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Interaction of nitrate and folate on the risk of breast cancer among postmenopausal women

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Abstract

Ingested nitrate can be endogenously reduced to nitrite, which may form *N*-nitroso compounds, known potent carcinogens. However, some studies have reported no or inverse associations between dietary nitrate intake and cancer risk. These associations may be confounded by a protective effect of folate, which plays a vital role in DNA repair. We evaluated the interaction of dietary and water nitrate intake with total folate intake on breast cancer risk in the Iowa Women's Health Study. Dietary intake was assessed at study baseline. Nitrate intake from public water was assessed using a historical database on Iowa municipal water supplies. After baseline exclusions, 34,388 postmenopausal women and 2,875 incident breast cancers were included. Overall, neither dietary nor water nitrate was associated with breast cancer risk. Among those with folate intake ≥400 μg/d, breast cancer risk was significantly increased in public water users with the highest nitrate quintile (HR=1.40, 95%CI=1.05–1.87) and private well users (HR=1.38, 95%CI=1.05–1.82) compared to public water users with the lowest nitrate quintile; in contrast, there was no association among those with lower folate intake. Our findings do not support a previous report of increased risk of breast cancer among individuals with high dietary nitrate but low folate intake.

Keywords

nitrate; folate; interaction; breast cancer; epidemiological

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Introduction

Breast cancer is the second leading cause of cancer death among women in the United States (1). Identifying risk factors and predictors of breast cancer is particularly important for primary and secondary prevention. Many epidemiologic studies have examined various dietary factors as potential modifiable risk factors or predictors of breast cancer. There are convincing or suggestive evidence for alcoholic drinks and total fat (postmenopausal breast cancer); however, the effects of other dietary factors are still not conclusive (2).

High intake of nitrate has been a growing concern in relation to cancer as ingested nitrate can be reduced in the oral cavity to nitrite, which can subsequently reacts with amines and amides in the stomach forming *N*-nitroso compounds (NOCs), most of which are known potent carcinogens (3, 4). Exposure to industrial NOCs was associated with DNA adduct formation and benign and malignant tumor incidence in mammary glands in animal studies, suggesting potential relevance for risk of breast cancer (5–7). Nitrate is a natural component of the diet, and nitrate and nitrite are added as preservatives to meats. Nitrate is also a common contaminant of drinking water as a result of excessive application of nitrogen fertilizers to crops, animal waste, pollution from inadequately treated municipal wastewater effluent and air pollution. Nitrogen from all these sources can leach into groundwater or run off to surface waters, which are sources of drinking water. Agricultural and urban nitrogen use has rapidly increased since 1950 and is currently estimated to exceed nitrogen fixed by natural sources by 30% (8). The maximum contaminant level (MCL) of 10mg/L (or 10 ppm) nitrate-nitrogen for public water supplies was determined based on the prevention of methemoglobinemia, an acute health effect (9). Research performed over the decade has demonstrated therapeutic indications from vasoprotective benefits of the short-term treatment with low-dose nitric oxide, a recycled product of nitrate *in vivo* (10). However, long-term effects of chronic intake of moderately high levels of nitrate (5–9 ppm nitrate nitrogen) from drinking water, in addition to dietary nitrate intake, on risk of chronic diseases such as cancer are still not clear.

A number of epidemiologic studies have reported no associations or inverse associations between dietary nitrate intake and cancer risk with most focusing on gastric cancer (11–15) and postulated that these results may be due to antioxidants, which co-exist with nitrate in foods (11, 13, 14, 16–18). The major dietary sources of nitrate are green leafy and root vegetables, which may contribute to up to 93% of total nitrate intake (18–20). Vegetables are also a major source of antioxidants such as vitamin C, which has been shown to inhibit the endogenous formation of NOCs in the stomach (16, 21, 22). Folate is also abundant in dietary sources of nitrate. NOCs are known to yield multiple DNA adducts and induce mutations in activated oncogenes (23–25). DNA adducts must be repaired to avoid proceeding to mutations. Folate plays an important role in DNA repair by providing one-carbon moieties for purine and pyrimidine nucleotide synthesis; therefore, deficient folate status may result in aberrant DNA synthesis and repair functions (26–28). Thus, no or inverse associations between nitrate intake and cancer risk reported by previous epidemiologic studies may be confounded by the protective effects of antioxidants or folate through the enhanced DNA repair system, as well as the inhibition of NOC formation by antioxidants.

Recently, a case-control study of breast cancer reported an interaction between dietary nitrate and folate intakes (29). Dietary nitrate intake was not associated with breast cancer risk, but increased intake of nitrate relative to folate, as a nitrate-folate ratio, was associated with elevated risk of breast cancer. Dietary nitrate intake was positively associated with risk of breast cancer only among women with low folate intake. These findings suggested that this dietary pattern may be a potential risk factor for breast cancer. However, this study had

several limitations, including a relatively small sample size (362 cases and 362 matched controls), potential biases related to the case-control study design, and lack of information on nitrate intake from drinking water. In the present study, we evaluated effect modification by folate on the association between nitrate intake and risk of breast cancer in the Iowa Women's Health Study.

Methods

The Iowa Women's Health Study (IWHS)

The IWHS is a prospective cohort study among women in Iowa, initiated in 1986; the research focus was on cancer incidence. Details of the study design of the IWHS have been described elsewhere (30). Briefly, a self-administered questionnaire was mailed to 99,826 women aged 55 to 69 who were randomly selected from the Iowa driver's license records. Of these women, 41,836 women (42%) who completed the baseline questionnaire comprise the IWHS cohort. The questionnaire contained questions on demographics, anthropometry, medical history, reproductive history, hormone use, family history of cancer, residence location, physical activity, smoking, alcohol consumption and usual dietary intake. Compared with respondents to the baseline questionnaire, non-respondents were slightly older and had a higher body mass index (BMI), but were otherwise comparable in terms of baseline demographic characteristics and lifestyle factors (31). The IWHS was approved by the Institutional Review Board of the University of Minnesota.

Dietary intake data

Dietary intake was assessed at study baseline using the Harvard food frequency questionnaire (FFQ) developed by Willett *et al.* Cohort participants were asked to report their usual intake frequencies of 127 food items during the past 12 months. There were 9 frequency levels of each food item, ranging from "never or less than once per month" to "6 or more per day". To enable participants to obtain a sense of scale, a commonly used portion size for each food item was specified. Dietary nutrient intake was calculated by multiplying the frequency of consumption of the specified unit of each food by the nutrient content of that unit of food. The use of dietary supplements was also asked. Total intake of folate, vitamin C and E were computed by combining intakes from foods and dietary supplements. This FFQ has been shown to be valid and reproducible in the study population (32).

To compute dietary intake of nitrate and nitrite, the nitrate and nitrite contents of FFQ foods were determined by reviewing the literature focusing on published reports for the United States and Canada. We computed means of the published values weighted by the number of samples analyzed as previously described (33, 34). The nitrate and nitrite contents of foods comprising a FFQ line item (for example, weighted means for canned, raw, and cooked tomatoes) were combined by weighting the food-specific nitrate and nitrite values by sex-specific intake amounts from the 1994–1996 Continuing Survey of Food Intake by Individuals (CSFII) (35). Dietary intakes of nitrate and nitrite were calculated by multiplying the nitrate or nitrite content of each line item by consumption frequency, and summing values across all line items. We also computed dietary intakes of nitrate and nitrite from plant and animal sources separately.

Nitrate intake from drinking water

The estimation of nitrate ingestion from public drinking water supplies was previously described in detail (36). Information on usual source of drinking water was collected in the second follow-up questionnaire in 1989. The cohort participants were asked the main source of drinking water at home (municipal water system, private wall, purchased bottled water from a store or dealer, and other) and how long they drank the type of water they indicated

(<1 year, 1–5 years, 6–10 years, 11–20 years, and >20 years). A total of 36,127 women (89% of eligible participants) responded to the questionnaire. The primary source of drinking water among these women was public water (76%) followed by private well (18%) and bottled water (6%). Of the women using municipal water, 82% and 69% used the municipal water supply for >10 years and >20 years, respectively. Of the women using a municipal water supply for >10 years, 79% resided in a total of 484 communities.

To estimate nitrate intake from drinking water, we used a historical database of Iowa municipal water supplies, which contains nitrate measurements from water samples collected during 3 time periods (1955–1964, 1976–1982, and 1983–1988). All water samples were analyzed at the University of Iowa Hygienic Laboratory. Water samples during 1955–1964 were analyzed using the phenoldisulfonic spectrophotometry method (37). The 1976–1988 water samples were analyzed using the cadmium reduction method (38). The mean values from the 3 time periods were averaged to calculate a mean nitrate concentration in each community's water supply for the entire 33-year period (1955–1988). There were no nitrate concentration data available for private wells.

Study population

Of the 41,836 cohort participants, we excluded women who reported at baseline: 1) history of cancer except non-melanoma skin cancer (N = 3,830); 2) premenopausal status (N = 547); or 3) previous mastectomy or partial breast resection (N = 354). For accuracy of dietary intake data, we also excluded 2,717 women for one or both of the following reasons; 1) left more than 30 items blank on the FFQ; or 2) reported implausible energy intake estimates (<600 or >5,000 kcal/day). As a result, a total of 34,388 postmenopausal women were included in the analysis of dietary nitrate intake.

To evaluate long-term exposure to nitrate from drinking water, we further excluded those who did not respond to or died before the 1989 follow-up survey and those who had used their public or private well water supply for 10 years or less. We also excluded women living in 47 communities that were served by multiple water sources and 41 communities for which no nitrate measurement data were available (36). After these exclusions, a total of 20,147 women (15,151 women using public water and 4,996 using private well water) were included in the drinking water analysis.

Through 2008, a total of 2,875 incident breast cancer cases were identified by annual computer match with the State Health Registry of Iowa's cancer database, a member of the National Cancer Institute's Surveillance, Epidemiology and End Results (SEER) program. The vital status of the cohort participants was determined via linkage with the State Health Registry of Iowa, supplemented with the National Death Index of the National Center for Health Statistics. Person-years of follow-up were assigned for each participant from the date of return of the baseline questionnaire to: 1) the date of first breast cancer diagnosis, 2) date of emigration from Iowa, 3) date of death, or 4) December 31, 2008, whichever came first.

Data Analysis

We used Cox proportional hazard regression modeling (SAS, PROC PHREG) to compute hazard ratios (HRs) and their 95% confidence intervals (CIs) for breast cancer. Associations between baseline demographic, lifestyle and dietary factors and breast cancer risk were evaluated to determine potential confounders for an association between nitrate intake and breast cancer risk. We assessed breast cancer risk in relation to nitrate intake from diet and drinking water separately, adjusting for confounders and biologically relevant risk factors for breast cancer including age (continuous), BMI (kg/m^2), waist-hip-ratio (WHR) (continuous), education (<high school, high school, >high school), smoking (never,

previous, current), alcohol intake, family history of breast cancer, age at menopause (continuous), age at first live birth (continuous), estrogen use, total energy intake (continuous), total intake of folate (except for analyses of the nitrate-folate ratio and nitrate-folate interaction), vitamin C and E and flavonoids (continuous), cruciferous vegetable and red meat intakes (servings/week, continuous). The distributions of dietary intake (folate, vitamin C and E, flavonoids, cruciferous vegetables and red meat) were markedly skewed towards high values; therefore, logarithmically transformed values were used as covariates in the analysis. HRs and 95% CIs were calculated for quintiles of nitrate intake using the lowest quintile as a reference group. We also tested for trends across quintile categories by using the median intake level in each quintile as a continuous variable in the models. We conducted similar analyses of dietary nitrite intake.

Nitrate intake from drinking water for public water supply users was computed by multiplying the average nitrate level in the public water supply by an estimated daily water consumption of 2 liters (L) per day; data on individual daily water consumption were not obtained. In addition, HRs and 95% CIs were computed for private well users compared to those in the lowest quintile of nitrate intake from public water. For public water supply users, total nitrate intake was also calculated by summing dietary and water nitrate intakes. Total and dietary nitrate intakes were highly correlated (Spearman correlation coefficient (r) = 0.93) because the primary nitrate source was diet for most women (percent of total nitrate intake from diet; median = 91.2%, interquartile range (IQR) = 78.9 – 97.0%). Therefore, we do not present results for total nitrate intake.

We performed three different types of analyses to evaluate an interaction of nitrate and folate. First, we repeated the analysis performed by Yang *et al* (29), the Korean case-control study that evaluated the association between a nitrate-folate ratio and breast cancer risk. Second, we evaluated an interaction between nitrate and folate for dietary and water nitrate separately, by including an interaction term for the nitrate intake quintiles and low (<400 μg/d = U.S. Recommended Daily Allowance (RDA)) and adequate or higher (≥400 μg/d) total folate intake, in models adjusted for potential confounders. Finally, we stratified the analysis of dietary and water nitrate intake by low and adequate or higher total folate to evaluate differential effects of folate on the nitrate-breast cancer association by intake levels.

All statistical procedures were performed using SAS version 9.2 (SAS Institute, Inc., Cary, NC). All reported p values were two-sided, and significance was defined as $p < 0.05$. This study had 80% power to detect HR of 1.20 in the highest quartile category of dietary nitrate intake compared with a reference group of women with total folate intake lower than the median (350.7 μg/d).

Results

The average age of the study population at baseline was 61.6 (standard deviation (SD), 4.2; range, 52–71). The study population was predominantly Caucasian (99.2%). Approximately 85% of the participants had completed high school or a higher level of education. Age, BMI, WHR, education, family history of breast cancer, age at menopause, age at first live birth and estrogen use were statistically significantly positively associated with breast cancer risk, while physical activity was inversely associated with breast cancer risk. Cigarette smoking, alcohol intake, age at menarche, and oral contraceptive use were not associated with breast cancer risk. The use of dietary supplements containing folic acid (folic acid or multivitamin) was reported by 8.3% and 69.7% of the women with low and adequate or higher total folate intake, respectively. There was a trend toward decreased risk of breast cancer with moderate total folate intake (400 – 600 μg/d) compared with low folate intake, but risk of breast cancer among those with high folate intake was not different from low folate intake. Total

energy, antioxidants (vitamin C and E and flavonoids), cruciferous vegetable and red meat intakes were not associated with breast cancer risk (data not shown).

The average dietary intakes of nitrate and nitrite were 123.5 mg/d and 1.2 mg/d, respectively. Nitrate intake from plant sources accounted for 97% of dietary nitrate intake. Approximately 63% of dietary nitrite intake was from plant sources. The major contributors to dietary nitrate intake included lettuce (23.2%), celery (16.6%), beets (5.4%), spinach (3.2%) and broccoli (2.9%), while the major contributors to dietary nitrite intake were red meat (beef, pork, and lamb; 11.2%), milk (11.0%), cereals (9.3%), apples (7.1%) and processed meat (salami, sausage, bologna, bacon, hot dog, etc.; 4.4%). Because major contributors of dietary nitrate intake were vegetables, which are also major sources of folate and antioxidants, we evaluated correlations of these nutrient intakes and dietary nitrate intake. Spearman correlation coefficients (r) for dietary nitrate intake with dietary folate, vitamin C, vitamin E and flavonoid intakes were 0.40, 0.36, 0.29 and 0.46, respectively (**Supplemental Table**).

Table 1 shows demographic, lifestyle and dietary factors by dietary nitrate intake quintiles. The prevalence of the completion of college or some college education, alcohol intake and moderate or high physical activity were 62%, 33% and 62% higher, respectively, while never cigarette smoking was 42% lower in the highest quintile of dietary nitrate intake than the lowest quintile. The prevalence of estrogen use was slightly higher in the highest vs. lowest quintile of dietary nitrate intake. Other demographic and lifestyle factors were not substantially different across quintiles of dietary nitrate intake. As expected, total energy intake, total folate, vitamin C and E, flavonoid and cruciferous vegetable intake were positively associated with dietary nitrate intake (p for tend <0.0001). Total folate and vitamin C and E intakes in the highest quintile of dietary nitrate were double and flavonoid intake was almost triple of that in the lowest quintile. Higher red meat intake was also associated with higher nitrate intake (p for tend <0.0001). The distributions of these baseline factors among public water users showed little associations with public water nitrate levels (36).

Overall, neither dietary nitrate nor nitrite intake was associated with risk of breast cancer after adjustment for potential cofounders (Table 2). There was a statistically significant inverse trend in breast cancer risk across quintiles of the nitrate to total folate ratio, but none of the risk estimates were statistically significant. The ratio of dietary nitrite intake to total folate intake was not associated with breast cancer risk (data not shown). Similarly, breast cancer risk was not associated with nitrate intake from drinking water or with private well use (Table 3).

There were no statistically significant interactions of nitrate intake from diet (in quintiles) and total folate intake (<400 μg/d or ≥400 μg/d) on risk of breast cancer (data not shown). A marginally statistically significant interaction between water nitrate intake and total folate intake (<400 μg/d or ≥400 μg/d) was observed in the highest (p for interaction = 0.055) and fourth (p for interaction = 0.053) quintiles of nitrate intake from public water. Among women with adequate or higher total folate intake (≥400 μg/d), breast cancer risk was statistically significantly increased in women using public water with the highest quintile of nitrate and in those using private wells compared to those using public water with the lowest quintile of water nitrate intake; whereas, such an association was not observed among women with low total folate intake (<400 μg/d) (Table 4). There was no effect modification by total folate intake on the association between dietary nitrate intake and breast cancer risk.

Discussion

In this large prospective cohort study, we did not observe associations between nitrate intake from diet or drinking water and breast cancer risk. Among women with adequate or higher total folate intake (≥400 μg/d), breast cancer risk was statistically significantly increased among those using public water with the highest quintile of nitrate and among private well users compared to those using public water with the lowest nitrate level. Effect modification by total folate intake was not observed in the association between dietary nitrate intake and risk of breast cancer.

High nitrate intake has been a concern in relation to risk of methemoglobinemia (or blue-baby syndrome), a potentially fatal illness in infants, and therefore nitrate levels in public water supplies have been regulated. However, health effects of chronic intake of moderately high nitrate intake have not been considered when regulatory levels have been determined. Approximately 8% of ingested nitrate from foods and water is endogenously reduced to nitrite, which can then react with amines and amides to form NOCs that may alkylate DNA forming DNA adducts (39). If DNA adducts are not removed by the DNA repair system, they may lead to chromosomal instability, which elevates genome-wide mutation rates (40).

The level of DNA adducts is influenced by the consumption of fruits and vegetables. In a large population-based study, DNA adduct level was negatively correlated with fruit and vegetable intakes (41). One potential mechanism that explains the preventive effect of fruits and vegetables against DNA adduct formation is that high folate levels in fruits and vegetables interfere with DNA adduct formation via enhanced function of the DNA repair system. The DNA repair system can identify DNA adducts and replace the entire portion of the damaged strand of the double helix with normal nucleotides (42).

In the previous case-control study in Korea, high intake of dietary nitrate relative to total folate was associated with increased risk of breast cancer (29). They also reported increased breast cancer risk related to higher dietary nitrate intake only among individuals with low folate intake. In the present study, we did not observe such an interaction of folate and nitrate intakes from foods or from drinking water. One possible reason for the differences between our results may be the lower dietary nitrate intake in our study population compared with the Korean study population. The average dietary nitrate in our study population was 123.5 mg/d, while the average in Korea was approximately 420 mg/d (29), which is approximately twice the Acceptable Daily Intake (ADI, 3.7 mg/kg of body weight; equivalent to 222 mg/d for a 60-kg person) established by the Joint Food and Agriculture Organization/World Health Organization (WHO) Expert Committee on Food Additives (43). The high dietary nitrate intake in Korea stems from frequent consumption of high nitrate vegetables including kimchi, traditional Korean fermented vegetables. The major vegetables in kimchi include cabbage and radishes that contain high levels of nitrate as well as NOC precursors and preformed NOCs (29, 44). According to the European Food Safety Authority, the WHO recommendation for fruit and vegetable consumption (400 gm/d) (45) would not exceed the ADI of nitrate, but high consumers of high-nitrate vegetables such as green leafy vegetables could exceed the ADI (46). In our study population, the average vegetable consumption was 26 servings/week (approximately 75% of the United States Department of Agriculture recommendation)(47), and the average consumptions of major contributors of dietary nitrate intake were lettuce (192 gm/week), celery (78 gm/week), and beets (34 gm/week).

Another hypothesized mechanism to explain the protective effect of fruit and vegetable intake against DNA adducts is the inhibition of oxidative damage by antioxidants contained in fruits and vegetables. Vitamin C and other antioxidants such as vitamin E and

polyphenols inhibit the endogenous formation of NOCs and DNA adducts (16, 48). When taken between 2 h before and 1 h after administration of nitrate and amino acids, vitamin C inhibited the formation of NOCs (49). There are other constituents of fruits and vegetables that potentially explain the protective effect of fruits and vegetables against cancer. Isothiocyanates, potent anti-carcinogens rich in cruciferous vegetables such as broccoli and cabbage, have shown to induce metabolic enzymes involved in the metabolism of dietary carcinogens and were associated with decreased risk of cancer (50, 51). In the present study, we adjusted for cruciferous vegetable intake as well as antioxidant intakes (vitamin C and E and flavonoids). When we did not adjust for intakes of folate, vitamin C and E, flavonoids and cruciferous vegetables, a statistically significant overall inverse association was observed between dietary nitrate intake and breast cancer risk, especially among women with low total folate intake (p for trend <0.05; data not shown). This inverse association might be due to confounding by antioxidants and other constituents of fruits and vegetables. In fact, such an inverse association with breast cancer risk was not observed in the water nitrate analysis. In addition, modest correlations of dietary antioxidant intakes with dietary nitrate intake (r = 0.29 – 0.46) and higher correlations with dietary folate intake (r = 0.33 – 0.72) might have caused residual confounding, which may partly explain lack of an interaction between dietary nitrate and folate intakes in our study.

The formation of NOCs and DNA adducts may be inhibited by antioxidants, and the repair of DNA adducts may be enhanced by an increased supply of folate. Nitrate intake from drinking water would, in theory, be more “carcinogenic” because water can be consumed without antioxidants and folate, but dietary sources of nitrate usually contain these nutrients abundantly. Some epidemiologic studies have shown increased risk of cancer related to higher nitrate levels in public water supplies (36, 52, 53), while others reported no associations (12, 34, 54). Nitrate levels in public water supplies are strictly monitored and regulated, and thus nitrate intake from drinking water usually represents only a small portion of total nitrate intake from both diet and water among public water supply users.

In our study population, dietary nitrate and total nitrate intakes were highly correlated (r = 0.93), and an average of only 14% of total nitrate intake was accounted for by nitrate intake from drinking water. On the other hand, nitrate levels in private well water are not monitored or regulated, and some wells are high in nitrate concentrations because private wells are often located in rural agricultural areas. Recent data indicate approximately 22% of domestic wells in U.S. agricultural area exceeded the MCL (55), and the Iowa Statewide Rural Well Water Survey from 1988 – 1989 showed that 18% of the well samples had higher nitrate levels than the MCL (56). Therefore, individuals using private well water could be considered as a group to have higher nitrate exposure in drinking water (acknowledging a wide range within the group).

In the current study, we found an increased risk of breast cancer in women with the highest nitrate intake from public water and in private well users compared to those using public water with the lowest nitrate level only among those with adequate or higher total folate intake (≥400 μg/d). The use of dietary supplements containing folic acid (folic acid or multivitamins) were higher (69.7%) in those with adequate or higher total folate intake compared to those with lower folate intake (8.3%). Among women with adequate or higher folate intake, the median of total folate intake was 689 μg/d (IQR = 596–805 μg/d) and 467 μg/d (IQR = 428–533 μg/d) in folic acid-containing supplement users and nonusers, respectively. Folate has been considered to have a double-edged effect on cancer development. Higher folate intake may be protective in the preneoplastic stage, whereas high folate intake could accelerate cancer development once neoplastic lesions appear (57). The increased risk of breast cancer in users of public water with the highest nitrate level and in private well users among those with adequate or higher total folate intake may be related

to a cancer promotion effect from excess folate intake and increased formation of NOCs. It should be also noted that elevated nitrate levels in water supplies can be an indicator of other potential carcinogenic contaminants in water. For example, high levels of atrazine, the most used herbicide in corn fields in U.S. and a hypothesized risk factor for breast cancer, have been reported in public water systems in Iowa (58, 59). Increased risk of breast cancer related to higher water nitrate might be not only related to nitrate but to other water contaminants such as atrazine (60, 61). We did not have actual nitrate measurements in private well water. Future studies are needed to evaluate a nitrate-folate interaction as well as the potential effect of nitrate intake on breast cancer risk in private well users.

A major strength of this study is a large prospective cohort study design. Dietary intake was assessed prior to breast cancer diagnoses and more than 2,800 accumulated breast cancer cases were available for analysis. In addition to dietary nitrate intake, we analyzed nitrate in drinking water for public water supply users using a historical analytical database on municipal water supplies. The present study also has limitations. The study population was predominantly elderly Caucasians, and thus the findings may not be generalizable to other populations. Dietary nitrate intake in this study population was low compared to the Korean study that evaluated this hypothesis, which might be, in part, why we observed different results. In addition to possible nondifferential misclassification, dietary intake assessed by a FFQ may not capture the information most relevant to breast cancer risk such as food preparation and storage methods as well as timing of food and water consumption. Dietary intake assessed at study baseline may have changed over the follow-up period, which may have caused nondifferential misclassification in dietary intake. We compared dietary intake at the baseline (1986) assessment and the 2004 assessment, and found the two measurements to be fairly well correlated suggesting little change in dietary intake patterns. Correlation coefficients for intakes of total calories, macronutrients, and folate with or without dietary supplements were 0.44, 0.39 – 0.42, 0.29 and 0.34, respectively. Nitrate intake from public drinking water was computed based on the annual average nitrate levels in public water supplies in each community and an estimated daily consumption of 2 L drinking water because information on the daily consumption of drinking water was not available. Therefore, water nitrate data used in this study did not reflect annual variations in nitrate levels in public water supplies or patterns of individual water consumption such as the amount and timing of water consumption. Lack of nitrate data in private wells is another limitation.

In summary, our findings do not support the previously reported increased risk of breast cancer among individuals with high dietary nitrate but low folate intakes. Among women with total folate intake of 400 μg/d or higher, risk of breast cancer was increased in users of public water with the highest quintile of nitrate and in private well users compared to women with the lowest quintile of nitrate from public water supplies. Future studies are warranted and should consider multiple potential confounders including intakes of antioxidants and other nutrients contained in nitrate-rich foods.

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Table 1

Baseline demographic, lifestyle, and dietary factors and dietary nitrate intake (quintiles)

	Total	Dietary nitrate intake quintiles					*p*
		Q1	Q2	Q3	Q4	Q5	
Age, mean (SD)	61.6 (4.2)	61.4 (4.2)	61.5 (4.2)	61.5 (4.2)	61.6 (4.2)	61.8 (4.2)	<0.0001
BMI, mean (SD)	27.0 (5.1)	26.8 (5.0)	26.9 (53.0)	27.1 (5.1)	27.0 (5.0)	27.1 (5.2)	<0.0001[a]
WHR, mean (SD)	0.84 (0.08)	0.84 (0.09)	0.83 (0.08)	0.84 (0.08)	0.84 (0.08)	0.83 (0.08)	<0.0001[a]
>High school education[c], %	39.8	29.7	36.6	39.8	44.6	48.2	<0.0001
Cigarette smoking, %							<0.0001
Never	65.6	62.6	65.3	66.3	67.6	66.4	
Former	19.4	17.6	18.4	19.3	19.4	22.2	
Current	15.0	19.8	16.3	14.4	13.0	11.4	
Alcohol intake, %	45.1	37.4	42.6	46.4	49.0	49.9	<0.0001
Physical activity, %							<0.0001
Low	47.5	60.6	52.9	46.8	41.1	36.0	
Moderate	27.5	22.9	26.5	28.3	30.3	29.6	
High	25.0	16.5	20.6	24.9	28.6	34.4	
Family history of breast cancer, %	23.0	22.5	22.3	23.9	23.1	23.3	0.14
Age at menarche, mean (SD)	12.8 (1.5)	12.9 (1.5)	12.9 (1.4)	12.8 (1.4)	12.8 (1.4)	12.8 (1.4)	<0.0001[a]
Age at menopause, mean (SD)	47.7 (6.4)	47.3 (6.5)	47.7 (6.3)	47.7 (6.3)	47.9 (6.4)	47.9 (6.3)	<0.0001[a]
Parity, %	91.0	90.4	91.3	91.1	91.0	91.2	0.41
Age at first live birth, mean (SD)	20.7 (7.6)	20.2 (7.7)	20.7 (7.5)	20.8 (7.6)	20.9 (7.6)	21.0 (7.6)	<0.0001[a]
Estrogen use, %	38.6	36.8	37.9	38.5	40.1	39.9	0.0002
Oral contraceptive use, %	19.3	19.0	19.3	19.1	19.3	19.7	0.81
Total energy (kcal), median	1,721	1,379	1,597	1,744	1,873	2,068	
Total folate (μg/d), median	350.7	242.4	296.8	335.2	378.1	468.9	<0.0001[b]
Vitamin C (mg/d), median	190.9	127.8	159.0	184.1	208.5	264.6	<0.0001[b]
Vitamin E (mg/d), median	9.7	6.5	8.1	9.2	10.6	13.1	<0.0001[b]
Flavonoids (mg/d), median	10.3	5.8	8.4	10.1	12.0	15.4	<0.0001[b]
Cruciferae (servings/week), median	2.5	1.5	2.0	2.5	3.5	5.0	<0.0001[b]

	Total	Dietary nitrate intake quintiles					*p*
		Q1	Q2	Q3	Q4	Q5	
Red meat (servings/week), median	5.0	4.0	5.0	5.0	5.5	6.0	<0.0001[b]

BMI, body mass index; WHR, waist-hip-ratio

[a] *p* value for trend in lsmeans from an age-adjusted linear regression model

[b] *p* value for trend from an age- and energy-adjusted linear regression model using log-transformed values.

[c] The completion of college or some college education.

Table 2

Dietary intake of nitrate and nitrite (quintiles) and breast cancer risk

	Q1 (reference)	Q2	Q3	Q4	Q5	p_{trend}
Nitrate						
Median (mg/d)	49.3	78.7	106.1	140.2	209.9	
Range (mg/d)	3.9 – 65.2	65.2 – 91.8	91.8 – 121.8	121.8 – 165.6	165.6 – 2,346.4	
Cases	604	541	575	601	554	
Person-years	123,277	125,273	124,907	125,466	124,333	
HR (95%CI)[a]	1.0	0.86 (0.76 – 0.98)	0.90 (0.79 – 1.02)	0.96 (0.84 – 1.10)	0.86 (0.74 – 1.01)	0.31
Nitrite						
Median (mg/d)	0.6	0.9	1.1	1.4	1.8	
Range (mg/d)	0.1 – 0.8	0.8 – 1.0	1.0 – 1.2	1.2 – 1.5	1.5 – 7.1	
Cases	532	604	573	589	577	
Person-years	123,423	124,696	124,948	124,903	125,284	
HR (95%CI)[a]	1.0	1.12 (0.98 – 1.28)	1.06 (0.92 – 1.22)	1.10 (0.94 – 1.28)	1.05 (0.86 – 1.29)	0.28
Nitrate-folate ratio						
Median	0.12	0.22	0.31	0.40	0.57	
Range	0.01 – 0.17	0.17 – 0.26	0.26 – 0.35	0.35 – 0.47	0.47 – 2.66	
Cases	569	575	608	560	563	
Person-years	122,949	124,816	124,405	126,670	124,415	
HR (95%CI)[a]	1.0	0.96 (0.84 – 1.10)	0.98 (0.84 – 1.14)	0.87 (0.75 – 1.02)	0.87 (0.74 – 1.03)	0.04

[a]Adjusted for age (continuous), total energy intake (continuous), BMI (continuous), WHR (continuous), education (<high school, high school, >high school), smoking (never, previous, current), physical activity level (low, moderate, high), alcohol intake (yes, no), family history of breast cancer (yes/no), age at menopause (continuous), age at first live birth (<20, 20 – <30, ≥30, nulliparous), estrogen use (never, ever), total intake of folate (except for nitrate-folate ratio), vitamin C and E and flavonoids, intakes of cruciferae and red meat.

Table 3

Nitrate intake from drinking water (quintiles) and breast cancer risk[a]

	Median (mg/2L)	Range (mg/2L)	Cases	Person-years	HR (95% CI)[b]
Water nitrate intake					
Q1	1.6	0 – 2.8	253	57,345	1.0 (reference)
Q2	4.1	3.0 – 7.9	255	56,101	1.07 (0.89 – 1.28)
Q3	9.4	8.0 – 14.1	244	57,163	0.96 (0.80 – 1.16)
Q4	21.2	14.3 – 33.3	250	54,775	1.05 (0.88 – 1.27)
Q5	57.8	33.5 – 145.3	286	57,902	1.14 (0.95 – 1.36)
p_{trend}					0.11
Private well users	N/A	N/A	463	96,326	1.14 (0.97 – 1.34)

[a]Analysis includes 20,147 women (15,151 public water supply users and 4,996 private well users) who responded to the 1989 follow-up survey, had used public water private well water supply for more than 10 years, and lived in communities which were served by one water source and had nitrate measurement data.

[b]Adjusted for age (continuous), total energy intake (continuous), BMI (continuous), WHR (continuous), education (<high school, high school, >high school), smoking (never, previous, current), physical activity level (low, moderate, high), family history of breast cancer (yes/no), estrogen use (never, ever), and total intakes of folate, vitamin C and E and flavonoids, intakes of cruciferae and red meat.

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Table 4

Dietary and water nitrate intakes (quintiles) in relation to breast cancer risk stratified by total folate intake

	Total folate intake			
	<400 μ/d		≥400 μg/d	
	Cases	HR (95%CI)[a]	Cases	HR (95%CI)[a]
Dietary nitrate (mg/d)				
Q1 (≤65.2)	456	1.0 (reference)	148	1.0 (reference)
Q2 (65.2 – 91.8)	374	0.81 (0.69 – 0.94)	167	1.00 (0.79 – 1.28)
Q3 (91.8 – 121.8)	398	0.93 (0.79 – 1.09)	177	0.82 (0.64 – 1.05)
Q4 (121.8 – 165.6)	311	0.83 (0.70 – 1.00)	290	1.18 (0.93 – 1.49)
Q5 (≥165.6)	192	0.83 (0.67 – 1.02)	362	0.94 (0.73 – 1.22)
p_{trend}		0.19		0.87
Water nitrate (mg/2L)[b]				
Q1 (≤2.8)	169	1.0 (reference)	84	1.0 (reference)
Q2 (3.0 – 7.9)	151	0.97 (0.77 – 1.22)	104	1.25 (0.92 – 1.68)
Q3 (8.0 – 14.1)	140	0.88 (0.70 – 1.12)	104	1.12 (0.83 – 1.52)
Q4 (14.3 – 33.3)	150	0.92 (0.73 – 1.16)	100	1.31 (0.97 – 1.78)
Q5 (≥33.5)	168	1.00 (0.79 – 1.25)	118	1.40 (1.05 – 1.87)
p_{trend}		0.71		0.04
Private well users	283	1.01 (0.83 – 1.24)	180	1.38 (1.05 – 1.82)

[a] Adjusted for age (continuous), total energy intake (continuous), BMI (continuous), WHR (continuous), education (<high school, high school, >high school), smoking (never, previous, current), physical activity level (low, moderate, high), alcohol intake (yes, no), family history of breast cancer (yes/no), age at menopause (continuous), age at first live birth (<20, 20 – <30, ≥30, nulliparous), and estrogen use (never, ever), and total intake of vitamin C and E and flavonoids, intakes of cruciferae and red meat.

[b] Analysis includes 20,147 women (15,151 public water supply users and 4,996 private well users) who responded to the 1989 follow-up survey, had used public water private well water supply for more than 10 years, and lived in communities which were served by one water source and had nitrate measurement data.