hispanic white (84.5%) and postmenopausal (65.7%). At enrollment, 27.5% of premenopausal and 30.4% of postmenopausal

women were obese (BMI≥30 kg/m^2). Most women (84.7%) had education levels higher than high school. The majority of participants (71.6%) had an annual household income ≥$50,000. Over one-third of participants were former smokers (35.8%) and only 8.1% were current smokers. Very few women had at least 7 drinks per week (13.6%) and 18.8% women never drank alcohol. Nearly one-third of women never drank regular coffee (29.0%) and 14.8% consumed ≥15 cups per week. Approximately even thirds of women reported an average daily sleep shorter than 7 hours (28.8%), 7-7.9 hours (37.7%), and ≥8 hours (33.4%). In age-adjusted models, being a former (vs. never) smoker, having a postmenopausal BMI ≥25 kg/m^2 (vs. <25 kg/m^2), completing graduate school (compared to high school or less), having an annual household income ≥100,000 per year (compared to <50,000), and high-level meat (compared to <0.70 ounce-equivalent/day) and higher total energy consumption (≥1,961.2 compared to <1,197.2 kcal/day) were associated with higher breast cancer risk.

Log-rank tests showed that breast cancer risk was not different by black tea consumption levels (p=0.19) but was significantly different by green tea consumption levels (p<0.01). Table B.2 presents HRs for association between tea consumption and breast cancer risk. Black tea consumption (80.8%) was more frequently reported than green tea (55.4%). Overall, 25.1% and 9.0% of women drank ≥5 cups/week of black and green tea, respectively. Drinking at least 5 cups of black tea per week was associated with a lower risk of breast cancer (HR=0.86, 95% CI 0.76, 0.98 compared to women who did not drink black tea, *p-trend*=0.01, *p-overall*=0.08). A similar association was observed for green tea consumption (HR=0.84, 95% CI 0.73, 0.97 for ≥5 vs. 0 cups/week of green tea, *p-trend*<0.01, *p-overall*<0.01).

There were a total of 2,089 ER+ and 371 ER- breast cancers in our sample. Overall, ≥5 cups/week of black tea, compared to 0, was inversely associated with ER+ breast cancer risk

(HR=0.87, 95% CI 0.76, 1.01, *p-trend*=0.04, *p-overall*=0.18). Green tea (≥5 vs. 0 cups/week, HR=0.83, 95% CI 0.70, 0.99, *p-trend*<0.01, *p-overall*=0.01) was also associated with a reduced risk of ER+ breast cancer (Table B.3). Black and green tea consumption were not significantly associated with ER- breast cancer risk, but the point estimates were similarly inverse. Joint Cox models showed no significant difference in associations between tea and breast cancer by ER status (*p-interaction*=0.78, for black tea; *p-interaction*=0.88, for green tea). We observed inverse but non-significant associations between triple negative breast cancer risk and ≥5 cups/week of black (HR=0.77, 95% CI 0.48, 1.23 vs. 0 cups/week of black tea, *p-trend*=0.12, *p-overall*=0.27) or green (HR=0.57, 95% CI 0.30, 1.07 vs. 0 cups/week of green tea, *p-trend*=0.06, *p-overall*=0.33) consumption.

Table B.4 presents effect measures of black and green tea consumption by menopausal status. We observed that consuming ≥5 cups/week of tea was not associated with premenopausal breast cancer risk (black tea: HR=0.89, 95% CI 0.67, 1.19, *p-trend*=0.27, *p-overall*=0.74; green tea: HR=0.99, 95% CI 0.71, 1.38, *p-trend*=0.91, *p-overall*=0.96). For postmenopausal breast cancer risk, consuming ≥5 cups/week of black (HR=0.86, 95% CI 0.75, 0.99, *p-trend*=0.03, *p-overall*=0.15) or green tea (HR=0.79, 95% CI 0.67, 0.94, *p-trend*<0.01, *p-overall*<0.01) was associated with a lower risk of breast cancer compared to non-consumers of each tea type. Log-likelihood ratio tests suggested no significant difference in effect measures by menopause status for black or green tea (*p-interaction*=0.98 for black tea, *p-interaction*=0.26 for green tea).

The association between tea consumption and breast cancer risk appeared qualitatively different between non-obese and obese women (Table B.5); however, these differences were not statistically significant (*p-interaction*=0.52 and 0.57 for black and green tea, respectively). Among non-obese (BMI <30 kg/m^2) women, the association between tea and breast cancer risk

was inverse: ≥5 cups/week of black (HR=0.83, 95% CI 0.71, 0.96, *p-trend*=0.01, *p-overall*=0.05) or green tea (HR=0.79, 95% CI 0.66, 0.94, *p-trend*<0.01, *p-overall*<0.01) was associated with an approximately 20% lower breast cancer risk compared to non-consumers of each tea type. Among obese women (BMI ≥30 kg/m^2), the association between black or green tea consumption and breast cancer risk appeared null (HR=0.96 for ≥5 vs. 0 cups per week of either black or green tea).

Regular coffee consumption (Table B.6) or alcohol consumption (Table B.7) did not appear to modify the association between black or green tea consumption and breast cancer risk. In analyses restricted to women with follow-up time longer than 6 months, ≥5 cups/week of black (HR=0.87, 95% CI 0.77, 0.99, *p-trend*=0.03) or green tea (HR=0.85, 95% CI 0.73, 0.98, *p-trend*<0.01), as compared to 0, was associated with a lower risk of breast cancer, consistent with our primary analyses (Table B.8).

5.4 Discussion

In our study, frequent (≥5 cups/week) black or green tea consumption was associated with a lower risk of breast cancer. Associations between tea consumption and breast cancer risk did not significantly differ by ER status, menopausal status, BMI, alcohol intake, or regular coffee consumption.

A previous meta-analysis [194] investigating associations between tea and breast cancer risk synthesized 13 and 4 epidemiologic studies for black and green tea, respectively. In their study, they observed an inverse association for green tea (RR=0.78, 95% CI 0.61, 0.98) which is consistent as ours. However, they obtained a null association for black tea (RR=0.98, 95% CI 0.88, 1.09) which differs from our results. Several factors could be associated with the

heterogeneous results regarding black tea. In 6 of 13 studies, the primary exposure was not specified as black or green tea; study estimates for any tea could have included herbal varieties that were not addressed in our analysis. Further, several studies [43, 54, 195] used low-level black tea consumption as the reference group, which attenuate the association between black tea consumption and breast cancer.

The inverse association we observed may be due, in part, to the polyphenols (e.g. EGCG) found in black and green tea. A previous animal study fed female mice on solution containing radio-labeled EGCG and found that mammary gland levels of EGCG substantially increased after 24 hours of ingestion [196]. This indicates the potential for polyphenols in black and green tea to be absorbed and transported in the human body.

Additional laboratory evidence supports an inverse association between green tea consumption and breast cancer risk [197]. By observing the viability of MDA‐MB‐231 breast cancer cells, Hong et al. [198] found that EGCG can induce breast tumor cell apoptosis and inhibit tumorigenesis by inactivating the β‐catenin signaling pathway. Okuda et al. [199] found that polyphenols in non-herbal tea could inhibit the mutagenic activity of chemical mutagens. This evidence suggests black and green tea may prevent breast cancer by inhibiting tumor initiation. Black and green tea may also reduce breast cancer risk by inhibiting tumor promotion. Laboratory studies [200-202] indicate that EGCG can inhibit angiogenesis by suppressing the expression of vascular endothelial growth factor (VEGF). Angiogenesis is an important factor for solid tumor growth since vascular networks facilitate nutrient transport and waste product removal [203]. Usually, solid tumors like early-stage breast cancer need new blood vessels to proliferate larger than 1micromiter in size [90]; therefore, black and green tea, which are rich in EGCG, may reduce breast cancer risk by inhibiting angiogenesis and tumor proliferation.

The clear definition and categorization of tea, which considered constituent heterogeneity and potential dose-response, is a strength of our analysis. We used a national cohort study with a larger overall sample size and number of breast cancer cases as compared to previous studies, which increases precision. Moreover, in sensitivity analysis, we generated a lag time period of 6 months in order to exclude women diagnosed of breast cancer shortly after enrollment to reduce the potential for undiagnosed breast cancer to influence tea consumption patterns. However, some limitations should be considered. At enrollment, black and green tea consumption was recalled retrospectively, which may have introduced some measurement error. In addition to frequency and serving size, the duration of tea consumption may also play an important role in cancer prevention; however, this information was not available. Moreover, overall tests in multivariable models for black tea obtained marginally significant or non-significant results, suggesting that outcomes regarding black tea should be interpreted with the caution that type I error could be introduced in analysis.

In conclusion, our study suggests that drinking 5 or more cups of black or green tea per week is associated with an approximate 15% lower risk of breast cancer as compared to 0 cups per week. Tea is an inexpensive, accessible, and safe beverage that may provide chemopreventive effects for breast cancer risk reduction.

CHAPTER 6. DISCUSSION

6.1 Main findings

In the first aim, we included 889 premenopausal women from a nested case-control study within the Sister Study for analysis. After adjusting for age, race, smoking status, BMI, physical activity, household income, education level, and energy intake, we observed urinary levels of F_2-IsoP and 15-F_{2t}-IsoP-M were not significantly different across green tea consumption levels (never, <1 cup/week, 1-<5 cups/week, ≥5 cups/week). Black tea consumption was not associated with F_2-IsoP, but drinking at least 5 cups of black tea per week was associated with a slightly increased level of 15-F_{2t}-IsoP-M, which was attenuated after adjustment for caffeine. We did not obtain any evidence to suggest that overweight or regular coffee consumption modified associations between tea and urinary F_2-IsoP or 15-F_{2t}-IsoP-M.

In the second aim, the full cohort of 49,214 women in the Sister Study were included for analysis. A total of 3,044 breast cancer cases were identified during the follow-up. Overall, as compared to non-drinkers of each type of tea, drinking at least 5 cups of black or green tea per week was associated with a reduced risk of breast cancer, and effect measures of black (HR=0.86, 95% CI 0.76, 0.98) and green tea (HR=0.84, 95% CI 0.73, 0.97) were very similar. We also examined if effect measures of black or green tea consumption significantly differed by several biologically important factors including estrogen receptor status, menopausal status, obesity, regular coffee drinking, and alcohol consumption . However, associations of black or green tea were not statistically different by any of these factors.

Together, these results suggest that while tea consumption may be inversely associated

with breast cancer risk, our data did not support an inverse association between tea consumption and urinary concentrations of F_2-isoprostanes, a biomarker of oxidative stress. However, as our oxidative stress analyses were limited to a single biomarker and premenopausal women; we cannot exclude the possibility that tea consumption is associated with lower oxidative in postmenopausal women.

6.2 Biological interpretation

Experimental and clinical evidence suggests that persistent oxidative stress may increase risk of cancer by causing molecular damage [25, 204-206]. Although we did not find a significant association between tea and urinary levels of F_2-isoprostanes, other biomarkers related to oxidative stress should be considered. Urinary F_2-isoprostanes were identified as having the most favorable qualities for oxidative stress measurement within the NIEHS Biomarkers of Oxidative Stress Study (BOSS) [207-210]. The BOSS used two animal models (rats and fetal pigs) to evaluate 18 proposed biomarkers of oxidative stress (GSH/GSSG, MDA, F_2-isoprostanes, ascorbic acid, protein carbonyls, methionine sulfoxide, tyrosine products, 8-OHdG, DNA strand breaks, M1G, lipid hydroperoxide, thiobarbituric acid reactive substances, vitamin C, vitamin E, Co-Enzyme Q9/10, uric acid, total antioxidant capacity, and cysteine/cysteine) in relation to oxidative insult with carbon tetrachloride, ozone, and lipopolysaccharides across multiple laboratories in the U.S. and internationally [207-210].

In addition to F_2-isoprostanes, 8-hydroxy-2' -deoxyguanosine (8-OHdG) may more directly reflect DNA damage compared with F_2-isoprostanes. Unlike F_2-isoprostanes, 8-OHdG is an end product of non-enzymatic DNA oxidation [109]. However, levels of 8-OHdG can be influenced by DNA repair capacity which makes 8-OHdG an indicator of the combined effects

of oxidative stress-associated damage and DNA repair capability. Furthermore, previous research did not report a substantial variation of F_2-isoprostanes [176], whereas such variation was identified in 8-OHdG [177]. In some previous studies, MDA was used as the biomarker of oxidative stress; however, MDA is more affected by dietary lipid consumption and can be generated from non-lipid sources such as bile pigments [211], which may cause measurement error.

Mechanisms in addition to oxidative stress, such as apoptosis and anti-angiogenesis, could be related to the chemopreventive effects of tea consumption. For example, *in vitro* studies suggest EGCG in black and green tea down-regulated telomerase in human breast carcinoma MCF-7 cells, leading to suppression of cell viability and induction of apoptosis [84, 212]. Other laboratory research found that green tea extract or EGCG could suppress angiogenesis of MDA-MB231 breast cancer cells by inhibiting VEGF transcripts and promoter activity [213].

Du et al.[214] investigated 10 polyphenols that were identified in non-herbal tea, and EGCG showed the most potent anti-proliferative effects among these chemicals. Although green tea contains a substantially higher amount of EGCG than black tea [13], our findings did not support a more substantial chemopreventive effect of green tea compared to black tea. Several reasons may partially explain this issue. For example, gallic acid is another type of tea polyphenol that was proved to have anti-tumor properties and exist at a higher concentration in black tea than green tea at the same temperature [215-217]. This suggests that, to examine if there is a potential difference between black and green tea in aspects of cancer prevention, more population-based studies are needed in combination with laboratory evidence.

6.3 Health significance

Oxidative stress is the imbalance between oxygen reactive species (ROS) and antioxidants, and persistent oxidative stress has been found to be associated with pathogenesis of cardiovascular disease, dementia, and cancer [23, 74, 147]. Previous experimental studies reported inverse associations between black or green tea consumption and oxidative stress, which provided evidence that tea drinking can lower oxidative stress levels [33, 67]. However, this anti-oxidative stress property of tea consumption may not exist in general population who consume a low dosage of tea for a relatively longer period of time. Unlike previous experimental studies, our results do not support the inverse association between tea and F_2-isoprostanes. This real world evidence suggests that black or green tea may not be a powerful anti-oxidative stress agent, but additional research is warranted to replicate our findings and consider other biomarkers of oxidative stress.

Breast cancer is the most commonly diagnosed malignancy among women in the US. In 2017, over 19 billion dollars were spent in breast cancer-related health service [218], suggesting a huge economic burden is associated with breast cancer. Tea is a favorable beverage and easily accessible product around the world. Moreover, tea has an extremely low toxicity and can be a complementary nutrient. All these make tea a potential and cost-effective primary preventive agent of breast cancer.

6.4 Future directions

Future epidemiologic studies investigating tea consumption and oxidative stress should try to address potential changes in consumption patterns over time. Tea consumption can be time-dependent, and multiple measurements can help researchers capture the change or trend of

drinking habits. In addition to FFQ, 24-hour recall and food diary should also be applied so that a validation comparison can be made across different types of measurement methods and researchers can observe whether retrospective recall is associated with measurement error. More information should be asked in aspects of tea consumption pattern such as the age women started drinking tea so that we can assess if duration of consumption is associated with oxidative stress; moreover, cumulative cup-years can be calculated if duration of consumption is measured to assess cumulative dosage. Since biomarkers of oxidative stress can be affected by many factors such as acute illness, physical activity, and recent medication use, epidemiologists should also consider repeated measures of oxidative stress biomarkers at different time points to obtain the average level of oxidative stress.

We hypothesize that the inverse associations between black or green tea consumption and breast cancer risk is related to polyphenols contained in tea, thus, future nutritional analysis should try to calculate the amount of different types of polyphenols in black or green tea. By doing this, epidemiologists can examine whether the association is mediated by these polyphenols. Because metabolism and absorption of tea can be affected by inherent physiologic conditions or genetic factors, such pathways should be considered in future studies as well.

Although drinking tea has very few side effects, there is still a possibility that extremely high level tea consumption may cause health problems. For example, excessive black tea consumption is found to inhibit non-haem iron absorption [219], suggesting that epidemiologists should also consider recording tea consumption-related adverse health events to evaluate the safety issues associated with tea consumption.

APPENDIX A: TABLES OF AIM 1

Table A.1: Characteristics of study participants and estimates of urinary isoprostanes of covariates.

Characteristics	N=889	**F_2-IsoP**			**15-F_{2t}-IsoP-M**		
	n (%)	GM (95% CI)‖	uGMD (95% CI)ψ	aGMD (95% CI)¶	GM (95% CI)‖	uGMD (95% CI)ψ	aGMD (95% CI)¶
Age (year)							
35-<40	61 (6.9)	1.61 (1.41, 1.83)	REF	REF	0.75 (0.67, 0.83)	REF	REF
40-<45	177 (19.9)	1.43 (1.33, 1.53)	-0.13 (-0.28, 0.02)	-0.09 (-0.24, 0.05)	0.72 (0.67, 0.77)	-0.04 (-0.17, 0.09)	-0.02 (-0.14, 0.10)
45-<50	377 (42.4)	1.44 (1.36, 1.51)	-0.11 (-0.25, 0.03)	-0.09 (-0.22, 0.05)	0.73 (0.70, 0.76)	-0.03 (-0.15, 0.09)	-0.02 (-0.13, 0.10)
≥50	274 (30.8)	1.41 (1.32, 1.51)	-0.13 (-0.28, 0.01)	-0.12 (-0.26, 0.02)	0.68 (0.65, 0.72)	-0.10 (-0.23, 0.02)	-0.09 (-0.21, 0.02)
Race							
Non-Hispanic white	776 (87.3)	1.45 (1.40, 1.51)	REF	REF	0.72 (0.69, 0.74)	REF	REF
Non-Hispanic black	56 (6.3)	1.25 (1.09, 1.44)	-0.14 (-0.28, 0.00)	-0.24 (-0.38, -0.10)	0.65 (0.58, 0.74)	-0.09 (-0.21, 0.03)	-0.20 (-0.31, -0.08)
Other	57 (6.4)	1.42 (1.27, 1.60)	-0.02 (-0.16, 0.12)	0.00 (-0.14, 0.13)	0.73 (0.65, 0.81)	0.01 (-0.11, 0.13)	0.03 (-0.08, 0.15)
BMI (kg/m^2)							
<25	406 (45.7)	1.29 (1.23, 1.36)	REF	REF	0.63 (0.60, 0.65)	REF	REF
25-<30	246 (27.7)	1.46 (1.38, 1.55)	0.13 (0.04, 0.21)	0.11 (0.03, 0.19)	0.70 (0.67, 0.74)	0.12 (0.05, 0.19)	0.10 (0.03, 0.17)
30-<35	122 (13.7)	1.50 (1.37, 1.65)	0.15 (0.05, 0.26)	0.10 (0.00, 0.21)	0.79 (0.74, 0.85)	0.24 (0.16, 0.33)	0.19 (0.11, 0.28)
35-<40	67 (7.5)	1.71 (1.49, 1.97)	0.27 (0.14, 0.40)	0.21 (0.07, 0.34)	0.94 (0.84, 1.05)	0.41 (0.30, 0.52)	0.37 (0.26, 0.48)
≥40	47 (5.3)	2.23 (1.90, 2.63)	0.54 (0.39, 0.69)	0.46 (0.30, 0.62)	1.17 (1.06, 1.30)	0.63 (0.51, 0.76)	0.57 (0.44, 0.70)
Missing	1 (0.1)	6.08 (-)			1.85 (-)		

Education level							
High school or less	107 (12.0)	1.57 (1.41, 1.76)	REF	REF	0.84 (0.77, 0.91)	REF	REF
Some college or undergraduate	543 (61.1)	1.42 (1.36, 1.48)	-0.09 (-0.20, 0.02)	-0.02 (-0.13, 0.09)	0.72 (0.69, 0.75)	-0.14 (-0.24, -0.05)	-0.06 (-0.15, 0.03)
Graduate school	239 (26.9)	1.42 (1.33, 1.52)	-0.10 (-0.22, 0.03)	0.00 (-0.13, 0.12)	0.65 (0.62, 0.69)	-0.25 (-0.35, -0.14)	-0.13 (-0.23, -0.03)
Annual household income ($)							
<50,000	144 (16.2)	1.64 (1.50, 1.80)	REF	REF	0.83 (0.76, 0.90)	REF	REF
50,000-<100,000	364 (40.9)	1.54 (1.46, 1.63)	-0.05 (-0.15, 0.04)	-0.02 (-0.12, 0.08)	0.76 (0.72, 0.79)	-0.08 (-0.17, 0.00)	0.00 (-0.09, 0.08)
≥100,000	360 (40.5)	1.29 (1.23, 1.35)	-0.24 (-0.34, -0.14)	-0.14 (-0.25, -0.04)	0.63 (0.60, 0.66)	-0.27 (-0.35, -0.18)	-0.10 (-0.18, -0.01)
Missing	21 (2.4)	1.11 (0.90, 1.38)			0.76 (0.63, 0.91)		
Smoking history							
Never	545 (61.3)	1.41 (1.36, 1.47)	REF	REF	0.70 (0.67, 0.72)	REF	REF
Former	267 (30.0)	1.42 (1.33, 1.52)	-0.01 (-0.09, 0.07)	-0.01 (-0.09, 0.06)	0.70 (0.67, 0.74)	0.01 (-0.06, 0.07)	-0.01 (-0.07, 0.05)
Current	77 (8.7)	1.69 (1.50, 1.92)	0.16 (0.03, 0.29)	0.07 (-0.06, 0.20)	0.88 (0.79, 0.97)	0.24 (0.14, 0.35)	0.13 (0.02, 0.23)
Regular (not decaf) coffee consumption (cups/wk)							
0	279 (31.4)	1.53 (1.44, 1.63)	REF	REF	0.73 (0.69, 0.78)	REF	REF
<10	257 (28.9)	1.37 (1.28, 1.46)	-0.12 (-0.21, -0.03)	-0.07 (-0.15, 0.02)	0.69 (0.66, 0.73)	-0.06 (-0.13, 0.02)	0.00 (-0.07, 0.07)
10-<15	203 (22.8)	1.37 (1.28, 1.48)	-0.12 (-0.22, -0. 03)	-0.09 (-0.18, 0.00)	0.69 (0.64, 0.73)	-0.06 (-0.15, 0.02)	-0.04 (-0.11, 0.04)
≥15	143 (16.1)	1.49 (1.36, 1.62)	-0.06 (-0.16, 0.05)	-0.04 (-0.15, 0.07)	0.76 (0.71, 0.81)	0.03 (-0.06, 0.12)	0.04 (-0.05, 0.12)

Missing	7 (0.8)	1.39 (0.97, 2.00)			0.63 (0.44, 0.92)		
Total energy intake (kcal/day)							
<1,230.40	222 (25.0)	1.41 (1.32, 1.51)	REF	REF	0.70 (0.66, 0.75)	REF	REF
1,230.40-<1,528.70	223 (25.1)	1.40 (1.31, 1.50)	-0.02 (-0.12, 0.08)	-0.02 (-0.12, 0.07)	0.68 (0.64, 0.72)	-0.03 (-0.12, 0.05)	-0.03 (-0.11, 0.05)
1,528.70-<1,974.90	221 (24.9)	1.46 (1.36, 1.56)	0.03 (-0.07, 0.12)	0.02 (-0.08, 0.12)	0.74 (0.69, 0.78)	0.05 (-0.04, 0.13)	0.03 (-0.05, 0.11)
≥1974.90	223 (25.0)	1.48 (1.38, 1.58)	0.02 (-0.07, 0.12)	-0.01 (-0.10, 0.09)	0.73 (0.69, 0.78)	0.05 (-0.04, 0.13)	0.00 (-0.08, 0.08)
Physical activity (MET-hour/week)							
<28.11	221 (24.9)	1.68 (1.57, 1.81)	REF	REF	0.82 (0.77, 0.87)	REF	REF
28.11-<44.16	221 (24.9)	1.40 (1.31, 1.49)	-0.18 (-0.27, -0.08)	-0.15 (-0.24, -0.06)	0.70 (0.66, 0.74)	-0.16 (-0.25, -0.08)	-0.12 (-0.20, -0.05)
44.16-<65.99	221 (24.9)	1.43 (1.34, 1.53)	-0.17 (-0.27, -0.07)	-0.13 (-0.23, -0.04)	0.70 (0.66, 0.74)	-0.17 (-0.25, -0.09)	-0.13 (-0.21, -0.05)
≥65.99	221 (24.9)	1.27 (1.19, 1.36)	-0.29 (-0.39, -0.20)	-0.22 (-0.31, -0.12)	0.65 (0.61, 0.69)	-0.24 (-0.33, -0.16)	-0.16 (-0.24, -0.08)
Missing	5 (0.4)	1.34 (0.86, 2.09)			0.76 (0.54, 1.07)		

Abbreviations: BMI: body mass index, MET: metabolic equivalent of task, Cr: creatinine, F_2-IsoP: F_2-isoprostane, 15-F_{2t}-IsoP-M: 2,3-dinor-5,6-dihydro-15-F_{2t}-isoprostane, GM: geometric mean, SD: standard deviation, uGMD: univariate geometric mean difference, aGMD: adjusted geometric mean difference, CI: confidence interval.

‖ Geometric mean was calculated using the whole sample (n=889).

ψ Univariate models were restricted to participants without missing values of the covariates and had the same size as adjusted models (n=856).

¶ aGMD was calculated in multivariable model adjusting for all variables in table 1.

Geometric mean difference and 95% CI were calculated on the basis of natural logarithm of F_2-IsoP and 15-F_{2t}-IsoP-M.

Table A.2: Association between tea consumption or caffeine intake and urinary F_2-IsoP or 15-$F2_{2t}$-IsoP-M

Characteristics	N=889 n (%)	**F_2-IsoP**			**15-F_{2t}-IsoP-M**		
		GM (95% CI)‖	uGMD (95% CI)ψ	aGMD (95% CI)¶	GM (95% CI)‖	uGMD (95% CI)ψ	aGMD (95% CI)¶
Black tea (cups/wk)							
0	165 (18.6)	1.50 (1.38, 1.63)	REF	REF	0.71 (0.66, 0.75)	REF	REF
<1	311 (35.0)	1.39 (1.32, 1.47)	-0.07 (-0.17, 0.03)	-0.05 (-0.14, 0.05)	0.70 (0.67, 0.74)	0.00 (-0.09, 0.08)	0.04 (-0.04, 0.12)
1-<5	187 (21.0)	1.38 (1.28, 1.48)	-0.08 (-0.19, 0.03)	-0.07 (-0.18, 0.04)	0.68 (0.64, 0.73)	-0.04 (-0.13, 0.06)	0.01 (-0.08, 0.10)
≥5	221 (24.9)	1.51 (1.40, 1.62)	0.01 (-0.09, 0.12)	0.01 (-0.09, 0.11)	0.76 (0.71, 0.81)	0.08 (-0.02, 0.17)	0.09 (0.01, 0.17)
Missing	5 (0.5)	1.62 (0.81, 3.24)			0.63 (0.34, 1.15)		
Green tea (cups/wk)							
0	408 (45.9)	1.47 (1.40, 1.55)	REF	REF	0.72 (0.69, 0.75)	REF	REF
<1	315 (35.4)	1.45 (1.37, 1.54)	0.00 (-0.08, 0.07)	0.02 (-0.06, 0.09)	0.72 (0.68, 0.76)	0.00 (-0.07, 0.06)	0.03 (-0.03, 0.09)
1-<5	96 (10.8)	1.29 (1.17, 1.43)	-0.12 (-0.24, -0.01)	-0.06 (-0.17, 0.06)	0.66 (0.61, 0.72)	-0.09 (-0.19, 0.01)	-0.02 (-0.11, 0.07)
≥5	67 (7.6)	1.41 (1.24, 1.60)	-0.04 (-0.18, 0.09)	0.02 (-0.11, 0.15)	0.70 (0.64, 0.78)	-0.02 (-0.14, 0.10)	0.05 (-0.06, 0.16)
Missing	3 (0.3)	1.17 (0.24, 5.77)			0.67 (0.21, 2.16)		
Caffeine intake (mg/day)							
<33.9	222 (25.0)	1.42 (1.33, 1.52)	REF	REF	0.67 (0.64, 0.71)	REF	REF
33.9-<111.2	223 (25.1)	1.46 (1.36, 1.57)	0.03 (-0.07, 0.13)	0.04 (-0.06, 0.13)	0.74 (0.69, 0.79)	0.09 (0.00, 0.17)	0.08 (0.01, 0.16)
111.2-<205.2	221 (24.9)	1.38	-0.03	0.00	0.69	0.03	0.05

		(1.29, 1.48)	(-0.13, 0.06)	(-0.10, 0.09)	(0.65, 0.74)	(-0.06, 0.11)	(-0.03, 0.13)
≥205.2	223 (25.0)	1.49	0.03	0.01	0.75	0.10	0.07
		(1.39, 1.59)	(-0.07, 0.12)	(-0.09, 0.11)	(0.71, 0.79)	(0.02, 0.18)	(-0.01, 0.16)
Black tea (cups/wk)§							
0				REF			REF
<1				-0.05			0.04
				(-0.14, 0.05)			(-0.04, 0.12)
1-<5				-0.07			0.00
				(-0.18, 0.04)			(-0.09, 0.09)
≥5				0.01			0.07
				(-0.10, 0.11)			(-0.02, 0.16)
Green tea (cups/wk)§							
0				REF			REF
<1				0.02			0.02
				(-0.06, 0.09)			(-0.04, 0.08)
1-<5				-0.06			-0.03
				(-0.17, 0.05)			(-0.13, 0.06)
≥5				0.01			0.03
				(-0.12, 0.14)			(-0.08, 0.14)

Abbreviations: F_2-IsoP: F_2-isoprostane, 15-F_{2t}-IsoP-M: 2,3-dinor-5,6-dihydro-15-F_{2t}-isoprostane, uGMD: univariate geometric mean difference, aGMD: adjusted geometric mean difference, CI: confidence interval

§The multivariable model additionally adjusted for caffeine intake.

‖Geometric mean was calculated using the whole sample (n=889).

ψ Univariate models were restricted to participants without missing values of the covariates (black tea: n=858, green tea: n=861, caffeine: n=863).

¶The multivariable model adjusted for age, race, smoking status, BMI, physical activity, household income, education level, and energy intake (black tea: n=858, green tea: n=861, caffeine: n=863).

Geometric mean difference and 95% CI were calculated on the basis of natural logarithm of F_2-IsoP and 15-F_{2t}-IsoP-M

Table A.3: Association between tea consumption and urinary F2-IsoP or 15-F2t-IsoP-M stratified by overweight

Tea consumption	F_2-IsoP aGMD and 95% CI					$15\text{-}F_{2t}$-IsoP-M aGMD and 95% CI				
	BMI<25 kg/m2		BMI≥25 kg/m2		Pint	BMI<25 kg/m2		BMI≥25 kg/m2		Pint
Black tea (cups/wk)	n (%)		n (%)			n (%)		n (%)		
0	70 (18.0)	REF	86 (18.4)	REF	0.45	70 (18.0)	REF	86 (18.4)	REF	0.92
<1	153 (39.2)	-0.11 (-0.25, 0.03)	151 (32.3)	0.01 (-0.13, 0.15)		153 (39.2)	0.02 (-0.09, 0.14)	151 (32.3)	0.01 (-0.12, 0.13)	
1-<5	74 (19.0)	-0.07 (-0.23, 0.09)	105 (22.4)	-0.07 (-0.22, 0.08)		74 (19.0)	-0.02 (-0.15, 0.12)	105 (22.4)	-0.04 (-0.17, 0.09)	
≥5	93 (23.8)	-0.01 (-0.17, 0.15)	126 (26.9)	0.03 (-0.12, 0.19)		93 (23.8)	0.05 (-0.08, 0.18)	126 (26.9)	0.07 (-0.07, 0.20)	
Green tea (cups/wk)	n (%)		n (%)			n (%)		n (%)		
0	169 (43.1)	REF	225 (48.0)	REF	0.67	169 (43.1)	REF	225 (48.0)	REF	0.85
<1	140 (35.7)	0.02 (-0.10, 0.13)	167 (35.6)	0.03 (-0.08, 0.13)		140 (35.7)	0.02 (-0.07, 0.11)	167 (35.6)	0.01 (-0.08, 0.10)	
1-<5	53 (13.5)	-0.05 (-0.21, 0.10)	41 (8.7)	-0.11 (-0.28, 0.07)		53 (13.5)	-0.04 (-0.16, 0.09)	41 (8.7)	-0.09 (-0.24, 0.06)	
≥5	30 (7.7)	0.05 (-0.14, 0.25)	36 (7.7)	-0.06 (-0.25, 0.13)		30 (7.7)	0.01 (-0.15, 0.16)	36 (7.7)	0.03 (-0.14, 0.19)	

Abbreviations: BMI: body mass index, F_2-IsoP: F_2-isoprostane, $15\text{-}F_{2t}$-IsoP-M: 2,3-dinor-5,6-dihydro-$15\text{-}F_{2t}$-isoprostane, aGMD: adjusted geometric mean difference, CI: confidence interval, Pint: p value of interaction

The multivariable model adjusted for age, race, smoking status, physical activity, household income, education level, energy intake, and caffeine intake.

Interaction was examined using log-likelihood ratio test by adding an interaction term between obesity and tea consumption into the adjusted multivariable model

Among women with BMI<25 kg/m2, 390 were included for black tea analysis and 392 were included for green tea analysis.

Among women with BMI≥25 kg/m2, 468 were included for black tea analysis and 469 were included for green tea analysis.

Table A.4: Association between tea consumption and urinary F2-IsoP or 15-F2t-IsoP-M stratified by coffee consumption

Tea consumption	F_2-IsoP aGMD and 95% CI					15-F_{2t}-IsoP-M aGMD and 95% CI				
	Non-consumer		Consumer		Pint	Non-consumer		Consumer		Pint
Black tea (cups/wk)	n (%)		n (%)			n (%)		n (%)		
0	67 (25.3)	REF	88 (15.0)	REF	0.74	67 (25.3)	REF	88 (15.0)	REF	0.55
<1	61 (23.0)	0.05 (-0.13, 0.22)	241 (41.1)	-0.08 (-0.21, 0.04)		61 (23.0)	-0.02 (-0.18, 0.13)	241 (41.1)	0.07 (-0.03, 0.17)	
1-<5	41 (15.5)	-0.03 (-0.22, 0.16)	137 (23.3)	-0.09 (-0.23, 0.05)		41 (15.5)	-0.01 (-0.18, 0.16)	137 (23.3)	0.01 (-0.10, 0.12)	
≥5	96 (36.2)	0.10 (-0.14, 0.35)	121 (20.6)	-0.05 (-0.20, 0.09)		96 (36.2)	-0.06 (-0.28, 0.15)	121 (20.6)	0.11 (-0.01, 0.22)	
Green tea (cups/wk)	n (%)		n (%)			n (%)		n (%)		
0	161 (60.1)	REF	232 (39.6)	REF	0.14	161 (60.1)	REF	232 (39.6)	REF	0.78
<1	75 (28.0)	0.12 (-0.01, 0.26)	229 (39.1)	0.01 (-0.09, 0.10)		75 (28.0)	0.02 (-0.10, 0.14)	229 (39.1)	0.04 (-0.03, 0.12)	
1-<5	17 (6.3)	0.02 (-0.22, 0.27)	76 (13.0)	-0.06 (-0.20, 0.07)		17 (6.3)	0.07 (-0.15, 0.29)	76 (13.0)	-0.04 (-0.15, 0.06)	
≥5	15 (5.6)	-0.20 (-0.46, 0.07)	49 (8.4)	0.05 (-0.11, 0.21)		15 (5.6)	0.00 (-0.23, 0.24)	49 (8.4)	0.03 (-0.09, 0.16)	

Abbreviations: F_2-IsoP: F_2-isoprostane, 15-F_{2t}-IsoP-M: 2,3-dinor-5,6-dihydro-15-F_{2t}-isoprostane, aGMD: adjusted geometric mean difference, CI: confidence interval, Pint: p value of interaction

The multivariable model adjusted for age, race, smoking status, BMI, physical activity, household income, education level, energy intake, and caffeine intake.

Interaction was examined using log-likelihood ratio test by adding an interaction term between coffee and tea consumption into the adjusted multivariable model

Among non-consumers, 265 were included for black tea analysis and 268 were included for green tea analysis.

Among consumers, 587 were included for black tea analysis and 586 were included for green tea analysis.

APPENDIX B: TABLES OF AIM 2

Table B.1: Participants characteristics of NIEHS Sister Study 2003-2009 at enrollment

Characteristics	Overall (n=49,214)	Had breast cancer‡ (n=3,044)	Person-year under observation	Age-adjusted HR
Age (year)				
35-<45	6,324 (12.9%)	299 (9.8%)	54405.9	REF
45-<55	16,898 (34.3%)	962 (31.6%)	143738.7	1.07 (0.88, 1.30)
55-<65	17,368 (35.3%)	1,142 (37.5%)	144889	1.01 (0.79, 1.29)
≥65	8,624 (17.5%)	641 (21.1%)	69368.7	0.99 (0.74, 1.33)
Race				
Non-Hispanic white	41,573 (84.5%)	2,637 (86.6%)	354552	REF
Non-Hispanic black	4,047 (8.2%)	209 (6.9%)	30148.7	0.98 (0.85, 1.13)
Other	3,584 (7.3%)	198 (6.5%)	27614.2	1.01 (0.87, 1.17)
Missing	10 (0.0%)	0		
BMI (kg/m^2) (premenopausal at enrollment)				
<25	7,363 (43.7%)	423 (44.7%)	64280.7	REF
25-<30	4,828 (28.7%)	288 (30.4%)	41096	1.05 (0.90, 1.21)
30-<35	2,589 (15.4%)	134 (14.2%)	21786.7	0.92 (0.75, 1.11)
35-<40	1,199 (7.1%)	59 (6.2%)	9939.2	0.89 (0.68, 1.17)
≥40	848 (5.0%)	43 (4.5%)	6827.2	0.95 (0.69, 1.30)
Missing	9 (0.1%)	0		
(postmenopausal at enrollment)				
<25	11,801 (36.5%)	696 (33.2%)	100047.3	REF
25-<30	10,712 (33.10%)	693 (33.1%)	88704.1	1.11 (1.00, 1.24)
30-<35	5,772 (17.8%)	420 (20.0%)	46912	1.29 (1.14, 1.45)
35-<40	2,538 (7.8%)	173 (8.3%)	20449.9	1.24 (1.05, 1.46)
≥40	1,532 (4.7%)	115 (5.5%)	12056.3	1.43 (1.18, 1.75)
Missing	4 (0.0%)	0		
Education level				
High school or less	7,528 (15.3%)	440 (14.5%)	61100.4	REF
Some college or undergraduate	29,874 (60.7%)	1,808 (59.4%)	250244.6	1.05 (0.94, 1.16)
Graduate school	11,805 (24.0%)	796 (26.2%)	100994	1.13 (1.01, 1.27)
Missing	7 (0.0%)	0		
Annual household income ($)				
0-<50,000	12,034 (24.5%)	728 (23.9%)	97808.3	REF
50,000-<100,000	19,348 (39.3%)	1,160 (38.1%)	163454.8	1.02 (0.93, 1.12)

≥100,000	15,909 (32.3%)	1,014 (33.3%)	135224.4	1.11 (1.01, 1.23)
Missing	1,923 (3.9%)	142 (4.7%)		
Smoking history				
Never	27,611 (56.1%)	1,634 (53.7%)	232524.6	REF
Former	17,594 (35.8%)	1,192 (39.2%)	147231.7	1.10 (1.02, 1.19)
Current	3,997 (8.1%)	218 (7.2%)	32540.1	0.99 (0.86, 1.14)
Missing	12 (0.0%)	0		
Alcohol consumption (drinks/week)				
0	9,266 (18.8%)	564 (18.5%)	75400.7	REF
<1	17,357 (35.3%)	1,016 (33.4%)	145582.2	0.97 (0.88, 1.08)
1-<7	15,840 (32.2%)	1,017 (33.4%)	134431.0	1.06 (0.96, 1.18)
≥7	6,669 (13.6%)	444 (14.6%)	56308.7	1.07 (0.94, 1.21)
Missing	82 (0.2%)	3 (0.1%)		
Regular (not decaf) coffee consumption (cups/wk)				
0	14,260 (29.0%)	867 (28.5%)	120083.8	REF
<10	15,991 (32.5%)	968 (31.8%)	133641.2	0.99 (0.91, 1.09)
10-<15	10,764 (21.9%)	671 (22.0%)	90465.6	1.02 (0.92, 1.13)
≥15	7,265 (14.8%)	480 (15.8%)	60631.3	1.09 (0.97, 1.21)
Missing	934 (1.9%)	58 (1.9%)		
Caffeine intake (mg/day)				
<31.6	12,299 (25.0%)	755 (24.8%)	103380.0	REF
31.6-<105.8	12,313 (25.0%)	764 (25.1%)	102773.8	1.02 (0.92, 1.12)
105.8-<205.0	12,308 (25.0%)	747 (24.5%)	103567.6	0.98 (0.89, 1.09)
≥205.0	12,294 (25.0%)	778 (25.6%)	102680.9	1.03 (0.93, 1.14)
Total energy intake (kcal/day)				
<1,197.2	12,307 (25.0%)	741 (24.3%)	101960.9	REF
1,197.2-<1,545.6	12,301 (25.0%)	707 (23.3%)	103567.0	0.94 (0.85, 1.05)
1,545.6-<1,961.2	12,303 (25.0%)	761 (25.0%)	103999.6	1.01 (0.92, 1.12)
≥1,961.2	12,303 (25.0%)	835 (27.4%)	102874.8	1.14 (1.04, 1.26)
Physical activity (MET-hour/week)				
<27.1	12,206 (24.8%)	787 (25.9%)	101791.8	REF
27.1-<44.4	12,169 (24.7%)	776 (25.5%)	101961.6	0.98 (0.89, 1.08)
44.4-<67.2	12,220 (24.8%)	744 (24.4%)	102841.4	0.93 (0.84, 1.03)
≥67.2	12,201 (24.8%)	720 (23.7%)	102447.6	0.90 (0.81, 1.00)
Missing	418 (0.9%)	17 (0.6%)		

Meat consumption¶ (ounce-equivalent/day)				
<0.70	12,280 (24.9%)	728 (23.9%)	102001.2	REF
0.70-<1.23	12,346 (25.1%)	738 (24.2%)	103819.4	1.01 (0.91, 1.12)
1.23-<2.01	12,287 (25.0%)	731 (24.0%)	103335.4	1.02 (0.92, 1.13)
≥2.01	12.301 (25.0%)	847 (27.8%)	103246.3	1.19 (1.08, 1.32)
Missing				
Sleep duration (hours/day)				
<7	14,156 (28.8%)	847 (27.8%)	116925.5	0.94 (0.86, 1.03)
7-<8	18,561 (37.7%)	1,205 (39.6%)	157252.8	REF
≥8	16,432 (33.4%)	987 (32.4%)	137714.7	0.92 (0.85, 1.00)
Missing	65 (0.1%)	5 (0.2%)		

Abbreviation: NIEHS: The National Institute of Environmental Health Sciences, BMI: body mass index, MET: metabolic equivalent of task, HR: hazard ratio, CI: confidence interval
ǂ Breast cancer included invasive breast cancer and non-invasive cancer
¶This included cured meat and red meat.

Table B.2: Hazard ratios and 95% confidence intervals of breast cancer across different levels of tea consumption in the total study population

	Overall (n=49,214)	Had breast cancer‡ (n=3,044)	Person-year under observation	HR[a] (95% CI)	HR[b] (95% CI)
Black tea (cups/week)					
0	8,966 (18.2%)	556 (18.3%)	73866.6	REF	REF
<1	16,566 (33.7%)	1,048 (34.4%)	139435	0.99 (0.89, 1.10)	0.97 (0.87, 1.08)
1-<5	10,884 (22.1%)	686 (22.5%)	91527.1	0.97 (0.87, 1.09)	0.93 (0.83, 1.05)
≥5	12,329 (25.1%)	722 (23.7%)	103835	0.90 (0.80, 1.01)	0.86 (0.76, 0.98)
Missing	469 (1.0%)	32 (1.1%)			
Green tea (cups/week)					
0	21,359 (43.4%)	1,314 (43.2%)	179751.7	REF	REF
<1	16,527 (33.6%)	1,107 (36.4%)	138691.1	1.09 (1.00, 1.18)	1.07 (0.99, 1.17)
1-<5	6,321 (12.8%)	358 (11.8%)	52629	0.91 (0.81, 1.03)	0.90 (0.80, 1.02)
≥5	4,409 (9.0%)	230 (7.6%)	36419.2	0.85 (0.73, 0.98)	0.84 (0.73, 0.97)
Missing	598 (1.2%)	35 (1.2%)			

Abbreviations: HR: hazard ratio, CI: confidence interval.
HR[a] is the age-adjusted hazard ratio, HR[b] is the multi-adjusted hazard ratio.
46,349 women were included for black tea analysis, and 46,223 women were included for green tea analysis.
Variables adjusted: race, body mass index, education, income, smoking status, alcohol consumption, energy intake, physical activity, meat consumption, sleep duration, and caffeine intake.
‡ Breast cancer included invasive and non-invasive breast cancer.

Table B.3: Hazard ratios and 95% confidence intervals of breast cancer across different levels of tea consumption by ER status

	ER+ breast cancer as outcome↑		ER- breast cancer as outcome¶		Triple negative breast cancer as outcome §	
	No. case/overall	aHR (95% CI)	No. case/overall	aHR (95% CI)	No. case/overall	aHR (95% CI)
Black tea (cups/week)						
0	376/8,419	REF	66/8,419	REF	36/8,403	REF
<1	728/15,618	0.98 (0.87, 1.11)	132/15,618	0.97 (0.84, 1.12)	67/15,591	0.99 (0.65, 1.49)
1-<5	466/10,284	0.95 (0.83, 1.08)	88/10,284	0.92 (0.74, 1.15)	54/10,269	1.16 (0.75, 1.80)
≥5	498/11,615	0.87 (0.76, 1.01)	81/11,615	0.83 (0.62, 1.12)	42/11,599	0.77 (0.48, 1.23)
Green tea (cups/week)						
0	895/20,087	REF	159/20,087	REF	93/20,056	REF
<1	774/15,659	1.08 (0.98, 1.18)	137/15,659	1.07 (0.93, 1.23)	71/15,628	0.99 (0.73, 1.36)
1-<5	244/5,927	0.90 (0.78, 1.03)	41/5,927	0.88 (0.69, 1.13)	24/5,922	0.86 (0.55, 1.36)
≥5	155/4,133	0.83 (0.70, 0.99)	27/4,133	0.81 (0.57, 1.15)	11/4,127	0.57 (0.30, 1.07)

Abbreviations: aHR: adjusted hazard ratio, CI: confidence interval.
Variables adjusted: race, body mass index, education, income, smoking status, alcohol consumption, energy intake, physical activity, meat consumption, sleep duration, and caffeine intake.
↑45,936 women were included for black tea analysis and 45,806 women were included for green tea analysis.
¶45,936 women were included for black tea analysis and 45,806 women were included for green tea analysis
§ ER-/PR-/HER2- breast cancer was treated as the outcome of interest. Other breast cancer and other outcomes at the end of follow-up were treated as censoring events in the model (45,862 women were included for black tea analysis and 45,733 women were included for green tea analysis).

Table B.4: Hazard ratios and 95% confidence intervals of breast cancer across different levels of tea consumption by menopausal status

	Premenopausal breast cancer		Postmenopausal breast cancer	
	No. case/overall	aHR (95% CI)	No. case/overall	aHR (95% CI)
Black tea (cups/week)				
0	111/2,689	REF	389/6,762	REF
<1	202/4,668	1.02 (0.81, 1.30)	757/12,786	0.96 (0.85, 1.09)
1-<5	125/2,990	1.00 (0.77, 1.30)	502/8,494	0.93 (0.81, 1.07)
≥5	113/3,086	0.89 (0.67, 1.19)	541/9,814	0.86 (0.75, 0.99)
Green tea (cups/week)				
0	259/6,296	REF	940/16,382	REF
<1	186/4,473	0.98 (0.81, 1.18)	824/12,850	1.09 (0.99, 1.20)
1-<5	65/1,642	0.93 (0.70, 1.22)	259/4,965	0.89 (0.77, 1.02)
≥5	42/1,019	0.99 (0.71, 1.38)	165/3,530	0.79 (0.67, 0.94)

Abbreviations: aHR: adjusted hazard ratio, CI: confidence interval.

Variables adjusted: race, body mass index, education, income, smoking status, alcohol consumption, energy intake, physical activity, meat consumption, sleep duration, and caffeine intake

For premenopausal breast cancer, 13,433 women were included for black tea analysis, and 13,430 women were included for green tea analysis.

For postmenopausal breast cancer, 37,856 women were included for black tea analysis, and 37,727 women were included for green tea analysis.

Table B.5: Subgroup analysis of associations between tea consumption and breast cancer risk stratified by obesity (BMI±30 kg/m^2)

	BMI<30 kg/m^2		BMI≥30 kg/m^2		
	No. case/overall	aHR (95% CI)	No. case/overall	aHR (95% CI)	P_{int}
Black tea (cups/week)					
0	369/5,997	REF	155/2,504	REF	0.52
<1	712/11,425	0.95 (0.84, 1.09)	283/4,328	0.98 (0.80, 1.20)	
1-<5	425/7,095	0.90 (0.78, 1.04)	221/3,281	1.03 (0.83, 1.27)	
≥5	449/8,066	0.83 (0.71, 0.96)	234/3,653	0.96 (0.77, 1.20)	
Green tea (cups/week)					
0	834/13,689	REF	405/6,583	REF	0.57
<1	744/11,442	1.05 (0.95, 1.16)	313/4,363	1.13 (0.97, 1.31)	
1-<5	235/4,301	0.87 (0.75, 1.01)	100/1,676	0.96 (0.77, 1.20)	
≥5	152/3,078	0.79 (0.66, 0.94)	66/1,091	0.96 (0.73, 1.25)	

Abbreviations: aHR: adjusted hazard ratio, CI: confidence interval, P_{int}: p value for interaction test.
Variables adjusted: race, body mass index, education, income, smoking status, alcohol consumption, energy intake, physical activity, meat consumption, sleep duration, and caffeine intake
Interaction was tested by log-likelihood ratio test.
For BMI<30 kg/m^2, 32,583 women were included for black tea analysis, and 32,510 women were included for green tea analysis.
For BMI≥30 kg/m^2, 13,766 women were included for black tea analysis, and 13,713 women were included for green tea analysis.

Table B.6: Subgroup analysis of associations between tea consumption and breast cancer risk stratified by regular coffee consumption

	Non-consumers		Consumers		
	No. case/overall	aHR (95% CI)	No. case/overall	aHR (95% CI)	P_{int}
Black tea (cups/week)					
0	238/3,759	REF	283/4,607	REF	0.54
<1	208/3,255	0.99 (0.82, 1.19)	769/12,238	0.96 (0.84, 1.10)	
1-<5	119/2,141	0.82 (0.66, 1.03)	519/8,069	0.97 (0.84, 1.12)	
≥5	241/4,234	0.82 (0.62, 1.08)	427/7,250	0.87 (0.75, 1.01)	
Green tea (cups/week)					
0	445/7,233	REF	777/12,722	REF	0.37
<1	211/3,505	0.97 (0.82, 1.14)	828/12,048	1.11 (1.00, 1.23)	
1-<5	78/1,381	0.89 (0.70, 1.14)	252/4,497	0.91 (0.79, 1.05)	
≥5	70/1,279	0.93 (0.71, 1.21)	141/2,798	0.80 (0.67, 0.96)	

Abbreviations: aHR: adjusted hazard ratio, CI: confidence interval, P_{int}: p value for interaction test.
Variables adjusted: race, body mass index, education, income, smoking status, alcohol consumption, energy intake, physical activity, meat consumption, sleep duration, and caffeine intake
Interaction was tested by log-likelihood ratio test.
For non-consumers, 13,389 women were included for black tea analysis, and 13,420 women were included for green tea analysis.
For consumers, 32,164 women were included for black tea analysis, and 32,102 women were included for green tea analysis.

Table B.7: Subgroup analysis of associations between tea consumption and breast cancer risk stratified by alcohol consumption.

	Non-alcohol drinkers		Alcohol drinkers		
	No. case/overall	aHR (95% CI)	No. case/overall	aHR (95% CI)	P_{int}
Black tea (cups/week)					
0	152/2,397	REF	372/6,104	REF	0.71
<1	145/2,375	0.90 (0.71, 1.13)	850/13,378	0.99 (0.87, 1.12)	
1-<5	92/1,616	0.84 (0.64, 1.10)	554/8,760	0.96 (0.84, 1.10)	
≥5	119/2,228	0.78 (0.59, 1.02)	564/9,491	0.88 (0.77, 1.01)	
Green tea (cups/week)					
0	273/4,579	REF	966/15,693	REF	0.05
<1	135/2,341	0.92 (0.74, 1.13)	922/13,464	1.10 (1.01, 1.21)	
1-<5	66/924	1.16 (0.88, 1.52)	269/5,053	0.86 (0.75, 0.98)	
≥5	38/738	0.82 (0.58, 1.16)	180/3,431	0.84 (0.72, 0.99)	

Abbreviations: aHR: adjusted hazard ratio, CI: confidence interval, P_{int}: p value for interaction test.
Variables adjusted: race, body mass index, education, income, smoking status, alcohol consumption, energy intake, physical activity, meat consumption, sleep duration, and caffeine intake
Interaction was tested by log-likelihood ratio test.
For non-alcohol drinkers, 8,616 women were included for black tea analysis, and 8,582 women were included for green tea analysis.
For alcohol drinkers, 37,733 women were included for black tea analysis, and 37,641 women were included for green tea analysis.

Table B.8: Hazard ratios and 95% confidence intervals of breast cancer across different levels of tea consumption excluding people with ≤6 months follow-up

	Overall (n=49,037)	Had breast cancer‡ (n=2,895)	Person-year under observation	HR[a] (95% CI)	HR[b] (95% CI)
Black tea (cups/week)					
0	8,928 (18.2%)	528 (18.3%)	69391.8	REF	REF
<1	16,500 (33.7%)	990 (34.3%)	131165.7	0.99 (0.88, 1.10)	0.96 (0.86, 1.08)
1-<5	10,838 (22.1%)	649 (22.5%)	86094.4	0.97 (0.86, 1.09)	0.93 (0.82, 1.05)
≥5	12,286 (25.1%)	687 (23.8%)	97678.1	0.90 (0.80, 1.02)	0.87 (0.77, 0.99)
Missing	467 (1.0%)	30 (1.0%)			
Green tea (cups/week)					
0	21,273 (43.4%)	1,243 (43.1%)	169087.9	REF	REF
<1	16,461 (33.6%)	1,054 (36.6%)	130442.6	1.09 (1.01, 1.19)	1.08 (0.99, 1.18)
1-<5	6,296 (12.8%)	336 (11.7%)	49473.7	0.90 (0.80, 1.02)	0.89 (0.79, 1.01)
≥5	4,394 (9.0%)	218 (7.6%)	34217.6	0.85 (0.73, 0.98)	0.85 (0.73, 0.98)
Missing	595 (1.2%)	33 (1.1%)			

Abbreviations: HR: hazard ratio, CI: confidence interval.
HR[a] is the age-adjusted hazard ratio, HR[b] is the multi-adjusted hazard ratio.
46,168 women were included for black tea analysis, and 46,043 women were included for green tea analysis.
Variables adjusted: race, body mass index, education, income, smoking status, alcohol consumption, energy intake, physical activity, meat consumption, sleep duration, and caffeine intake.
‡ Breast cancer included invasive and non-invasive breast cancer.

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