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Ingested nitrate and nitrite and bladder cancer in Northern New England

Kathryn Hughes Barry[1,*,2], **Rena R. Jones**[1], **Kenneth P. Cantor**[1], **Laura E. Beane Freeman**[1], **David C. Wheeler**[3], **Dalsu Baris**[1,*], **Alison T. Johnson**[4], **G. M. Monawar Hosain**[5], **Molly Schwenn**[6,*], **Han Zhang**[7], **Rashmi Sinha**[8], **Stella Koutros**[1], **Margaret R. Karagas**[9], **Debra T. Silverman**[1], **Mary H. Ward**[1]

[1]Occupational and Environmental Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD

[2]Department of Epidemiology and Public Health, University of Maryland School of Medicine, and Program in Oncology, University of Maryland Marlene and Stewart Greenebaum Comprehensive Cancer Center, Baltimore, MD

[3]Department of Biostatistics, Virginia Commonwealth University, Richmond, VA

[4]Vermont Department of Health, Burlington, VT

[5]Bureau of Public Health Statistics and Informatics, Department of Health and Human Services, Concord, NH

[6]Maine Cancer Registry, Augusta, ME

[7]Biostatistics Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD

[8]Metabolic Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD

[9]Department of Epidemiology, Geisel School of Medicine at Dartmouth, Hanover, NH

Abstract

BACKGROUND—*N*-nitroso compounds are hypothesized human bladder carcinogens. We investigated ingestion of *N*-nitroso compound precursors nitrate and nitrite from drinking water and diet and bladder cancer in the New England Bladder Cancer Study.

METHODS—Using historical nitrate measurements for public water supplies and measured and modeled values for private wells, as well as self-reported water intake, we estimated average

Address for correspondence and reprints: Mary H. Ward, Ph.D., Occupational and Environmental Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, 9609 Medical Center Dr., Room 6E138, Rockville, MD 20850, wardm@mail.nih.gov.

*former affiliation

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Data availability

Investigators may contact Dr. Debra Silverman, Chief of the Occupational and Environmental Epidemiology Branch at the National Cancer Institute for access to data and code for replication.

nitrate concentrations (mg/L NO_3-N) and average daily nitrate intake (mg/day) from 1970 to diagnosis/reference date (987 cases and 1180 controls). We estimated overall and source-specific dietary nitrate and nitrite intakes using a food frequency questionnaire (1037 cases and 1225 controls). We used unconditional logistic regression to estimate odds ratios (OR) and 95% confidence intervals (CI). We evaluated interactions with factors that may affect *N*-nitroso compound formation (i.e., red meat, vitamin C, smoking), and water intake.

RESULTS—Average drinking water nitrate concentration above the 95th percentile (>2.07 mg/L) compared with the lowest quartile (≤0.21 mg/L) was associated with bladder cancer (OR=1.5, 95% CI: 0.97–2.3; p-trend=0.01); the association was similar for average daily drinking water nitrate intake. We observed positive associations for dietary nitrate and nitrite intakes from processed meat (highest vs. lowest quintile OR for nitrate=1.4, 95% CI: 1.0–2.0; p-trend=0.04; OR for nitrite=1.5, 95% CI: 1.0–2.1; p-trend=0.04, respectively), but not other dietary sources. We observed positive interactions between drinking water nitrate and red meat (p-interaction 0.05) and processed red meat (0.07).

CONCLUSIONS—Our results suggest the importance of both drinking water and dietary nitrate sources as risk factors for bladder cancer.

Keywords

bladder cancer; diet; disinfection by-products; drinking water contaminants; nitrate; nitrite; trihalomethanes

INTRODUCTION

Nitrate and nitrite occur naturally in drinking water.[1] Anthropogenic inputs (e.g., nitrogen fertilizers) in the United States increased ten-fold between 1950 and the early 1980s, which resulted in increased nitrate concentrations in drinking water aquifers.[2,3] The U.S. Environmental Protection Agency (EPA) regulates nitrate in public drinking water supplies, with a maximum contaminant level (MCL) of 10 mg/L NO_3-N based on risk of methemoglobinemia (“blue baby syndrome”), an acute health effect. In contrast to public water supplies, private wells are not regulated and often have higher levels.[3,4] In addition to exposure through drinking water, nitrate and nitrite are ingested through diet. The predominant sources of nitrate are green leafy and root vegetables, which also contain antioxidants. For nitrite, the highest levels are found in processed meats.[1,5]

Nitrosating agents derived from nitrite react with amines and amides (i.e., from meat and fish) to form *N*-nitroso compounds in a process called endogenous nitrosation.[1,4] Endogenous nitrosation is inhibited in the presence of antioxidant nutrients (e.g., vitamin C).[6–8] The International Agency for Research on Cancer (IARC) classified nitrate and nitrite as probable human carcinogens (Group 2A) when ingested under conditions that result in endogenous formation of *N*-nitroso compounds.[1]

Epidemiologic evidence for an association between drinking water nitrate and bladder cancer is mixed. Two case–control studies in Spain and Iowa[9,10] and a prospective cohort study in the Netherlands[11] observed no association between drinking water nitrate levels and bladder cancer. In contrast, a prospective cohort study among postmenopausal women in

Iowa identified a positive association[12] that persisted with additional follow-up and improved exposure assessment.[13] Previous studies have estimated exposures from public water supplies. To our knowledge, no previous bladder cancer studies have incorporated measurements from private wells. There is growing epidemiologic evidence of an association of dietary nitrate and nitrite from processed meat sources with bladder cancer; some, but not all, studies have identified positive associations with higher intake.[13–15]

In the present analysis within the New England Bladder Cancer Study (NEBCS), we estimated associations between bladder cancer and exposure to nitrate and nitrite from dietary sources, and nitrate from drinking water, using measurements from both public water supplies and private wells. As was done by IARC, we evaluated drinking water and dietary nitrate separately because of the expected different associations by source.[1] We also evaluated the potential for effect modification of these associations by factors influencing endogenous nitrosation, including smoking and dietary consumption of red and processed meat and vitamin C.

METHODS

Study Population

The NEBCS is a population-based case–control study of bladder cancer conducted in Maine, New Hampshire and Vermont.[16] Cases were patients aged 30–79 with newly diagnosed, histologically confirmed urinary bladder carcinoma from 2001–2004 for Maine and Vermont residents and 2002–2004 for New Hampshire residents, who were identified through hospital pathology departments and hospital and state cancer registries. Of 1,878 eligible bladder cancer patients, we interviewed 1,213 (65%). We frequency-matched controls to cases on state, sex, and age at diagnosis (5-year groups).[16] Controls were randomly selected from Department of Motor Vehicles records (<65 years) or from Center for Medicare and Medicaid Services beneficiary records (≥65 years). Participation rates were 65% for both sources, resulting in 1,418 interviewed controls. All participants gave written consent. The study was approved by institutional review boards of the National Cancer Institute, the US Geological Survey, Westat, Inc., Geisel School of Medicine at Dartmouth and departments of health in Maine, New Hampshire, and Vermont.

Drinking water source

The NEBCS drinking water exposure assessment has been described in detail.[17–19] Participants provided lifetime residential and occupational histories and residential and workplace drinking water sources as part of a computer-assisted personal interview. We asked participants to estimate their usual adult water intake and the percent of water consumed from the home tap.[17,18]

Nitrate in public water supplies (PWS)

We assigned nitrate concentrations for PWS and private well exposure–years from 1970 to diagnosis/reference date using several methods (see eTable 1). We focused on this period because of substantial increases in nitrogen fertilizer after the 1950s,[2,20] and because there were few PWS measurements before 1970. For PWS exposure–years, we used measurement

data from the three study states (86% of exposure–years) and an additional 18 states (13%) to estimate average nitrate (mg/L NO_3-N). We also obtained information on historical and current PWS sources (surface, groundwater, mixed) for the three study states and Massachusetts (which contributed the next largest exposure–years, about 6%), hereafter termed the four core states. Only current water source was available for the other 17 states. We assigned nitrate levels for finished water samples (i.e., from a tap in the water distribution system), where available; otherwise, we assigned pre-treatment measurements. We imputed nitrate concentrations below the limit of detection (~9% of measurements in the four core states, ~28% in the 17 other states) from a log-normal distribution using state-specific models[21] adjusting for water source (surface/groundwater).

We computed annual average concentrations for 17% of PWS exposure–years. For years without measurements, but no change in source (e.g., well depth(s) did not change), we assigned the average of the PWS measurements in the same decade (about 16% of exposure–years) or nearest decade (about 39%). For the 17 states without information on historical source, we assumed that the source did not change (about 9%). When extrapolation was not possible (e.g., because the source changed), we applied population-weighted source-specific averages for the state in the same decade or nearest available decade (about 12%). For groundwater sources, these were depth-specific averages. After these assignments, about 9% of the PWS exposure–years remained unassigned. These included PWS with no measurements for the same source in the state (0.01%), unknown PWS in the 21 states (about 6%), and PWS in other states (about 3%).

Nitrate in private wells

Private wells accounted for 36% of the exposure–years(. We measured nitrate in water samples from interview homes with private wells (2001–2004). Water was sampled from the kitchen tap and was shipped on ice overnight to the U.S. Geological Survey (USGS) National Water Quality Laboratory in Denver, where samples were acidified and kept refrigerated until analysis (within 2 weeks of sampling). Nitrite plus nitrate was measured by the cadmium reduction method (USGS method I2445–90)[22] with a minimum reporting level of 0.037 mg/L NO_3-N. Water samples were also obtained from 448 private wells at former residences in the three study states.[19] We assigned the nitrate measurements to all years that the participant used the private source from 1970 onward (65% of private well exposure–years). We did not have nitrate measurements for workplace private wells.

To estimate nitrate concentrations for residential private wells in the study area with no samples (28% of exposure–years on wells), we developed a continuous random forest model using private well measurements from this study (N=1695) in 2001–2004 (see eText 1 and eTable 2 for details of the random forest modeland top 20 variables).

Drinking water nitrate exposure metrics

We computed two exposure metrics based on measurements for PWS and measured and modeled private well values. First, we computed the average nitrate concentration (mg/L NO_3-N) by summing annual nitrate estimates for residences and workplaces and dividing by the total years with a nitrate estimate. Second, we computed average daily intake (mg/d

NO_3-N) by multiplying each participant's average nitrate concentration by their usual daily water intake in adulthood. We computed a weighted average of residential and workplace data using percent water intake from the home tap (median: 93%), assuming the rest was from the workplace.

Dietary nitrate and nitrite intake

As described,[23] participants completed a modified version of the 124-item dietary history questionnaire[24] that inquired about usual dietary intakes over the past 5 years. We estimated dietary nitrate and nitrite intakes using a database of nitrate and nitrite levels in foods[5] and computed a weighted average of levels in foods contributing to each line item based on sex-specific intakes from the 1994–1996 Continuing Survey of Food Intake by Individuals.[25] We summed nitrite intake across plant and animal sources separately, since risk may differ by source. Vegetable and fruits are major sources of dietary nitrate; intake from plants was similar to total intake so only total dietary nitrate is presented.[26] We separately computed nitrate and nitrite intakes from processed meats, which included red processed meat (ham, bacon, sausage, hot dog and cold cuts) and white processed meat (turkey sausages and hot dogs, poultry cold cuts). We estimated intakes of other nutrients (e.g., dietary vitamin C) as previously described.[23] We also computed total vitamin C intake from diet and supplements.

Statistical analysis

We used logistic regression models to compute odds ratios (OR) and 95% confidence intervals (CI) for drinking water and dietary nitrate and nitrite and bladder cancer. Our models of drinking water nitrate included 987 cases and 1,180 controls with ≥70% of exposure–years with nitrate estimates from PWS and private wells. We also separately evaluated predominantly private well users (≥70% of exposure–years on private wells; 252 cases, 355 controls) and predominantly PWS users (≥70% exposure–years on PWS; 495 cases, 543 controls). We restricted analyses of dietary nitrate and nitrite to those without extreme energy intakes (i.e., removing ≤1st or ≥99th percentile of total kcal) (1037 cases, 1225 controls).

For our drinking water metrics, we modeled quartile and 95th percentile cutpoints determined among controls with the lowest quartile as the reference group, except for the private well analyses (highest quantile was the 90th percentile due to smaller numbers, and reference was ≤50th percentile since the 50th percentile equaled the 25th). For the dietary analyses, we evaluated quintiles of dietary nitrate and nitrite intakes overall and by source. We determined cutpoints among controls and used the lowest quintile as the reference group. We computed p-values for trend using semi-continuous variables defined as the median for each category among controls. We evaluated the linearity of the relationship of average nitrate concentration and daily intake with bladder cancer risk using cubic splines in our regression models. These nonparametric analyses showed no evidence of nonlinear relationships.

We adjusted all models for age (30–54, 55–64, 65–74 and ≥75 years), sex, race (white/other/refused/don't know), Hispanic ethnicity (yes/no/don't know), study state (New Hampshire, Maine, Vermont), smoking (never, former, occasional, current, don't know) and high-risk

occupation[27] (yes, no, never worked). Additional adjustment for pack–years of smoking did not appreciably alter findings, and this variable was not included in the final models. Final drinking water models were also adjusted for average total trihalomethanes concentration (≤15.7 μg/L, >15.7–26.8 μg/L, >26.8–37.1 μg/L, >37.1–45.7 μg/L, >45.7 μg/L) because total trihalomethanes were associated with bladder cancer in the NEBCS[18] and were inversely associated with average nitrate concentration in our study (Spearman rho=−0.31, p<0.0001). Average nitrate concentration models were also adjusted for total water intake (≤1.09 L/d, >1.09–1.53 L/d, >1.53–2.24 L/d, >2.24–3.79 L/d and >3.79 L/d). Additional adjustment for cumulative arsenic lagged 40 years or dug well use before 1960, previously associated with bladder cancer in the NEBCS,[17] did not appreciably influence findings, nor did dietary nitrate or nitrite from processed meat, and these variables were not included in the final models.

In addition to the base covariates, we adjusted dietary nitrate and nitrite models for dietary vitamin B12 (per 1,000 kcal – continuous), previously associated with bladder cancer in the NEBCS,[23] dietary vitamin C intake (per 1,000 kcal – continuous) and total energy intake (kcal - continuous). We additional adjusted models of processed meat nitrate and nitrite for total meat intake (per 1,000 kcal – continuous). Additional adjustment for average total trihalomethanes concentration or cumulative arsenic lagged 40 years did not appreciably alter findings and we did not include these variables in the final models.

We evaluated interactions between the average drinking water and dietary nitrate and nitrite metrics and factors related to endogenous nitrosation, including smoking (never/former/current), dietary and total vitamin C intake divided at the median among controls (65 mg/d and 119 mg/d, respectively). For average nitrate concentration in drinking water, we also evaluated interactions with red meat intake (i.e. both processed and unprocessed, median=31 g/d), and processed red meat (median=6.5 g/d). We also evaluated interactions between the drinking water metrics and average total trihalomethanes concentration (median=15.7 μg/L) and total water intake (median=1.5 L/d) to evaluate risk patterns by these factors. We computed p-values for interaction using the likelihood ratio test, comparing nested models with and without the interaction terms. We investigated joint effects using a common reference group including the lowest nitrate exposure category with the lowest risk subgroup for the modifier (e.g., never smokers, >median vitamin C). All analyses were conducted in SAS version 9.3 (SAS Institute, Cary, NC), except for the random forest models, which were conducted in R.[28] All tests were two-sided.

RESULTS

Characteristics were similar between the sub-populations included in our drinking water and dietary analyses (Table 1), and between each sub-population and the full study population as previously reported.[16] Briefly, the majority of participants were non-Hispanic whites; most participants resided in Maine and New Hampshire. While cases and controls were similar with respect to age, sex, and state of residence (matching factors), cases were more likely than controls to be smokers.[16] Cases also tended to have less education, more high-risk occupations, increased total energy intake, and increased water intake compared with controls.

We observed a positive association between bladder cancer and average drinking water nitrate concentration above the 95th percentile (>2.07 mg/L NO_3-N; median among controls=3.3 mg/L NO_3-N) compared to the lowest quartile (≤0.21 mg/L NO_3-N) (OR=1.5, 95% CI: 0.97–2.3; p-trend=0.01) (Table 2). We observed a similar association for >95th percentile of average daily drinking water nitrate intake (>4.59 mg) compared to the lowest quartile (≤0.30 mg) (OR=1.4, 95% CI: 0.89–2.2; p-trend=0.06; Table 2). We additionally restricted to those with ≥80% and ≥90% of exposure–years with nitrate estimates, with similar results for the 80% group and slightly stronger associations among the 90% group (eTable 3).

We also evaluated risk separately among those with predominantly private well or PWS sources in the exposure period (see eTable 4). Among the subgroups with ≥70% exposure–years either on private wells or on PWS, the ORs for the highest average nitrate concentrations (private wells: >90th percentile >2.38 mg/L; PWS: >95th percentile >1.52 mg/L) compared to the lowest quantile were elevated (private wells, OR=1.7, 95% CI: 0.88–3.4; PWS, OR=1.4, 95% CI: 0.71–2.5); p-values for trend were 0.26 and 0.13, respectively. Because the average nitrate concentration distribution differed somewhat between these groups, we reanalyzed the PWS group with the cutpoints for private well users. Associations were elevated, although imprecise, in the top two exposure categories (>0.83–2.38 mg/L OR=1.3, 95% CI 0.84–2.0; >2.38 mg/L OR=1.8, 95% CI 0.62–5.0) and were similar to the ORs for these categories among private well users (eTable 4). We observed somewhat stronger associations for PWS users (p-trend=0.01) than private well users for average daily nitrate intake from water (p-trend=0.15).

For dietary nitrate and nitrite, there were no trends for overall nitrate or nitrite intakes (p-trend=0.38 and 0.92) or nitrite intakes from animal or plant sources (Table 3). However, we observed positive trends associated with nitrate (p-trend=0.04) and nitrite (p-trend=0.04) intakes from processed meat, and the ORs comparing the highest vs. lowest quintiles were increased (nitrate OR=1.4, 95% CI: 1.01–2.0; nitrite OR=1.5, 95% CI: 1.0–2.1; Table 3).

We observed positive interactions between average drinking water nitrate concentration (p-interaction= 0.05) and red meat and processed red meat consumption (p-interaction=0.07,). Compared to those with the lowest water nitrate concentration and with red meat or processed red meat intake below the median, those with average nitrate levels >95th percentile and ≥median intakes had an increased risk of bladder cancer (red meat OR=2.6, 95% CI: 1.3–5.1; processed red meat OR=3.5, 95% CI: 1.8–6.9) (Table 4). Compared to those with the lowest nitrate concentration and total water intake below the median, those with average nitrate concentration >95th percentile and total water intake ≥median had an increased risk of bladder cancer (OR= 2.1, 95% CI: 1.1–3.8; p-interaction=0.05) (see eTable 5). There was no association with bladder cancer for the other categories. There was no evidence of interaction (p>0.20) for average drinking water nitrate with average total trihalomethanes concentrations or dietary vitamin C intake or with total vitamin C or smoking (data not presented). In addition, there was no evidence of interaction for the dietary nitrate or nitrite metrics with dietary vitamin C intake, total vitamin C or smoking (data not presented).

DISCUSSION

In one of the largest studies to date of bladder cancer in relation to drinking water and dietary nitrate/nitrite, we observed a positive association between bladder cancer and average drinking water nitrate concentration >95th percentile compared to the lowest quartile. Risk was also increased for average daily drinking water nitrate intake >95th percentile. Results were similar to our overall findings when we examined average nitrate concentrations among the subgroups that primarily used PWS or private wells. Average nitrate concentrations among private well users were slightly higher than those who primarily used PWS. We observed evidence of a positive interaction between average drinking water nitrate concentration and total red meat and processed red meat consumption, but not other factors that influence endogenous nitrosation. We also observed positive associations between bladder cancer and dietary intakes of nitrate and nitrite from processed meat, but no trend for other sources.

Our finding of a positive association between drinking water nitrate and bladder cancer is similar to that of a prospective cohort study among postmenopausal women in Iowa.[12,13] In the most recent report from the Iowa cohort, based on 130 cases, the authors observed an increased risk of bladder cancer for average nitrate concentration in the highest quartile (>2.97 mg/L NO_3-N) compared to the lowest quartile (<0.47 mg/L) (Hazard Ratio=1.47, 95% CI: 0.91–2.38; p-trend=0.11),[13] and an increased risk with ≥4 years above 5 mg/L NO_3-N compared to no exposure above this level. Although nitrate concentrations in the Iowa cohort tended to be higher than in our study, our highest category (>2.07 mg/L NO_3-N) was only slightly lower than the highest Iowa study category. A Spanish case–control study (n=531 cases) did not observe an association for those with concentrations >5–10 mg/L NO_3 (>1.10–2.26 mg/L NO_3-N) or >10 mg/L NO_3 compared with concentrations ≤5mg/L NO_3, but there was an elevated risk for those with >20 years exposure to the highest levels (>9.5 mg/L NO_3 equivalent to >2.1 mg/L NO_3-N).[9] In contrast to our study, a Netherlands cohort with 889 cases (highest category ≥7.7 mg/L NO_3-N, reference <0.9 mg/L NO_3-N),[11] and a case–control study in Iowa with 808 cases (highest category ≥3.09 mg/L NO_3-N, reference <0.6 mg/L NO_3-N)[10] did not observe an association with bladder cancer. None of the previous studies incorporated nitrate measures from private wells.

The Iowa cohort[13] and Spanish case–control study[9] adjusted for total trihalomethanes concentration, but neither observed appreciable differences in their findings. In contrast, adjustment for total trihalomethanes resulted in a modest strengthening of our drinking water nitrate findings, likely related to an inverse association with nitrate in our study (rho= −0.31). We expect that the inverse correlation was due to the assignment of zero total trihalomethanes in private wells,[18] whereas the previous studies did not incorporate private well data.[9,13] The Iowa Women's Health Study and Spanish study observed some evidence of effect modification by total trihalomethanes,[9,13] whereas we observed little evidence in our study. Although cumulative arsenic in drinking water lagged 40 years was associated with bladder cancer in the NEBCS,[17] we did not observe a difference in results when we adjusted for this exposure. Other studies have considered nitrate to be an indicator of potential exposure to endocrine disrupting chemicals from wastewater,[29] which are not regulated in drinking water supplies. We cannot exclude the possibility of confounding by

specific endocrine disruptors or other water contaminants that we did not assess, although we did have data on the only water contaminants that have been consistently linked to bladder cancer.

Notably, we observed positive interactions between average drinking water nitrate concentrations and red meat and processed red meat consumption with respect to bladder cancer. There is biologic plausibility because higher red meat consumption can increase endogenous nitrosation.[1] However, in contrast to our study, the Spanish study did not observe evidence of effect modification by red or processed meat consumption.[9] Other factors thought to be modifiers of endogenous nitrosation (vitamin C and smoking) did not modify our observed associations for drinking water nitrate. Our result for vitamin C is consistent with other findings.[9–11,13] Our finding of no effect modification by smoking is consistent with the Iowa case–control study,[10] but contrary to the Iowa Women's Health Study cohort, which observed the strongest association for drinking water nitrate and bladder cancer for current smokers,[13] and the Netherlands cohort, which observed some evidence of an association among ever smokers.[11]

Previously in the NEBCS, intake of processed meat in the highest compared to the lowest quartile was associated with an increased risk of bladder cancer (OR=1.28, 95% CI: 1.00–1.65) that was stronger for red processed meat (OR=1.41, 95% CI: 1.08–1.84).[23] We report positive associations between both nitrate and nitrite from processed meat and bladder cancer of a similar magnitude (ORs between 1.4 and 1.5), suggesting that the nitrate and nitrite were driving the association with processed meat. Similarly, the Los Angeles Bladder Cancer Study and the NIH-AARP Diet and Health Study found a positive association between nitrate/nitrite from processed meat and bladder cancer.[14,15] In contrast, the Iowa Women's Health Study did not observe an association for nitrate or nitrite from any dietary sourcey.[13] We did not observe evidence of effect modification of dietary nitrate or nitrite associations with bladder cancer by smoking in our study, which is consistent with findings of the NIH-AARP study,[15] but differs from the Los Angeles case–control study (stronger association among never smokers).[14] Similar to our study, neither NIH-AARP nor the Iowa Women's Health Study observed evidence of effect modification by vitamin C[13,15].

Our study comprehensively evaluated the association between nitrate and nitrite and bladder cancer risk by studying both drinking water and dietary sources. We evaluated these sources separately because of their different potential for the endogenous nitrosation. Human feeding studies have showed reduced *N*-nitroso compound formation when nitrate is ingested together with antioxidants, which occurs when the nitrate source is vegetables.[1] Further strengths included the collection of lifetime drinking water source histories, availability of nitrate measurements from public and private water sources, and data on other drinking water contaminants that have been associated with bladder cancer.[17,18] We also conducted modeling to predict nitrate levels in private wells for which we did not have measurements; however, limitations were the lack of historical nitrate data and under-prediction of higher nitrate concentrations.

There are no data available on the temporal stability of nitrate concentrations in private wells in New England, and we were unable to verify whether our extrapolation of private well

nitrate levels measured in the early 2000's back to 1970 was appropriate. Most current wells were in bedrock[30] that is often fractured in northern New England. Water flow through fractured bedrock complicates modeling of contaminants. A further limitation of our study was that we only evaluated water nitrate over the prior 30 years and it is possible that longer or earlier periods of exposure may be important. However, widespread nitrate contamination of water supplies was not common before the 1960s.[20] We also did not have data on historical water sources for PWS in states outside of the four core states; however, the assumption that water sources did not change only affected 9% of the exposure–years on PWS.

In summary, our findings suggest that nitrate concentrations in drinking water below the current regulatory limit (10 mg/L NO_3-N) are associated with bladder cancer, and these associations may be stronger among those with higher red or processed red meat consumption. Our study also adds to growing evidence of an association between nitrate and nitrite intakes from processed meat and bladder cancer and suggests the importance of both drinking water and dietary nitrate sources in bladder cancer risk.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1.

Characteristics of the populations included in the drinking water and dietary nitrate analyses in the New England Bladder Cancer Study

	Population included in main drinking water analyses[a]		Population included in dietary analyses[b]	
	Cases	Controls	Cases	Controls
Characteristic				
State, n (%)				
Maine	487 (49)	614 (52)	522 (49)	665 (52)
Vermont	179 (18)	202 (17)	187 (18)	226 (18)
New Hampshire	321 (33)	364 (31)	356 (33)	376 (30)
Age (years), n (%)				
30-54	157 (16)	204 (17)	158 (15)	195 (15)
55-64	257 (26)	279 (24)	273 (26)	306 (24)
65-74	370 (37)	456 (39)	403 (38)	504 (40)
75 and older	203 (21)	241 (20)	231 (22)	262 (21)
Sex, n (%)				
Male	750 (76)	863 (73)	809 (76)	928 (73)
Female	237 (24)	317 (27)	256 (24)	339 (27)
Race, n (%)				
White	931 (94)	1111 (94)	1007 (95)	1198 (95)
Other	55 (6)	67 (6)	58 (5)	67 (5)
Don't know	1	1	0	1
Refused	0	1	0	1
Hispanic status, n (%)				
Yes	20 (2)	21 (2)	20 (2)	20 (2)
No	967 (98)	1158 (98)	1045 (98)	1244 (98)
Don't know	0	1	0	3
Education, n (%)				
Less than high school	222 (22)	205 (17)	220 (21)	199 (16)
High School graduate	325 (33)	361 (31)	337 (32)	376 (30)
Vocational	80 (8)	90 (8)	79 (7)	98 (8)
Some college	134 (14)	193 (16)	157 (15)	197 (16)
College graduate	118 (12)	162 (14)	146 (14)	200 (16)
Post-graduate	108 (11)	169 (14)	126 (12)	197 (16)
Smoking status, n (%)				
Non-smoker	149 (15)	405 (34)	162 (15)	425 (34)
Former	508 (52)	571 (48)	564 (53)	631 (50)
Occasional	17 (2)	35 (3)	18 (2)	39 (3)
Current	312 (32)	169 (14)	320 (30)	171 (14)
Don't know	1	0	1	1
High-risk occupation, n (%)				

Characteristic	Population included in main drinking water analyses[a]		Population included in dietary analyses[b]	
	Cases	Controls	Cases	Controls
Yes	534 (55)	365 (31)	585 (55)	406 (32)
No	444 (45)	808 (69)	474 (45)	852 (68)
Missing	9	7	6	9
Average TTHM concentration, n (%)				
0-15.7	474 (49)	575 (50)	503 (49)	611 (50)
>15.7-26.8	234 (24)	285 (25)	258 (25)	299 (25)
>26.8-37.1	150 (16)	176 (15)	156 (15)	185 (15)
>37.1-45.7	52 (5)	59 (5)	51 (5)	63 (5)
>45.7	61 (6)	65 (6)	59 (6)	61 (5)
Missing	16	20	38	48
Red meat intake (g/d), n (%)				
0-31	360 (46)	467 (50)	441 (47)	562 (50)
>31	417 (54)	473 (50)	495 (53)	561 (50)
Missing	210	240	129	144
Total energy intake (kcal/d)	1922 ± 936	1812 ± 842	1877 ± 780	1797 ± 679
Dietary vitamin C intake (mg), mean (sd)	71 ± 45	70 ± 38	72 ± 48	71 ± 39
Dietary vitamin B12 intake (mcg), mean (sd)	2.5 ± 1.0	2.6 ± 1.0	2.5 ± 1.0	2.5 ± 1.0
Total water intake (L/d)	2.0 ± 1.2	1.8 ± 1.1	2.0 ± 1.3	1.8 ± 1.1

[a] Included participants with a drinking water nitrate estimate for >=70% exposure-years from 1970 to diagnosis/reference date from residential and workplace public water supplies and measured and modeled private wells; those with missing water intake were excluded

[b] Included participants that completed the Diet History Questionnaire (DHQ); those with total energy in the 1st or 99th percentiles were excluded

sd indicates standard deviation.

Table 2.

Drinking water nitrate from public water supplies and measured and modeled private wells and the risk of bladder cancer among New England Bladder Cancer Study participants with a nitrate estimate for >=70% of exposure-years from 1970 to diagnosis/reference date (987 cases and 1180 controls)[a]

Average nitrate concentration (mg/L NO_3-N)[b]	**Cases**	**Controls**	**OR (95% CI)**[c]	p-trend[d]
≤0.21 mg/L	259	293	1.0 Ref.	
>0.21-0.37 mg/L	212	294	0.81 (0.61-1.1)	
>0.37-0.68 mg/L	229	294	0.89 (0.68-1.2)	
>0.68-2.07 mg/L	221	241	1.0 (0.79-1.4)	
>2.07 mg/L	66	58	1.5 (0.97-2.3)	
p-trend[d]				0.01
Average daily nitrate intake (mg NO_3-N)[b]	**Cases**	**Controls**	**OR (95% CI)**[c]	
≤0.30 mg	214	295	1.0 Ref.	
>0.30-0.62 mg	227	295	1.1 (0.87-1.5)	
>0.62-1.29 mg	244	295	1.1 (0.86-1.5)	
>1.29-4.59 mg	243	236	1.5 (1.1-1.9)	
>4.59 mg	59	59	1.4 (0.89-2.2)	
				0.06

[a] Both residential and workplace measures were included where available, as well as measured and modeled private well values; participants with missing water intake were excluded

[b] Exposure categories represent quartiles, with the top category reflecting an additional cut at 95th percentile

[c] Adjusted for age, gender, smoking status, high-risk occupation, race, ethnicity, state, and average TTHM concentration for all models, as well as total water intake (L/d) for average nitrate concentration model

[d] Based on median values for quantiles of drinking water nitrate entered into the model as a continuous variable; median for top 95th percentile average nitrate was 3.64 mg/L

CI indicates confidence interval, OR odds ratio.

Table 3.

Dietary nitrate and nitrite intakes (mg/day per 1000 kcal) by source and the risk of bladder cancer among New England Bladder Cancer Study participants (1037 cases and 1225 controls)[a]

Total dietary nitrate quintiles[b]	Cases/Controls	OR (95% CI)[c]	p-trend[d]
≤21.19 mg/d	227/247	1.0 Ref.	
>21.19-28.28 mg/d	230/245	1.2 (0.88-1.5)	
>28.28-36.10 mg/d	225/243	1.2 (0.92-1.6)	
>36.10-47.21 mg/d	183/246	1.0 (0.75-1.4)	
>47.21 mg/d	172/244	0.95 (0.69-1.3)	
			0.38
Total dietary nitrite quintiles[b]			
≤0.48 mg/d	222/243	1.0 Ref.	
>0.48-0.56 mg/d	212/245	1.0 (0.77-1.4)	
>0.56-0.63 mg/d	202/244	1.0 (0.74-1.3)	
>0.63-0.72 mg/d	217/248	1.1 (0.80-1.4)	
>0.72 mg/d	184/245	0.97 (0.71-1.3)	
			0.92
Dietary nitrite from plant sources quintiles[b]			
≤0.27 mg/d	219/245	1.0 Ref.	
>0.27-0.33 mg/d	224/242	1.1 (0.86-1.5)	
>0.33-0.39 mg/d	223/245	1.2 (0.91-1.6)	
>0.39-0.47 mg/d	200/246	1.1 (0.84-1.5)	
>0.47 mg/d	171/247	0.93 (0.68-1.3)	
			0.55
Dietary nitrite from animal sources quintiles[b]			
≤0.15 mg/d	213/241	1.0 Ref.	
>0.15-0.19 mg/d	184/243	0.84 (0.63-1.1)	
>0.19-0.24 mg/d	189/250	0.88 (0.66-1.2)	
>0.24-0.30 mg/d	224/247	1.1 (0.80-1.4)	
>0.30 mg/d	227/244	1.1 (0.79-1.5)	
			0.20
Dietary nitrate from processed meat quintiles[b]			
≤0.26 mg/d	142/205	1.0 Ref.	
>0.26-0.43 mg/d	147/208	1.0 (0.75-1.4)	
>0.43-0.66 mg/d	205/220	1.3 (0.96-1.8)	
>0.66-0.98 mg/d	186/220	1.2 (0.87-1.7)	

Total dietary nitrate quintiles[b]	Cases/Controls	OR (95% CI)[c]	p-trend[d]
>0.98 mg/d	223/224	1.4 (1.0-2.0)	
			0.04
Dietary nitrite from processed meat quintiles[b]			
≤0.04 mg/d	139/208	1.0 Ref.	
>0.04-0.06 mg/d	158/208	1.2 (0.87-1.7)	
>0.06-0.09 mg/d	185/218	1.3 (0.94-1.8)	
>0.09-0.14 mg/d	217/228	1.5 (1.1-2.0)	
>0.14 mg/d	204/215	1.5 (1.0-2.1)	
			0.04

[a] Participants who completed the food frequency questionnaire; those in the 1st or 99th percentile of total energy intake were excluded

[b] Defined among controls

[c] Adjusted for age, gender, smoking status, high-risk occupation, race, ethnicity, state, dietary vitamin C intake (per 1,000 kcal - continuous), dietary vitamin B12 (per 1,000 kcal – continuous), total energy intake (kcal - continuous) and total water intake (L/d - continuous); models for nitrate/nitrite from processed meat were additionally adjusted for total meat intake (per 1,000 kcal - continuous)

[d] Based on median values for quintiles of dietary nitrate/nitrite intake entered into the model as a continuous variable

CI indicates confidence interval, OR odds ratio.

Table 4.

Joint effects of average drinking water nitrate concentration and red meat intake on bladder cancer among New England Bladder Cancer Study participants with a nitrate estimate for >=70% of exposure-years from 1970 to diagnosis/reference date (777 cases and 940 controls)[a]

	Total red meat intake				
	< 31 g / day		≥ 31 g / day		
Average nitrate concentration (mg/L NO_3-N)[b]	Cases/Controls	OR (95% CI)[c]	Cases/Controls	OR (95% CI)[c]	P-int[c,d]
≤0.21 mg/L	103/110	1.0 Ref	97/124	0.82 (0.55-1.2)	
>0.21-0.37 mg/L	77/113	0.75 (0.50-1.2)	86/117	0.80 (0.52-1.2)	
>0.37-0.68 mg/L	78/124	0.78 (0.67-1.6)	108/105	1.1 (0.71-1.7)	
>0.68-2.07 mg/L	84/91	1.0 (0.33-1.4)	89/107	0.88 (0.57-1.4)	
>2.07 mg/L	18/29	0.69 (0.55-1.2)	37/20	2.6 (1.3-5.1)	
					0.05
	Processed red meat intake				
	< 6.5 g / day		≥ 6.5 g / day		
Average nitrate concentration (mg/L NO_3-N)[b]	Cases/Controls	OR (95% CI)[c]	Cases/Control	OR (95% CI)[c]	P-int[c,d]
≤0.21 mg/L	79/115	1.0 Ref.	121/119	1.5 (1.0-2.3)	
>0.21-0.37 mg/L	75/116	1.1 (0.67-1.7)	88/114	1.1 (0.72-1.8)	
>0.37-0.68 mg/L	88/127	1.2 (0.76-1.8)	98/102	1.4 (0.92-2.2)	
>0.68-2.07 mg/L	73/85	1.5 (0.92-2.4)	100/113	1.3 (0.81-2.0)	
>2.07 mg/L	21/28	1.1 (0.55-2.2)	34/21	3.5 (1.8-6.9)	
					0.07

[a] Average nitrate concentrations in residential and workplace public water supplies and measured and modeled private wells; participants with missing water intake, extreme energy intake (1^{st} or 99^{th} percentiles of total kcal) or missing dietary data were excluded

[b] Exposure categories represent quartiles, with the top category reflecting an additional cut at 95th percentile

[c] Adjusted for age, gender, smoking status, high-risk occupation, race, ethnicity, state, average total trihalomethane concentration and total water intake (L/d)

[d] P-value for interaction computed from likelihood ratio test comparing nested models with and without interaction terms

CI indicates confidence interval, OR odds ratio.