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Nitrate and nitrite ingestion and risk of ovarian cancer among postmenopausal women in Iowa

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Nitrate and nitrite are precursors in the endogenous formation of *N*-nitroso compounds (NOC), potential human carcinogens. We evaluated the association of nitrate and nitrite ingestion with postmenopausal ovarian cancer risk in the Iowa Women's Health Study. Among 28,555 postmenopausal women, we identified 315 incident epithelial ovarian cancers from 1986 to 2010. Dietary nitrate and nitrite intakes were assessed at baseline using food frequency questionnaire data. Drinking water source at home was obtained in a 1989 follow-up survey. Nitrate-nitrogen (NO_3-N) and total trihalomethane (TTHM) levels for Iowa public water utilities were linked to residences and average levels were computed based on each woman's duration at the residence. We computed multivariable-adjusted hazard ratios (HR) and 95% confidence intervals (CI) using Cox proportional hazards regression. We tested interactions of nitrate with TTHMs and dietary factors known to influence NOC formation. Ovarian cancer risk was 2.03 times higher (CI = 1.22–3.38, $p_{trend} = 0.003$) in the highest quartile ($\geq$2.98 mg/L) compared with the lowest quartile ($\leq$0.47 mg/L; reference) of NO_3-N in public water, regardless of TTHM levels. Risk among private well users was also elevated (HR = 1.53, CI = 0.93–2.54) compared with the same reference group. Associations were stronger when vitamin C intake was <median ($p_{interaction} = 0.01$ and 0.33 for private well and public supplies, respectively). Dietary nitrate was inversely associated with ovarian cancer risk ($p_{trend} = 0.02$); whereas, dietary nitrite from processed meats was positively associated with the risk ($p_{trend} = 0.04$). Our findings indicate that high nitrate levels in public drinking water and private well use may increase ovarian cancer risk among postmenopausal women.

Ovarian cancer has the highest mortality rate among all cancers of the female reproductive system.[1] Given its poor prognosis, identifying risk factors is critical to decrease mortality from ovarian cancer. However, the etiology of this malignancy is poorly understood. A large variation in ovarian cancer incidence among countries[2] and the increased risk of ovarian cancer among immigrants to the United States from other countries with low ovarian cancer incidence such as Japan[3,4] suggest a role of environmental factors, including diet. However, few modifiable risk factors have been identified to date.

Nitrate is a common contaminant of drinking water. Nitrogen from nitrogen fertilizer applications and animal and

Key words: nitrate, nitrite, ovarian cancer, diet, drinking water, disinfection byproducts

Abbreviations: BMI: body mass index; CI: confidence interval; CSFII: Continuing Survey of Food Intake by Individuals; DBP: disinfection byproduct; FFQ: food frequency questionnaire; HAA: haloacetic acid; HR: hazard ratio; IWHS: Iowa Women's Health Study; MCL: maximum contaminant level; NOC: *N*-nitroso compounds; RDI: recommended daily intake; TTHM: total trihalomethane.

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What's new?

While environmental factors such as diet are thought to have a role in ovarian cancer, few such factors have been identified. In the present study, the ingestion of nitrate and nitrite was investigated for possible involvement in ovarian cancer. Among postmenopausal women, risk of ovarian cancer was found to be positively associated with elevated nitrate levels in public drinking water supplies and with nitrite intake from processed meats. Elevated nitrate levels in private well water was linked to increased ovarian cancer risk among women with reduced vitamin C intake.

human waste can contaminate surface and groundwater drinking water sources. The maximum contaminant level (MCL) for public water supplies in the United States is 10 mg/L nitrate-nitrogen (NO_3-N) and is based on preventing methemoglobinemia or blue-baby syndrome in infants.[5] However, the long-term effects of chronic intake of moderately high levels (*i.e.*, $\geq$5 mg/L) of nitrate from drinking water on cancer risk are still not clear.[6,7] Nitrate is also a natural component of plants and is found at high levels in certain vegetables.[7] Nitrate and nitrite salts are also added as preservatives to processed meats such as bacons and hot dogs to prevent bacterial growth and to add color and flavor.[7]

About 5% of ingested nitrate is endogenously reduced to nitrite by bacteria in the oral cavity.[7] Under the acidic conditions in the stomach, nitrite is converted to nitrous acid, which can then be converted to nitrosating agents. Once formed, nitrosating agents can react with amines and amides to form nitrosamines and nitrosamides (collectively called *N*-nitroso compounds [NOCs]). Most NOCs are potent animal carcinogens[8] and ingested nitrate and nitrite are considered probable human carcinogens (2A) under conditions that result in endogenous nitrosation.[7] Nitrosamides directly alkylate DNA and may induce tumors in many organs, whereas nitrosamines must be activated by specific cytochrome P450 enzymes to be carcinogenic.[7] The organ specificity of tumor induction may therefore stem from tissue-specific cytochrome P450 enzymes, which vary in level across organs and species. Cytochrome P450 enzymes have been found in ovarian epithelial tissue of animals.[9,10] Certain nutrients are known to influence endogenous NOC formation in the stomach. Antioxidants, especially vitamin C, reduce the endogenous NOC formation in humans.[7] In contrast, heme iron, which is found mostly in red meats, has been shown to enhance total NOC formation.[11] However, epidemiologic evidence of such interactions on cancer risk is still evolving.

The Iowa Women's Health Study (IWHS) is a large ongoing prospective cohort study started in 1986. In prior analyses, we observed an increased risk of ovarian cancer among women who reported drinking public water with elevated nitrate levels; however, the association was not statistically significant based on a relatively small number of cases ($n = 82$).[12] With an additional 12 years of follow-up, we evaluated whether nitrate and nitrite intake from diet and drinking water (public supplies and private wells) were associated with ovarian cancer risk. We further evaluated whether the association between nitrate and nitrite intake and ovarian cancer risk was modified by dietary factors that may inhibit (vitamin C and E) or enhance (red meats) endogenous NOC formation and by levels of disinfection byproducts (DBPs) in drinking water.

Materials and Methods

The Iowa Women's Health Study (IWHS)

The study design of the IWHS has been described in detail.[13] In brief, a self-administered questionnaire was mailed to 99,826 women, aged 55–69 years, randomly selected from the Iowa State's driver's license list in 1986. Of these women, 41,836 (42%) completed the baseline questionnaire assessing a study participant's demographics, anthropometry, lifestyle, familial history of cancer, medical and reproductive histories, and dietary intake. Respondents and non-respondents were comparable in terms of baseline characteristics.[14] Five follow-up questionnaires (1987, 1989, 1992, 1998 and 2004) have been administered *via* mail. The IWHS was approved by the Institutional Review Boards of the University of Minnesota and the University of Iowa. Return of the completed questionnaire was considered as a subject's consent to study participation.

Dietary intake assessment

Dietary intake at baseline was assessed using the Harvard food frequency questionnaire (FFQ). Study participants were asked their usual intake frequency of 126 food items and the use of dietary supplements over the previous 12 months. The FFQ has been shown to have good validity and reproducibility for major macro- and micronutrients in the IWHS.[15] Nutrient intakes were computed by multiplying the frequency of consumption of each food by the nutrient content. Total intakes of vitamin C and E were calculated by combining intake from foods and dietary supplements.

The nitrate and nitrite contents of foods were determined from a literature review focusing on published reports for U.S. or Canadian populations as previously described.[16,17] We computed means of nitrate and nitrite values for foods weighted by the number of samples and accounting for preparation (raw, cooked and canned) when possible. Nitrate and nitrite contents of FFQ line items were computed by weighting the food-specific values by sex-specific intake amounts from the 1994–1996 Continuing Survey of Food Intake by Individuals (CSFII).[18] For each study participant, we

computed nitrate and nitrite intake overall and from plant and animal sources separately, including from processed meats only.

Water nitrate and DBP estimation

Information on drinking water was collected in a follow-up questionnaire mailed in 1989. Participants were asked the main source of drinking water at their current residence (municipal water system, rural water system, bottled water, private well water, other) and how long they had been drinking water from the indicated water source (<1, 1–5, 6–10, 11–20, >20 years). Of the 36,127 women completing the questionnaire (89% response rate), 27,409 (78%) reported public (municipal or rural) water and 6,634 (19%) reported private well water. Of the 27,409 women reporting public water, 22,375 (82%) reported using their water source for ≥11 years and 19,282 (70%) used it for >20 years. Of the 6,634 private well water drinkers, 5,862 (88%) used their water source for ≥11 years and 4,953 (75%) used it for >20 years. Information on tap water consumption at home and work was not collected.

We estimated nitrate and DBP levels in drinking water supplies using an historical municipal water supply monitoring database for Iowa. The database included NO_3-N measurements from finished water samples (1955–1988). NO_3-N levels in water samples were analyzed at the University of Iowa Hygienic Laboratory using standard methods.[19,20] Total trihalomethanes (TTHMs) and the sum of five haloacetic acids (HAA5) are the regulated DBPs.[21] TTHMs are the sum of four trihalomethanes (chloroform, bromoform, bromodichloromethane and dibromochloromethane). HAA5 is the sum of monochloro-, dichloro-, trichloro-, monobromo- and dibromoacetic acids.

A detailed description of the exposure assessment of DBPs in drinking water, developed in the context of another study, may be found elsewhere.[22] Routine monitoring of TTHMs started in the mid-1980s, and HAA5 in the mid-1990s. Annual average estimates for each DBP before these time periods were based on expert assessments, which considered measured TTHMs and HAA5 concentrations available in databases and historical information on water source, disinfection (pre-, intermediate and/or post-treatment; use of chlorine and/or chloramines) and other water treatment practices (*e.g.*, filtration, coagulation, sedimentation, softening), as well as selected water quality parameters.[22,23] Of the 356 Iowa public water utilities that served ≥ 1,000 persons at the time of estimation, we selected 34 that represented six categories of source water (surface water, shallow groundwater with high levels of brominated THMs, shallow groundwater with low levels of brominated THMs, nonalluvial groundwater with high levels of brominated THMs, nonalluvial groundwater with low levels of brominated THMs, and mixed surface/groundwater systems). We estimated DBP levels for these 34 utilities, considering measured data, changes in source water and/or treatment/disinfection practices over time, and water quality data. Whenever a utility significantly changed its historical treatment/disinfection process or source water, new DBP estimates were made. These annual estimates of 34 representative utilities were assigned to other utilities that used the same water source and similar water treatment and disinfection scheme.

Our study participants included in the water contaminant analyses lived in a total of 473 cities. We estimated the median duration of reported drinking water source categories (1–5, 6–10, 11–20, >20 years) as 4, 8, 16 and 40 years, respectively, based on complete water source history data from female controls of comparable ages in population-based case–control studies conducted during the same time period in Iowa.[24] For each median duration, we computed the means for NO_3-N and DBPs and the number of years in the time period for which the annual estimates exceeded half the MCL (5 mg/L and 40 μg/L for NO_3-N and TTHMs, respectively). In the previous analysis,[12] average NO_3-N levels (1955–1988) were assigned to each participant regardless of duration at their water source. In this study, we assigned average NO_3-N levels depending on their residential cities as well as the duration of using the reported water source. The NO_3-N estimates for each woman in the current study were highly correlated with our previous estimates (Spearman correlation coefficient, $r = 0.94$).

Statistical analysis

We excluded women who met the following criteria at baseline (numbers of subjects are not exclusive): (1) previous cancer diagnosis ($n = 3,830$); (2) premenopausal at baseline ($n = 569$); (3) history of bilateral oophorectomy ($n = 8,064$); and (4) an incomplete FFQ (left ≥ 30 items blank) or implausible energy intake (<600 or >5,000 kcal/day) ($n = 3,102$). In addition, we excluded ovarian cancers other than common epithelial cancers, including cancers of germ cell, sex-cord-stromal and others ($n = 27$), resulting in 28,555 women in the analysis for dietary nitrate and nitrite. We further limited drinking water analyses to women who provided drinking water information and reported using their water source for ≥11 years. In addition, we excluded women who lived in cities with public water systems that derived <75% from the same water source. The latter exclusion should increase the validity of the exposure measurement, as contaminant levels can vary between surface and groundwater sources as well as by depth of groundwater sources.[12] As a result, 17,216 women (13,051 drinking public water and 4,164 drinking private well water) remained in the drinking water analyses.

Incident common epithelial ovarian cancers (1986–2010) were identified *via* the annual linkage with the State Health Registry of Iowa's cancer registry, which is part of the Surveillance, Epidemiology and End Results program of the National Cancer Institute. Diagnosis date, type, stage and morphology of each incident cancer were obtained. Vital status (the date and cause of death) is annually identified through the linkage with the State Health Registry of Iowa, supplemented with the National Death Index. Person-years

were computed from the date of return of the baseline questionnaire to the date of first ovarian cancer diagnosis, bilateral oophorectomy (self-reported), emigration from Iowa (<0.5% annually), death or December 31, 2010, whichever came first.

Pair-wise correlations among NO_3-N and eight DBPs were evaluated using Spearman correlation coefficients (r). The eight DBPs were highly correlated ($r = 0.67$–0.98; Supporting Information Table S1) and we used TTHMs, the sum of the most prevalent DBP class measured, as a surrogate for total halogenated DBPs. Categorical variables were generated for water NO_3-N and TTHM levels (quartiles) and dietary nitrate and nitrite intake (quintiles). Because the range of nitrite intake from processed meats was narrow, we created a 4-level categorical variable (0, >0–0.09, 0.1–0.19, ≥0.2 mg/d) based on its distribution. We compared selected baseline characteristics by NO_3-N levels in public water and private well water use. Hazard ratios (HR) and 95% confidence intervals (CI) were computed using Cox proportional hazards regression as the measure of association with the lowest level as a reference group. We selected *a priori* several baseline characteristics that are risk or protective factors for ovarian cancer as covariates in the multivariable-adjusted model. These covariates included age (continuous), body mass index (BMI, continuous), familial history of ovarian cancer, number of live births (nulliparous, 1–2, 3–4, ≥5), age at menarche (≤ or >12), age at menopause (<45, 45–49, 50–54, ≥55), age at first live birth (<20, 20–24, 25–29, ≥30), oral contraceptive use (never, ever), estrogen use (never, ever) and history of unilateral oophorectomy. In the drinking water analyses, we mutually adjusted for NO_3-N and TTHMs levels (continuous) to evaluate the independent effect of each contaminant. Dietary nitrate and nitrite analyses were additionally adjusted for total energy intake and dietary factors (continuous) that were associated with ovarian cancer risk and were moderately correlated with dietary nitrate or nitrite intake in our study population (cruciferous vegetables, $r = 0.53$ and red meat, $r = 0.48$). Logarithmically transformed values were used for NO_3-N and TTHM levels and dietary factors as covariates, as their distributions were markedly skewed. We tested trends for associations across exposure levels using the median in each category as continuous variables. Because NO_3-N measurements in private well water were not available, ovarian cancer risk among private well water drinkers was compared with the risk among women in the lowest quartile of nitrate in public water. We tested interactions between water NO_3-N and TTHM levels as well as between nitrate (from drinking water or diet) and total vitamin C, E and red meat intake by stratified analyses (≤ or > median) and by including interaction terms (*i.e.*, cross products of dichotomous variables for vitamin C, E and red meats and median in nitrate or nitrite quartile or quintile as continuous variables) in regression models. We performed sensitivity analyses limited to women who reported using the same water source for >20 years. Statistical significance for all analyses was defined as $p < 0.05$.

Results

Mean age of study participants at baseline was 61.6 years (standard deviation, SD = 4.2 years). During the follow-up, 315 incident common epithelial ovarian cancers were identified. Of these, 190 ovarian cancers were included in water nitrate analysis (145 using public water supplies and 45 using private wells). Mean (SD) age at diagnosis was 73.2 (7.7) years. Higher risk for ovarian cancer was observed among women with a familial history of ovarian cancer, no history of unilateral oophorectomy, who were nulliparous and had fewer live births. Oral contraceptive use and ages at menarche and menopause were not associated with ovarian cancer risk; nor were demographic and lifestyle factors such as farm residence, age, BMI, cigarette smoking, physical activity, or alcohol intake. Median NO_3-N and TTHM levels for women drinking from public water supplies were 1.08 mg/L (range: 0.01–25.34 mg/L) and 4.59 μg/L (range: 0–200.88 μg/L), respectively. NO_3-N levels were not correlated with TTHMs or other DBP estimates ($r = -0.03$-0.29) (Supporting Information Table S1). A history of unilateral oophorectomy was slightly more prevalent among women with elevated NO_3-N levels in public water (Table 1). Other factors and dietary intake were not different across NO_3-N levels in public water. More than 90% of women who reported drinking private well water lived on a farm (72%) or in non-farm rural areas (19%) while about 95% of public water drinkers lived in towns. Compared with public water drinkers, more women on private well water had lower education levels, never smoked, had no history of unilateral oophorectomy and never used estrogens or oral contraceptives. Intakes of total calories and red meats (energy-adjusted) were higher among private well water drinkers than public water drinkers. In contrast, total vitamin C intake and energy-adjusted intakes of dietary nitrate and fruits and vegetables were slightly lower among private well users than public water drinkers.

Women who consumed water containing elevated NO_3-N levels were at higher risk for ovarian cancer ($HR_{Q4\ vs.Q1} = 2.14$, CI = 1.30–3.54, $p_{trend} = 0.002$; Table 2). This association did not change substantially by adjusting for TTHM levels. Longer duration of exposure to NO_3-N at levels exceeding half the MCL (5 mg/L) was associated with higher risk for ovarian cancer ($p_{trend} = 0.02$). Women who had ingested water with NO_3-N exceeding 5 mg/L for ≥4 years were at 1.6 times higher risk for ovarian cancer compared with women with no exposure to NO_3-N exceeding 5 mg/L (CI = 1.06–2.41). In contrast, neither average TTHM levels in public water nor years of exposure to TTHM levels exceeding half the MCL (40 μg/L) were associated with ovarian cancer risk. When stratified by low or high TTHM levels (≤ or >median, 4.60 μg/L), there was no evidence of interaction of NO_3-N with TTHMs (data not shown). None of the individual DBPs was associated with ovarian cancer risk (Supporting Information Table S2). Although not statistically significant, ovarian cancer risk was higher among private well users compared with those with the lowest NO_3-N levels in public water (HR = 1.53, CI = 0.93–2.54). Similar

Table 1. Demographic, lifestyle, reproductive and dietary factors among 17,216 women and by mean nitrate levels in public water and private well water use

		Mean nitrate (mg/L nitrate–nitrogen) levels in public water				
	All	0.01–0.472	0.473–1.08	1.09–2.97	2.98–25.34	Private well water
N	17,216	3,263	3,269	3,504	3,015	4,165
Age, years (mean ± SD)	61.6 ± 4.2	61.8 ± 4.2	61.7 ± 4.2	61.7 ± 4.2	61.7 ± 4.2	61.2 ± 4.1
BMI, kg/m^2 (mean ± SD)	26.9 ± 5.0	26.8 ± 5.0	26.7 ± 4.9	26.6 ± 5.0	26.8 ± 5.0	27.4 ± 5.1
Education, ≥ high school (%)	83.8	83.7	84.3	83.5	86.1	81.8
Residence location (%)						
Farm	19.6	3.3	3.3	2.1	2.5	71.9
Rural area (not farm)	6.2	1.7	2.3	1.4	3.0	19.1
Town	74.2	95.0	94.4	96.5	94.5	9.0
Smoking, ever (%)	34.3	37.2	38.9	40.3	37.2	21.3
Physical activity, low (%)	47.3	46.6	47.0	47.8	47.1	47.8
Unilateral oophorectomy (%)	9.8	11.3	10.2	9.8	9.7	8.7
Estrogen use, ever (%)	31.8	33.3	32.4	33.6	33.3	27.6
Oral contraceptive use (%)	19.8	20.7	21.0	19.5	19.0	19.1
Age at menarche ≥ 13 years (%)	57.4	58.4	57.4	56.7	56.6	58.0
Age at menopause ≥ 50 years (%)	53.8	51.9	53.2	52.5	53.0	57.3
Number of live births (mean ± SD)	3.1 ± 1.9	3.1 ± 2.0	3.0 ± 1.9	2.9 ± 1.8	2.9 ± 1.8	3.5 ± 2.0
Age at first live births, years (mean ± SD)	21.0 ± 7.7	20.7 ± 8.0	20.8 ± 7.8	20.7 ± 8.1	21.0 ± 7.8	21.5 ± 6.8
Total calorie intake, kcal (median)	1,731	1,699	1,693	1,702	1,694	1,839
Total vitamin C intake, mg/d (median)	188	189	189	188	192	186
Total vitamin E intake, mg/d (median)	9.5	9.5	9.5	9.5	9.5	9.5
Energy-adjusted intake[1] (median)						
Nitrate, mg/d	60.8	61.0	61.1	61.7	61.5	59.2
Fruits and vegetables, servings/wk	23.6	23.5	23.9	23.9	23.8	23.0
Red meat, servings/wk	3.0	2.9	1.9	2.9	2.8	3.5
Processed meat, servings/wk	0.7	0.7	0.7	0.7	0.7	0.7

[1]Intake adjusted for 1,000 kcal/d of total energy intake.

elevated risks were observed among private well drinkers who lived on a farm (HR = 1.49, CI = 0.87–2.55) or in rural areas or towns (HR = 1.64, 95% CI = 0.83–3.24). These associations remained unchanged after adjusting for dietary nitrate and nitrite intake. When limiting analyses to women who reported using the same water source for >20 years, all observed associations became slightly stronger.

The association between higher nitrate levels in public water and ovarian cancer was stronger among women with low vitamin C intake (≤ median, 190 mg/d, $p_{trend} = 0.005$) compared with those with high intake (> median, $p_{trend} = 0.12$); however, the interaction was not statistically significant ($p_{interaction} = 0.33$, Table 3). The elevated risk among private well water drinkers was observed only among women with low vitamin C intake (HR = 3.30, CI = 1.44–7.56, $p_{interaction} = 0.01$). We also attempted to use different cutpoints for total vitamin C intake including the recommended daily intake (RDI) for non-smoking adult women (=70 mg/d) and the first quartile of total vitamin C intake in our study population (=125 mg/d). Similar stronger positive associations between water nitrate and ovarian cancer risk were observed among women with lower vitamin C intake (data not shown); however, CIs in the low vitamin C intake group were wide due to small numbers of ovarian cancer cases. A stronger association between NO_3-N levels in public water or private well use and ovarian cancer risk was observed among women with high *vs.* low red meat intake although the interaction was not statistically significant.

Mean (SD) dietary nitrate and nitrite intakes were 123.3 mg/d (83.4 mg/d) and 1.2 mg/d (0.5 mg/d), respectively. Total dietary nitrate intake and nitrate intake from plants (*e.g.*, high nitrate vegetables such as lettuce, celery, beets, spinach and broccoli) were highly correlated ($r = 0.99$). On average, about 38% of dietary nitrite intake came from

Table 2. Exposures to nitrate–nitrogen (NO_3–N) and total trihalomethanes (TTHMs) in public water and ovarian cancer risk

				HR (95% CI)		
	Median	*N*	Cases	Age-adjusted	Model 1[1]	Model 2[2]
NO_3-N (mg/L)						
0.01–0.472	0.31	3,263	23	1.0	1.0	1.0
0.473–1.08	0.75	3,269	32	1.41 (0.82–2.41)	1.36 (0.80–2.34)	1.27 (0.73–2.21)
1.09–2.97	1.68	3,504	41	1.66 (1.00–2.76)	1.55 (0.92–2.59)	1.45 (0.85–2.44)
2.98–25.34	3.81	3,015	49	2.34 (1.42–3.84)	2.14 (1.30–3.54)	2.03 (1.22–3.38)
p_{trend}				0.0005	0.002	0.003
Private well water	–	4,165	45	1.50 (0.91––2.49)	1.53 (0.93–2.54)	–
Years of NO_3-N >5 mg/L[3]						
0	0	9,206	91	1.0	1.0	1.0
1–3	1	1,871	22	1.20 (0.75–1.91)	1.05 (0.64–1.72)	1.08 (0.65–1.77)
≥ 4[4]	8	1,974	32	1.66 (1.11–2.49)	1.60 (1.06–2.41)	1.52 (1.00–2.31)
p_{trend}				0.01	0.02	0.05
TTHMs (μg/L)						
0–0.89	0.47	3,112	27	1.0	1.0	1.0
0.90–4.59	1.95	3,612	33	1.07 (0.64–1.78)	1.10 (0.65–1.86)	1.08 (0.64–1.82)
4.77–14.31	10.67	3,524	55	1.82 (1.15–2.89)	1.86 (1.146– 3.00)	1.64 (1.00–2.70)
14.50–200.88	76.32	2,803	30	1.27 (0.76–2.14)	1.31 (0.77–2.24)	1.24 (0.73–2.13)
p_{trend}				0.78	0.74	0.80
Years of TTHMs >40 μg/L[3]						
0	0	9,838	110	1.0	1.0	1.0
> 0–35	3	1,442	17	1.05 (0.63–1.76)	1.00 (0.59–1.70)	0.99 (0.59–1.68)
≥ 36[4]	40	1,771	18	0.93 (0.56–1.53)	0.90 (0.54–1.50)	0.91 (0.55–1.52)
p_{trend}				0.84	0.69	0.72

[1]Adjusted for age, BMI, family history of ovarian cancer, number of live births (0, 1–2, 3–4, ≥ 5), age at menarche (≤ or >12), age at menopause (< 45, 45–49, 50–54, ≥ 55), age at first live birth (< 20, 20–24, 25–29, ≥ 30), oral contraceptive use (never, ever), estrogen use (never, ever) and history of unilateral oophorectomy.
[2]Additionally mutually adjusted for logarithmically transformed values of NO_3–N or TTHMs levels in public water.
[3]Half the maximum contaminant level (MCL) determined by the U.S. Environmental Protection Agency.
[4]The median years of exposures to a half of MCL among women who exposed during the reported duration of exposure.

animal sources and 15% came from processed meats. Higher dietary nitrate intake was observed among IWHS participants reporting higher age, BMI, education level, alcohol intake, physical activity level and estrogen use.[25] Women reporting higher dietary nitrate intake also reported higher intake of total calories, cruciferous vegetables, red meats and vitamins C and E. Higher dietary nitrate intake was associated with lower ovarian cancer risk ($HR_{Q5\ vs.Q1} = 0.61$, CI = 0.40–0.95; $p_{trend} = 0.02$, Table 4). Dietary nitrite intake was not associated with ovarian cancer risk. Similarly, neither dietary nitrite intake from plant nor animal sources was associated with ovarian cancer risk. However, higher nitrite intake from processed meats was marginally associated with higher ovarian cancer risk after adjusting for confounders ($p_{trend} = 0.04$). On a continuous scale, the risk was 12% (CI = 4–20%) higher with each 0.1 mg increment in nitrite intake from processed meats. These associations did not change by additional adjustment for total vitamin C and E intakes. There was no interaction between dietary nitrate or nitrite intake and total vitamin C, E or red meat intakes.

Discussion

We found higher risk for epithelial ovarian cancer among women drinking water from public supplies with higher nitrate levels, regardless of TTHM levels. Ovarian cancer risk also appeared higher among women drinking private well water compared with the lowest $NO\text{-}N_3$ quartile in public water supplies, and we observed a statistically significant interaction with vitamin C intake. Higher dietary nitrate intake was associated with lower risk for ovarian cancer, whereas higher nitrite intake from processed meats was associated with higher risk.

Epidemiologic studies of dietary nitrate intake have predominantly evaluated stomach cancer and many studies reported null associations or inverse trends.[7,26] One explanation for these findings is the potential interaction between

Table 3. Ovarian cancer risk in relation to nitrate–nitrogen (NO_3-N) levels in drinking water stratified by high or low total vitamin C and red meat intakes

	Vitamin C ≤190 mg/d				Vitamin C >190 mg/d				
	N	Cases	HR (95% CI)[1]	p_{trend}	*N*	Cases	HR (95% CI)[1]	p_{trend}	$p_{interaction}$
NO_3-N (mg/L)									
0.01–0.472	1,625	7	1.0	0.005	1,638	16	1.0	0.12	0.33
0.473–1.08	1,629	14	1.85 (0.74–4.65)		1,640	18	1.16 (0.59–2.29)		
1.09–2.97	1,762	26	3.17 (1.37–7.32)		1,742	15	0.83 (0.40–1.70)		
2.98–25.34	1,467	24	3.39 (1.45–7.95)		1,548	25	1.60 (0.85–3.02)		
Private well water[2]	2,125	29	3.30 (1.44–7.56)	–	2,040	16	0.77 (0.38–1.54)	–	0.01
	Red meats ≤5 servings/wk				**Red meats >5 servings/wk**				
	N	Cases	HR (95% CI)[1]	p_{trend}	*N*	Cases	HR (95% CI)[1]	p_{trend}	$p_{interaction}$
NO_3-N (mg/L)									
0.01–0.472	1,812	13	1.0	0.18	1,451	10	1.0	0.002	0.14
0.473–1.08	1,853	21	1.61 (0.81–3.22)		1,416	11	1.04 (0.43–2.50)		
1.09–2.97	2,032	26	1.69 (0.86–3.30)		1,472	15	1.36 (0.60–3.06)		
2.98–25.34	1,788	25	1.82 (0.93–3.57)		1,227	24	2.59 (1.23–5.48)		
Private well water[2]	1,629	15	1.34 (0.64–2.82)	–	2,536	30	1.68 (0.82–3.44)	–	0.63

[1]Adjusted for age, BMI, family history of ovarian cancer, number of live births (0, 1–2, 3–4, ≥5), age at menarche (≤ or >12), age at menopause (< 45, 45–49, 50–54, ≥55), age at first live birth (< 20, 20–24, 25–29, ≥30), oral contraceptive use (never, ever), estrogen use (never, ever) and a history of unilateral oophorectomy.
[2]HR and 95% CI were computed with the lowest quartile of nitrate among public water drinkers as a reference group.

nitrate and antioxidants, which are abundant in major dietary sources of nitrate such as green leafy and root vegetables.[27,28] Antioxidants, such as vitamins C and E, inhibit NOC formation by reducing nitrite to nitric oxides, and thus decreasing the level of NOCs and NOC-induced DNA adducts.[29,30] Therefore, a potentially carcinogenic effect of dietary nitrate intake may be reduced or eliminated by the protective effects of high antioxidant intake from fruits and vegetables. Indeed, dietary nitrate intake was highly correlated with total vegetable intake ($r = 0.84$), and moderately correlated with antioxidant intakes ($r = 0.36$–0.46) in our study.

Carcinogenic effects of NOCs in the ovary have been shown in animal studies.[9,10] However, to date, NOCs and their precursors nitrate and nitrite have been evaluated in relation to ovarian cancer risk in only a few epidemiologic studies. Ovarian cancer risk was evaluated in relation to dietary nitrate intake in two prospective cohort studies and these studies found no associations.[12,31] Dietary nitrite intake and ovarian cancer was assessed in only one prior cohort study.[31] In that study, total nitrite intake and nitrite intake from plant sources were not associated with epithelial ovarian cancer risk, but higher nitrite intake from animal sources was associated with higher risk ($HR_{Q5\ vs.\ Q1} = 1.34$, CI = 1.05–1.69, $p_{trend} = 0.02$). Processed meats contain added nitrate and nitrite as well as high amounts of amines and amides, precursors of NOCs. Ingestion of nitrate in combination with nitrosatable precursors has been shown to increase the formation of NOCs.[32] Furthermore, red and processed meats contain heme iron, a component of myoglobin, which promotes the formation of NOCs.[11] Therefore, nitrate and nitrite added to processed meats may result in exogenous and endogenous NOC formation. Three large prospective cohort studies have found statistically non-significant trends towards positive associations between processed meat intake and ovarian cancer.[33–35] Meta-analysis of four prospective cohort studies found a borderline positive exposure response between processed meat intake and ovarian cancer risk (HR = 1.05, CI = 0.98–1.14 for an intake increment of 100 g per week).[36]

Unlike dietary nitrate, nitrate from drinking water is not accompanied by micronutrients that could inhibit endogenous nitrosation. Therefore, nitrate from drinking water could result in more endogenously formed NOCs than nitrate from foods. Previous epidemiologic studies, including our study,[12] have shown associations between nitrate levels in public water and the risk of cancer, including bladder,[12] stomach and colorectal cancers.[6,7] However, ovarian cancer has been assessed in relation to nitrate in public water only in our previous analysis in the IWHS, as one of multiple cancer outcomes.[12] In our previous analysis including 82 incident ovarian cancers, we observed a positive association between higher nitrate levels in public water supplies and the risk of ovarian cancer ($HR_{Q4\ vs.\ Q1} = 1.86$, CI = 0.82–4.26); however, this association did not reach statistical significance level. In the current study, we found a statistically significant more than two-fold risk for ovarian cancer among women in the highest (median = 3.81 mg/L) compared in the lowest (median = 0.31 mg/L) NO_3-N quartiles in public water supplies.

Table 4. Dietary nitrate and nitrite intake and ovarian cancer risk among 28,555 women

				HR (95% CI)	
	Median	*N*	Cases	Model 1[1]	Model 2[2]
Nitrate (mg/d)					
Total intake					
Q1: 3.87–65.43	49.5	5,711	59	1.0	1.0
Q2: 65.44–92.04	78.9	5,711	73	1.18 (0.83–1.68)	1.05 (0.73–1.50)
Q3: 92.05–121.96	106.2	5,711	54	0.86 (0.58–1.26)	0.72 (0.48–1.06)
Q4: 121.97–165.48	140.2	5,711	74	1.21 (0.84–1.74)	0.96 (0.66–1.41)
Q5: 165.54–2,083.52	209.2	5,711	55	0.85 (0.56–1.27)	0.61 (0.40–0.95)
p_{trend}				0.37	0.02
Per 10 mg/d	–	–	–	0.99 (0.98–1.01)	0.98 (0.96–1.00)
Nitrite (mg/d)					
Total intake					
Q1: 0.11–0.80	0.7	5,709	62	1.0	1.0
Q2: 0.81–1.02	0.9	5,716	52	0.84 (0.56–1.26)	0.80 (0.53–1.21)
Q3: 1.021–1.23	1.1	5,716	65	1.12 (0.73–1.72)	1.04 (0.68–1.59)
Q4: 1.239–1.53	1.4	5,703	70	1.26 (0.79–2.02)	1.14 (0.71–1.82)
Q5: 1.537–7.13	1.8	5,711	66	1.20 (0.68–2.12)	1.03 (0.58–1.84)
p_{trend}				0.24	0.50
Per 0.1 mg/d	–	–	–	1.00 (0.97–1.04)	0.99 (0.95–1.03)
Animal sources					
Q1: 0–0.26	0.2	5,638	63	1.0	1.0
Q2: 0.26–0.36	0.3	5,689	44	0.68 (0.45–1.02)	0.72 (0.48–1.08)
Q3: 0.36–0.47	0.4	5,597	83	1.29 (0.89–1.88)	1.39 (0.96–2.02)
Q4: 0.47–0.61	0.5	5,668	59	0.89 (0.59–1.37)	0.98 (0.64–1.50)
Q5: 0.61–3.47	0.7	5,648	66	1.04 (0.64–1.67)	1.18 (0.72–1.91)
p_{trend}				0.45	0.25
Per 0.1 mg/d	–	–	–	1.04 (0.98–1.11)	1.06 (1.00–1.13)
Processed meats					
0	0	4,872	54	1.0	1.0
> 0–0.09	0.04	19,770	212	0.94 (0.69–1.28)	1.01 (0.74–1.38)
0.1 – 0.19	0.13	2,537	32	1.15 (0.73–1.82)	1.27 (0.80–2.01)
≥ 0.2	0.26	1,135	17	1.46 (0.82–2.58)	1.65 (0.93–2.94)
p_{trend}				0.10	0.04
Per 0.1 mg/d	–	–	–	1.10 (1.03–1.19)	1.12 (1.04–1.20)
Plant sources					
Q1: 0.04–0.47	0.4	5,701	64	1.0	1.0
Q2: 0.47–0.61	0.5	5,717	62	0.88 (0.61–1.28)	0.82 (0.56–1.19)
Q3: 0.61–0.76	0.7	5,712	57	0.87 (0.59–1.28)	0.77 (0.52–1.14)
Q4: 0.76–0.98	0.9	5,721	67	1.01 (0.67–1.51)	0.86 (0.57–1.29)
Q5: 0.98–6.39	1.2	5,704	65	0.96 (0.60–1.52)	0.77 (0.48–1.24)
p_{trend}				0.79	0.54
Per 0.1 mg/d	–	–	–	0.99 (0.95–1.03)	0.97 (0.92–1.01)

[1]Adjusted for age, BMI, family history of ovarian cancer, number of live births (0, 1–2, 3–4, ≥ 5), age at menarche (≤ or >12), age at menopause (< 45, 45–49, 50–54, ≥ 55), age at first live birth (< 20, 20–24, 25–29, ≥ 30), oral contraceptive use (never, ever), estrogen use (never, ever), history of unilateral oophorectomy and total energy intake (logarithmically transformed).
[2]Additionally adjusted for logarithmically transformed values of cruciferous vegetable and red meat intake.

For the first time, we found evidence suggesting a higher risk for ovarian cancer among women who were private well water drinkers. In Iowa, agricultural application of nitrogen is the major source of environmental nitrate contamination. Nitrate levels can be high in private wells in agricultural areas because of their location close to crop fields treated with nitrogen fertilizer and livestock manure, and because private wells are not regulated and may not be routinely monitored. In the United States, the average NO_3-N levels in streams and groundwater in agricultural areas are over 3 mg/L whereas average levels in urban areas and areas with mixed land use are about 1.5 mg/L and 1 mg/L, respectively.[6] About 22% of private wells in agricultural areas in the United States exceed the nitrate MCL (10 mg/L NO_3-N).[6] A survey of rural private wells in Iowa in 1988–1989 found that 18% of wells exceeded the MCL for nitrate. In addition, 37% of these rural private wells had levels greater than 3 mg/L, typically considered indicative of anthropogenic pollution.[37] We observed similarly elevated risk of ovarian cancers among private well users in farm and non-farm areas. Most of Iowa land is used for agriculture with row crops and grasslands covering 90% and urban areas accounting for only 1% of the state surface area.[38] Therefore, private wells located in non-farm rural areas or towns are likely to be in close proximity to farms and thus impacted by the agricultural use of nitrogen fertilizers. Nitrate levels in private well water are determined by many factors including geological characteristics and agricultural practices.[37] Well depth is the best predictor of well-water nitrate contamination with higher nitrate levels found in shallower wells. NO_3-N levels in 35% of private wells less than 15 m deep exceeded the MCL (about 28% of private wells in Iowa are less than 15 m deep).[37,39] Unfortunately, information on well depth was not collected in our study.

It should be noted that elevated nitrate levels may be an indicator of contamination with other chemicals or bacteria.[40] In agricultural areas, wells with elevated nitrate levels may also have elevated levels of herbicides, some of which are suspected carcinogens. For example, atrazine, a triazine herbicide, is one the most frequently detected pesticides in Iowa groundwater, and occupational exposure is a hypothesized risk factor for ovarian cancer.[41,42] Exposures to pesticides *via* drinking water are likely to be substantially lower than occupational exposures but few studies have been conducted. Atrazine and its metabolites have been detected in Iowa public water supplies, although levels are usually below the MCL and detections are not as frequent as for nitrate.[43] The 1988–1989 state-wide survey revealed that pesticides were present in about 5% of private wells in Iowa.[37] DBPs in drinking water have been associated with higher risk for bladder cancer and possibly other sites.[44] We evaluated, for the first time, DBPs in drinking water in relation to ovarian cancer and found only non-significant, uneven elevations of risk for the DBP metrics in our analysis. Evaluation in other populations would be valuable.

Ovarian cancer is a relatively rare cancer, but a large sample size as well as a long follow-up period enabled us to study 190 cases in relation to water contaminants. Emigration from Iowa rarely occurred in our cohort (<0.5% annually), enabling a nearly complete follow-up of the cohort and likely detection of most incident ovarian cancers. The attainment of water nitrate and DBP data through a linkage with a historical public water monitoring database is another strength of our study. In addition, reported duration of water source use enabled us to estimate the length of exposure to water contaminants, which is a key factor in exposure assessment. The majority of our cohort participants lived in the same address for more than 10 years at the post-enrollment drinking water data collection, which enabled us to estimate long-term exposures to nitrate and DBPs in drinking water. Our study has limitations as well. Dietary intake was assessed at cohort baseline and may have changed during the long follow-up period. However, dietary intakes assessed at cohort baseline and at the 2004 follow-up survey were reasonably correlated (*e.g.*, $r = 0.44$ for total calorie, 0.39–0.42 for macronutrients, 0.36 for total vegetables and 0.24 for processed meat products) and earlier exposures are likely to be the most relevant for cancer risk. Potential misclassification of dietary intake assessed using a FFQ is also probable. Furthermore, dietary intake assessment by a FFQ cannot capture important information related to the nitrate content and NOC formation such as food storage and cooking methods. Because information on study participants' daily water consumption was not available, patterns in individuals' water consumption such as the amount and timing as well as water consumption outside of their home (*e.g.*, work) was not taken into account in our exposure assessment. In addition, we did not have information on other factors that may influence nitrate metabolism to include in our analyses. For example, factors that affect the number of nitrate-reducing bacteria in saliva, such as mouthwash use and oral hygiene, may alter the rate of nitrate–nitrite conversion by saliva.[7] Similarly, proton-pump inhibitor use increases the pH in the stomach and may increase NOC formation.[45] Finally, study included only postmenopausal white women; therefore, interpretation of our results is limited to this population, and future studies should evaluate these exposures among all women including premenopausal women and other ethnic groups with ovarian cancer.

In conclusion, this study indicates that nitrate from public drinking water may be associated with higher risk of ovarian cancer among postmenopausal women. Our results suggest that postmenopausal women who drink private well water may be at higher risk for ovarian cancer, especially with low vitamin C intake. Our findings also support the hypothesis that dietary nitrite intake from processed meats increases ovarian cancer risk. Additional confirmatory studies with a larger number of ovarian cancer cases are warranted and could result in a novel target for ovarian cancer risk reduction.

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