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Nitrate exposure from drinking water and dietary sources among Iowa farmers using private wells

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Abstract

Nitrate levels are increasing in water resources across the United States and nitrate ingestion from drinking water has been associated with adverse health risks in epidemiologic studies at levels below the maximum contaminant level (MCL). In contrast, dietary nitrate ingestion has generally been associated with beneficial health effects. Few studies have characterized the contribution of both drinking water and dietary sources to nitrate exposure. The Agricultural Health Study is a prospective cohort of farmers and their spouses in Iowa and North Carolina. In 2018–2019, we assessed nitrate exposure for 47 farmers who used private wells for their drinking water and lived in 8 eastern Iowa counties where groundwater is vulnerable to nitrate contamination. Drinking water and dietary intakes were estimated using the National Cancer Institute Automated Self-Administered 24-Hour Dietary Assessment tool. We measured nitrate in tap water and estimated dietary nitrate from a database of food concentrations. Urinary nitrate was measured in first morning void samples in 2018–19 and in archived samples from 2010–2017 (minimum time between samples: 2 years; median: 7 years). We used linear regression to evaluate urinary nitrate concentrations in relation to total nitrate, and drinking water and dietary intakes separately. Overall, dietary nitrate contributed the most to total intake (median: 97%; interquartile range

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8. Author contributions:

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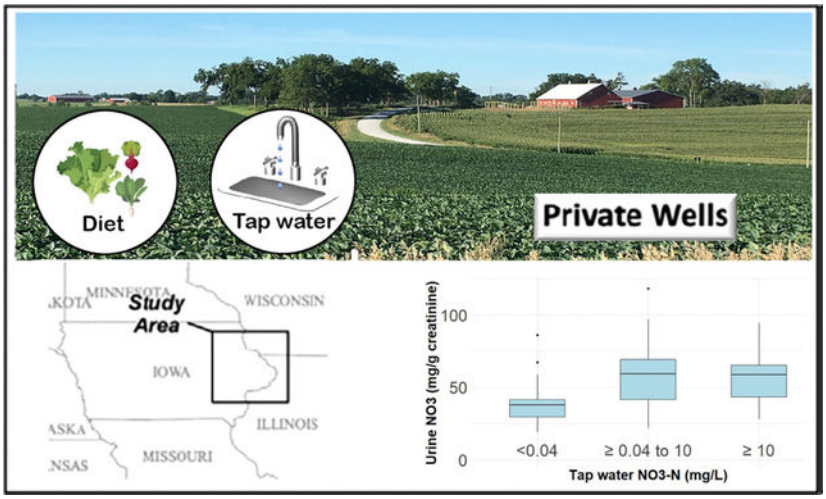
[IQR]: 57 – 99%). Among 15 participants (32%) whose drinking water nitrate concentrations were at/above the U.S. Environmental Protection Agency MCL (10 mg/L NO_3-N), median intake from water was 44% (IQR: 26–72%). Total nitrate intake was the strongest predictor of urinary nitrate concentrations (R^2 = 0.53). Drinking water explained a similar proportion of the variation in nitrate excretion (R^2 = 0.52) as diet (R^2 = 0.47). Our findings demonstrate the importance of both dietary and drinking water intakes as determinants of nitrate excretion.

Graphical Abstract



Keywords

nitrate; drinking water; private wells; biomarkers; diet; Iowa; water quality

1. Introduction

Since the 1950s, nitrate levels have increased in many drinking water sources due to human activities, especially nitrogen fertilizer use, animal feeding operations, and fossil fuel combustion (Dubrovsky et al., 2010). Private wells in agricultural areas are particularly vulnerable to nitrate contamination due to their proximity to agricultural nitrogen sources and septic systems. The U.S. Environmental Protection Agency (EPA) regulates and requires monitoring of nitrate in public water supplies. However, there are no regulations for private drinking water sources (U.S. EPA, 2013). About 42 million Americans (~15% of the population) currently rely on private wells for their main drinking water source (Dieter et al., 2018). It is not known how many private wells are regularly tested and treated (Levin et al., 2023).

The EPA maximum contaminant level (MCL) for nitrate of 10 mg/L as nitrate-nitrogen (NO_3-N; equivalent to about 44 mg/L as NO_3) was established to prevent infant methemoglobinemia, a potentially fatal health condition (U.S. EPA, 2013); however, other health risks were not considered (Ward et al., 2018a). When nitrate concentrations in drinking water are below the MCL, the majority of nitrate intake comes from the diet

(IARC, 2010). Foods that naturally contain high levels of nitrate include vegetables such as spinach, lettuce, celery, and beetroot (IARC, 2010; Inoue-Choi et al., 2016). The EPA sets a reference dose (RfD) for daily nitrate intake at 1.6 mg NO_3-N/day (U.S. EPA, 2013). The RfD was set based on early clinical signs of methemoglobinemia (excess of 10%) for infants aged 0–3 months old from ingestion of infant formula made with nitrate contaminated tap water (U.S. EPA, 2013). In the human body, ingested nitrate is reduced to nitrite, which reacts with amines and amides to form N-nitroso compounds (NOC), a process called endogenous nitrosation. Most NOC are animal carcinogens and cause tumors in multiple organs (IARC, 2010). The IARC considers nitrate and nitrite to be probable carcinogens when ingested under conditions that result in endogenous nitrosation (IARC, 2010). Vitamin C and other dietary antioxidants reduce the rate of endogenous nitrosation when ingested around the same time as nitrate (Mirvish et al., 1995). In contrast, the heme iron in red meat can increase NOC formation in the gastrointestinal tract (IARC, 2010). Therefore, nitrate ingested via drinking water is likely more of a concern for cancer risk than dietary sources because water does not contain antioxidants that inhibit NOC formation (IARC, 2010).

Epidemiological research has found evidence of both beneficial and adverse health effects of nitrate depending on the sources of exposure. There is some evidence that the protective cardiovascular benefits of fruit and vegetable consumption are due to the dietary nitrate levels (Ahluwalia et al., 2016). In contrast, adverse health effects, including specific cancers and adverse reproductive outcomes, are associated with nitrate exposure from drinking water sources, often at nitrate levels below the MCL (Clemmensen et al., 2023; Ward et al., 2018b). Because of the potentially different health effects, it is important to characterize the relative contributions of nitrate sources and to evaluate predictors of exposure.

Urinary nitrate is a biomarker of recent nitrate exposure because 60% to 70% of ingested nitrate is excreted in urine with a physiological half-life of approximately 5 hours (Bartholomew & Hill, 1984). In the absence of inflammation, the majority of nitrate in the body comes from ingested sources; however, endogenous synthesis of nitrate occurs and contributes to nitrate excretion (Green et al., 1981; IARC, 2010). Only a few studies (in the United States, Canada, and Europe) have evaluated the contribution of drinking water and dietary intakes to nitrate exposure in populations using private wells with elevated nitrate concentrations in their drinking water (Chilvers et al., 1984; Levallois et al., 2000; Mirvish et al., 1992; Møller et al., 1989). The primary aim of our study was to assess the contributions of diet and drinking water to total nitrate intake in a population using private wells as their drinking water source. Additionally, we evaluated predictors of urinary nitrate concentrations and assessed temporal variability. To address these objectives, we studied a subgroup of farmers from the Agricultural Health Study (AHS) who lived in an agricultural area of Iowa where private wells are vulnerable to nitrate contamination.

2. Methods

2.1 Study Population

We conducted a cross-sectional exposure study of male farmers in 2018–19 who were selected from participants in the Biomarkers of Exposure and Effect in Agriculture (BEEA) study within the AHS. The AHS is a prospective cohort study of over 89,000

licensed pesticide applicators and their spouses from Iowa and North Carolina for whom information on drinking water source and agricultural exposures was collected at enrollment (1993 to 1997) and at several follow-up interviews (Manley et al., 2022). Full questionnaires available at https://www.aghealth.nih.gov/collaboration/questionnaires.html. The BEEA study is a molecular epidemiologic subcohort that includes 1,681 male AHS pesticide applicators (Hofmann et al., 2015). Blood, urine, and dust samples were collected as part of a home visit in 2010 to 2017, but no tap water samples were collected.

As previously described (Bradley et al., 2023; D. A. Thompson et al., 2023), 50 participants were recruited from 8 counties in eastern Iowa (Buchanan, Cedar, Delaware, Dubuque, Jackson, Johnson, Jones, and Linn) where drinking water is sourced from aquifers vulnerable to contamination (hydrogeology is alluvial or karst topography) from intensive agricultural activities (row crop farming and animal feeding operations). Criteria for selection included living in the same residence (30+ years) since AHS enrollment and using a private well as their main drinking water source. To focus on participants with high exposure potential, well depths less than 150 feet or predicted nitrate concentrations greater than 5 mg/L as determined by a nitrate model developed for Iowa private wells were also required (Wheeler et al., 2015). Based on additional aims of the study to assess other water contaminants (Bradley et al., 2023; D. A. Thompson et al., 2023), enrollees also had to have been actively farming in the last 12 months, have grown crops on at least 50 acres of farmland, applied pesticides to 75% of their crops, and have grown major income-producing crops (corn and soybeans) as determined at the time of the BEEA interview. Three participants ultimately did not participate due to home visit delays (n=2) and hazardous winter road conditions at the time of sampling (n=1). All participants provided written consent to participate, and the study was approved by Institutional Review Boards at the National Cancer Institute (NCI) and the University of Iowa. The involvement of the CDC laboratory did not constitute engagement in human subjects research.

2.2 Interviews and Sample Collection

Urine collection kits, instructions, a self-administered questionnaire, and links to the NCI Automated Self-Administered 24-hour (NCI ASA-24) Dietary Assessment Tool were sent to all participants before the scheduled home visit (December 2018 to February 2019). The NCI ASA-24 assessment tool has been found to provide similar results to standard 24-hour recalls administered by interviewers (Pannucci et al., 2018; F. E. Thompson et al., 2015). Study participants also completed a self-administered questionnaire that included questions about farming practices, well depth, usual consumption patterns over the last 12 months of tap water, bottled water, beverages made with tap water (coffee, tea, and other beverages), alcohol, and a lifetime smoking history. Participants used the NCI ASA-24 dietary assessment tool to record food and beverages consumed during the prior 24-hour period (Subar et al., 2007). Additional information on participant characteristics (e.g., body mass index [BMI]) was obtained from the original BEEA interviews (conducted between 2010 and 2017). During the home visit, researchers reviewed the informed consent policies, NCI ASA-24 dietary assessment tool, and the self-administered questionnaire with all participants.

Researchers collected, acidified, and immediately chilled (on ice) an untreated cold tap water sample from each participants' kitchen sink (without flushing, pre-treatment, or screen removal) (Bradley et al., 2023; D. A. Thompson et al., 2023). Each participant was instructed to collect up to three urine samples (before bedtime, as needed during the night, and first morning void) in 90 ml collection containers starting the night before the home visit. Urine samples were chilled for transport to the University of Iowa, aliquoted, and then frozen at −80° C. Additionally, first morning void urine samples that were collected previously from these participants during the BEEA home visits between 2010 and 2017 (Hofmann et al., 2015) were sent to the Centers for Disease Control and Prevention (CDC) for analysis together with the samples collected in 2018–2019.

2.3 Laboratory Analyses

Tap water samples were shipped overnight on ice to the U.S. Geological Survey (USGS) National Water Quality Laboratory in Denver, Colorado. Samples were analyzed for nitrate using the nitrate reductase nitrate-nitrogen method with a reporting level of 0.01 mg/L as nitrate-nitrogen (Patton & Kryskalla, 2011). Quality-assurance/quality-control included five field and and five laboratory blanks (Bradley et al., 2023). Urine samples were sent frozen to the CDC for analyses of nitrate and creatinine. Nitrate was analyzed by ion chromatography tandem mass spectrometry with a limit of detection of 0.5 mg NO_3/L and average coefficient of variation (spiked urine samples) of <5% (Valentín-Blasini et al., 2005). Creatinine concentrations were measured using an Enzymatic Roche Cobas 6000 system (Li, 2019) and were used to adjust for urine dilution. Creatinine was previously measured in the original BEEA samples by the Advanced Research and Diagnostics Laboratory at the University of Minnesota using the same method.

2.4 Dietary and Drinking Water Nitrate Intake

We estimated dietary nitrate intake in the prior 24-hours using the responses from the NCI ASA-24 dietary assessment tool and an NCI database of food nitrate levels (Inoue-Choi et al., 2016). Water intake was estimated using the NCI ASA-24 dietary assessment tool that recorded a participant's beverage consumption including whether water was from the tap or bottled. We assumed nitrate concentrations in bottled water to be 0.5 mg/L NO_3-N based on measurements of mean nitrate concentrations in major bottled water brands sold in Iowa (Weyer et al., 2014). Nitrate intake from tap water and drinks made with tap water (coffee, tea, and other beverages) were estimated by multiplying the nitrate concentration in each participant's tap water sample by their total tap water intake. Nitrate concentrations in drinking water were lognormally distributed. A single imputation (range: >0 to <0.04 mg/L NO_3-N) was substituted for concentrations below the detection limit (<0.04 mg/L NO_3-N) based on the measured values and assuming a lognormal distribution (Lubin et al., 2004). Nitrate-N concentrations in water were converted to NO_3 before multiplying by tap water intake. We computed total nitrate (NO_3 mg/day) intake from diet and water by summing the two intake variables.

Total nitrate intake was also re-calculated using usual water intake and 24-hour dietary intake. We computed usual nitrate intake from drinking water using the questionnaire responses about tap water intake over the prior 12 months. At the time of the interview,

participants were asked how many drinks (in cups) per day, week, or month they consumed on average during the past 12 months, including tap water, bottled water, coffee, tea, and other beverages made with tap water. Usual tap water intake information was converted to a daily intake and multiplied by the nitrate concentration in a participant's tap water sample to compute usual water nitrate intake.

2.5. Nitrate excretion estimates using the Cockroft-Gault method

We estimated daily (24-hour) excretion from a participant's creatinine-adjusted urinary nitrate concentration using the formula (Mage et al., 2004):

$$nitrate\ excretion\ (mg\ NO_3) = spot\ urinary\ nitrate\ (mg/g\ Creatinine\ ^*creatinine\ excretion\ (g/day)$$

Creatinine excretion (24-hour) was computed using the Cockroft-Gault equation: $creatinine\ excretion\ (g/day) = 10^{-6} * k * [140 - age\ (yr)]^* wt\ (kg)^{1.5} * ht\ (cm)^{0.5}$ where k is a constant defined as $k_{\text{males}} = 1.93$ ($k_{\text{females}} = 1.64$), age is in years (yr), wt is weight in kilograms, and ht (height) is in centimeters.

2.5 Statistical Analysis

We describe frequency distributions (medians and interquartile ranges [IQR]) or percentages for participant characteristics including age at the 2018–2019 interview, smoking status, BMI at the BEEA interview, characteristics of the drinking water well, and distributions of the 24-hour drinking water and dietary nitrate intakes and total intakes based on 24-hour and usual nitrate (mg NO_3/day) intakes from tap water. We describe the distributions of urinary nitrate concentrations (mg/g creatinine) by well depth and by well nitrate concentrations. We computed the percent of the study population whose water samples were at or exceeded the MCL and the percentages of total nitrate intake that came from drinking water and dietary intakes overall and in this subgroup.

Urinary nitrate concentrations (mg NO_3/ml) and drinking water, dietary, and total nitrate intakes were transformed using the natural log because the data were right skewed. We used linear regression models in PROC GLM in SAS (version 9.4, SAS Institute Inc., Cary, North Carolina, USA) to estimate the proportional increase in urinary nitrate concentrations (NO_3 mg/ml) for each 1-unit increase in the natural log of the nitrate intake (mg NO_3). We evaluated drinking water, dietary, and total nitrate intake in the prior 24-hour period in separate models, as well as drinking water and dietary nitrate in the same model, adjusting for creatinine and age as continuous variables. We evaluated BMI and smoking status as possible confounders using a change in estimate approach. These variables did not change the parameter estimates by >10% and were not included in the final models. We present the parameter estimates, 95 percent confidence intervals (CI) and the model R-squared values.

Finally, we analyzed the intra- and inter-individual variability of the natural log transformed creatinine-adjusted urinary nitrate concentrations using SAS PROC MIXED (version 9.4). We evaluated variability in urinary nitrate concentrations between the 44 evening, 24 overnight, and 47 morning samples. Twenty-three participants provided 3 samples (evening, overnight, morning), 22 provided 2 samples (21 evening, morning; 1 overnight, morning),

and 2 participants provided only one sample (morning). Additionally, we compared creatinine-adjusted urinary nitrate concentrations between this 2018–2019 samples and the earlier BEEA samples. All 47 participants had at least 1 BEEA sample; 5 had 2 BEEA samples. We calculated the temporal variability accounting for the years between the samples (median = 6.7 years, IQR: 4.6–7.8; range 1.9–8.1). We computed the intraclass correlation coefficients (ICC) to describe the ratio of the between person variance to the total (between and within person) variance. We considered findings to be statistically significant if $p<0.05$.

3. Results

Demographics, characteristics of the private wells, and nitrate intake distributions are shown in Table 1. All participants were non-Hispanic white men. The mean age of participants was 64 years, and most participants (81%) were never smokers. Based on the original BEEA interview, 23% of participants were normal weight; 43% had a BMI of 25–30 kg/m^2 (overweight), and 34% had a BMI of ≥30 kg/m^2 (obese). Four households (8.5%) treated their well water using reverse osmosis, which removes nitrate and some other contaminants; six (12.8%) used a charcoal filter, which does not remove nitrate. Nitrate was not detected (< 0.04 mg/L NO_3-N) in tap water for 15 households (32%); whereas another 15 households (32%) had nitrate concentrations above the EPA MCL (10 mg/L NO_3-N). Three of the four households that reported using reverse osmosis treatment had nitrate concentrations greater than 9 mg/L NO_3-N in their tap water indicating that the treatment system was not adequately reducing nitrate concentrations.

The median intake from water was 0.9 mg NO_3/day (IQR: 0.3–37.5 mg/day) and the median 24-hour nitrate intake from diet was 30.4 mg NO_3/day (IQR: 17.9–88.1 mg/day). Intake from water and diet were not significantly correlated (Spearman rho=0.09). The median 24-hour total nitrate intake was 63.9 mg NO_3 (IQR: 23.0–157.6). Median usual water intake (L/day) based on intake in the prior 12 months was slightly higher than the 24-hour intake (usual: 1.2 L/day, IQR: 1.1–1.7; 24-hr: 1.0 L/day, IQR: 0.6–1.4), resulting in higher estimates of usual drinking water nitrate intake (2.2 mg/day, IQR: 0.3–51.9). Our estimate of median 24-hour nitrate excretion using the Cockroft-Gault equation was 87 mg/day (IQR: 57.9–118.6).

The percentages of total nitrate from drinking water and diet sources, as estimated by the 24-hour recalls, are shown in Table 2. The median percentage from diet was about 97 percent (IQR: 57.4%–99.5%); whereas the median from drinking water was about 2.7 percent (IQR: 0.5%–42.6%). Based on usual water intake, the percent contribution of drinking water intake was higher (median = 6.8%, IQR: 1.1%–58.5%). The low median contribution of water nitrate intake was most likely due to the 32% of participant wells with nitrate levels below the detection limit (<0.04 mg/l). Among the participant wells that had nitrate concentrations at or above the MCL (n=15; 32%), the median percentage of nitrate intake from water sources was 44% (IQR:26.2%–72.0%).

The distribution of urinary nitrate concentrations is shown in Table 3. The median urinary nitrate concentration was 51.9 mg/g creatinine (IQR: 36.6–67.3). Median urinary nitrate

concentrations were higher for participants with well depths less than 100 feet (58.8 mg/g, IQR: 42.0–69.1) compared to participants with well depths greater than 150 feet (40.1 mg/g, IQR: 28.0–63.3). Participants with non-detectable nitrate concentrations in their tap water (<0.04 mg/L) had lower urinary nitrate concentrations (median=38.9 mg/g creatinine, IQR: 28.0–58.8) compared to participants with well water nitrate concentrations greater than the MCL (median=61.8 mg/g, IQR: 43.6–79.1).

The results of the linear regression models of creatinine-adjusted urinary nitrate concentrations in relation to nitrate intakes are shown in Table 4. Dietary nitrate intake was a borderline significant predictor of urinary nitrate concentrations (p=0.07). A 2.7-fold increase in dietary intake (one-unit change on the natural log scale) predicted a 13% increase in urinary nitrate (R^2 = 0.47); whereas nitrate intake from water was associated with a smaller percentage increase but was a significant predictor (p=0.008) and explained a similar (and slightly higher) proportion of the variance in urinary nitrate compared with dietary intake (R^2 = 0.52). Total 24-hour nitrate intake from drinking water and diet combined was the strongest predictor of urinary nitrate concentrations (p=0.04; R^2 = 0.53); a 2.7-fold increase predicted a 19% increase in urinary nitrate concentrations (95% CI: 1.06–1.34). We observed similar parameter estimates when water and dietary intakes were mutually adjusted for each other and when we modeled water and total nitrate based on usual tap water intake.

The results of the temporal variability analysis among the morning, evening, and middle-of-the-night urine samples showed high repeatability (intraclass correlation coefficient [ICC] = 0.83). The adjusted within person temporal variability between the two samples (first morning voids) collected a median of about 7 years apart was higher, and we observed lower stability over this longer time period (ICC =0.39). Results were similar when we included the five additional repeated BEEA samples (ICC=0.36); whereas, limiting to the 13 participants with samples collected within 5 years resulted in a higher ICC (0.53).

4. Discussion

Our study of Iowa farmers with high potential nitrate exposure through their private wells revealed important information about the relative contribution of drinking water and diet to nitrate exposure. Drinking water was a substantial contributor to total nitrate intake when nitrate levels in the tap water were at or above the MCL. We found that total nitrate intake from diet and water combined was a strong predictor of urinary nitrate concentrations in a first morning void. Although diet accounted for the majority of nitrate intake for most participants, nitrate ingestion from water explained a similar percentage of the variance in nitrate excretion.

Study participants had a wide range of nitrate levels in their private drinking water wells ranging from non-detectable (<0.04 mg/L) to 29.5 mg/L NO_3-N, with 32% above the MCL. Our finding of a substantial contribution of drinking water to total nitrate intake when levels were at/near the MCL is consistent with previous studies of populations in agricultural areas (Chilvers et al., 1984), (Levallois et al., 2000), (Møller et al., 1989). Only a few prior studies have evaluated 24-hour dietary and drinking water intakes and other determinants of urinary nitrate excretion. A Canadian study of 187 private well users in rural Quebec

(Levallois et al., 2000) found that 24-hour diet and drinking water nitrate intakes (modeled together) explained a lower percentage of the variability in 24-hr nitrate excretion (R^2=0.29) than we found in our study (R^2=0.55). Further, urinary nitrate concentrations increased with increasing BMI, whereas we did not observe a significant relationship between BMI and nitrate excretion. The reasons for these differences in our study results are not clear. Some difference between our studies include the fact that exposure to high nitrate concentrations in drinking water was somewhat less common in the Canadian study than in our study (25% >10 mg/L NO_3-N versus 32%). Further, the Canadian study included both men and women who were younger and who were more likely to be smokers than our study population of male farmers. A Danish study (Møller et al., 1989) of a rural men and women drinking from community supplies with a range of nitrate concentrations (not detected to >20 mg/L NO_3-N) found similar increases in urinary nitrate excretion for dietary and drinking water and nitrate intakes based on duplicate diet measurements (Møller et al., 1989). Among well-water users in East Anglia, England (Chilvers et al., 1984), nitrate intake from water predicted urinary nitrate excretion independent of the levels of dietary nitrate intake similar to our findings of independent effects of diet and water intakes.

Among 44 male farmers in Nebraska (Mirvish et al., 1992), half of whom drank well water with nitrate concentrations >=10 mg/L NO_3-N, there was a strong association between ingestion of water >=10 mg/L and 24-hour urinary nitrate concentrations (diet nitrate intake was kept low by design). Nitrate concentrations in the well water and urine were also strongly associated with N-nitrosoproline excretion (Mirvish et al., 1992), providing support for the importance of nitrate ingestion from rural drinking water supplies on the endogenous formation of N-nitroso compounds.

Daily mean intake of nitrate from dietary sources is estimated to be about 40–100 mg NO_3 for Americans, although values can vary substantially between different populations (Mensinga et al., 2003). Daily mean intake was estimated to be 52–80 mg NO_3/day in the United States and United Kingdom based on average food consumption patterns from the Global Environmental Measuring Survey studies (IARC, 2010). In a case-control study in Iowa (Ward et al., 2003), usual nitrate intakes were estimated using a food frequency questionnaire (FFQ); median nitrate intake from diet was 84 mg NO_3/day (IQR:59–119) for men. Each of these dietary nitrate intakes is higher than the median of 30 mg NO_3/day among our participants. Some of the differences in nitrate intake between our population and prior studies may be due to the use of a FFQ to estimate intakes (usually over the prior year) compared with our use of a 24-hour recall. Comparisons of nitrate intakes using FFQs and 24-hr recalls are limited. In a subset of the NIH-AARP Diet and Health Study cohort, nitrate intake from two 24-hour recalls conducted from March to September were compared to intakes using the NCI Diet History Questionnaire (FFQ) (Inoue-Choi et al., 2016). There were only slight differences between the two dietary recall strategies among men (FFQ= 68.9 mg/d; 24-HR = 65.0 mg/d; (Inoue-Choi et al., 2016)).

Our single 24-hour intake estimates may have underestimated the usual intake (over the prior 12 months) due to the timing of the study visits. Interviews were conducted in the winter and intake of leafy green vegetables and other vegetables that are major contributors to dietary nitrate, may have been lower than at other times of the year when these vegetables

are more available (IARC, 2010; Inoue-Choi et al., 2016). Further, participants' 24-hour water intake was lower than their reported usual daily water intake, which may be related to reduced water consumption in winter compared to warmer months of the year.

Our estimate of median total nitrate excretion was higher than our estimate of 24-hour nitrate intake. This may be due to the known limitation of the Cockroft-Gault equation, which overestimates lean body mass of obese individuals resulting in higher estimates of creatinine excretion and an overestimation of nitrate excretion (Duraković, 1986). Additionally, although the equation includes age, creatinine excretion may be overestimated in elderly populations because declining muscle mass and renal function with age reduces creatinine clearance (Duraković, 1986). Further, our analysis also assumed that nitrate excretion using a first morning void urine sample represented the excretion rate in the prior 24-hour period.

Our study had several strengths. We evaluated a population with a large range in exposure to nitrate in drinking water due to their use of unregulated private wells in an agriculturally intensive and hydraulically vulnerable region of Iowa (Bradley et al., 2023). We used 24-hour dietary recalls, which are considered a reliable method for determining recent dietary and water intake (Subar et al., 2007) and we measured nitrate concentrations in participants' tap water. Finally, this study had two first morning voids urine samples collected a minimum of two years apart for assessing temporal variability. Nitrate excretion was moderately stable between sample collections (median: 7 years apart), indicating that urinary nitrate concentrations may be reasonably representative of long-term nitrate intake especially for populations for whom drinking water is an important source of exposure. However, studies in different populations and with larger sample sizes would be beneficial to characterize the temporal variability in nitrate excretion.

The major limitations of our study are the small sample size and the fact that participants were not representative of the AHS population or private well users in general. The inclusion criteria (well depths less than 150 feet or predicted nitrate concentrations greater than 5 mg/L (Bradley et al., 2023) resulted in higher nitrate exposure than the estimated 12% of Iowa private well users in the cohort with nitrate concentrations at/above the MCL (Manley et al., 2022). Our study also used first morning void urine samples instead of 24-hour samples, which are considered the gold standard for estimating urinary nitrate excretion (Bartholomew & Hill, 1984). However, we found low intraindividual variability in nitrate concentrations between the morning and evening samples and a high ICC, confirming that first morning void samples are likely representative of at least the prior 12-hour period.

Additionally, we relied on estimates of average nitrate levels in food from the existing literature to estimate nitrate intake, which does not account for variation in concentrations within foods. Nitrate levels in vegetables can vary due to levels of inorganic nitrates used in fertilizers and the nitrogen fertilizer applications rates (Anjana & Iqbal, 2007). Cooking methods also affect nitrate levels. For example, boiling leafy green vegetables in water devoid of nitrate has been found to reduce nitrate levels by up to 50 percent (EFSA, 2008).

5. Conclusion

Our study adds to the limited existing literature about nitrate intake from drinking water and dietary sources among populations drinking from private wells that are vulnerable to nitrate contamination. We confirmed the importance of nitrate from drinking water as a predictor of nitrate excretion and that intake from drinking water makes a substantial contribution to total intake when levels are at/above the MCL. Our findings are important for informing the design and interpretation of studies of nitrate ingestion and human health. Additionally, because nitrate ingestion promotes the formation of carcinogenic NOC compounds when dietary antioxidants are not present in sufficient concentrations, nitrate ingested via drinking water is more of a health concern than that from dietary sources (IARC, 2010). Future research to understand the relative importance of drinking water and dietary intakes to nitrate excretion in larger and more diverse populations would be beneficial, especially in areas with nitrate contamination of drinking water supplies.

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Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

7. Data availability

The data underlying this investigation will be provided upon request as described for the BEEA subcohort on the AHS website: https://aghealth.nih.gov/collaboration/studies.html.

9. References

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Highlights

- Few studies of nitrate exposure evaluated exposure among users of unregulated private wells
- Water and dietary intakes were independent predictors of nitrate excretion
- Water and dietary nitrate intakes explained a similar amount of variation in excretion
- At levels at/above regulatory limit, water was a major contributor to exposure

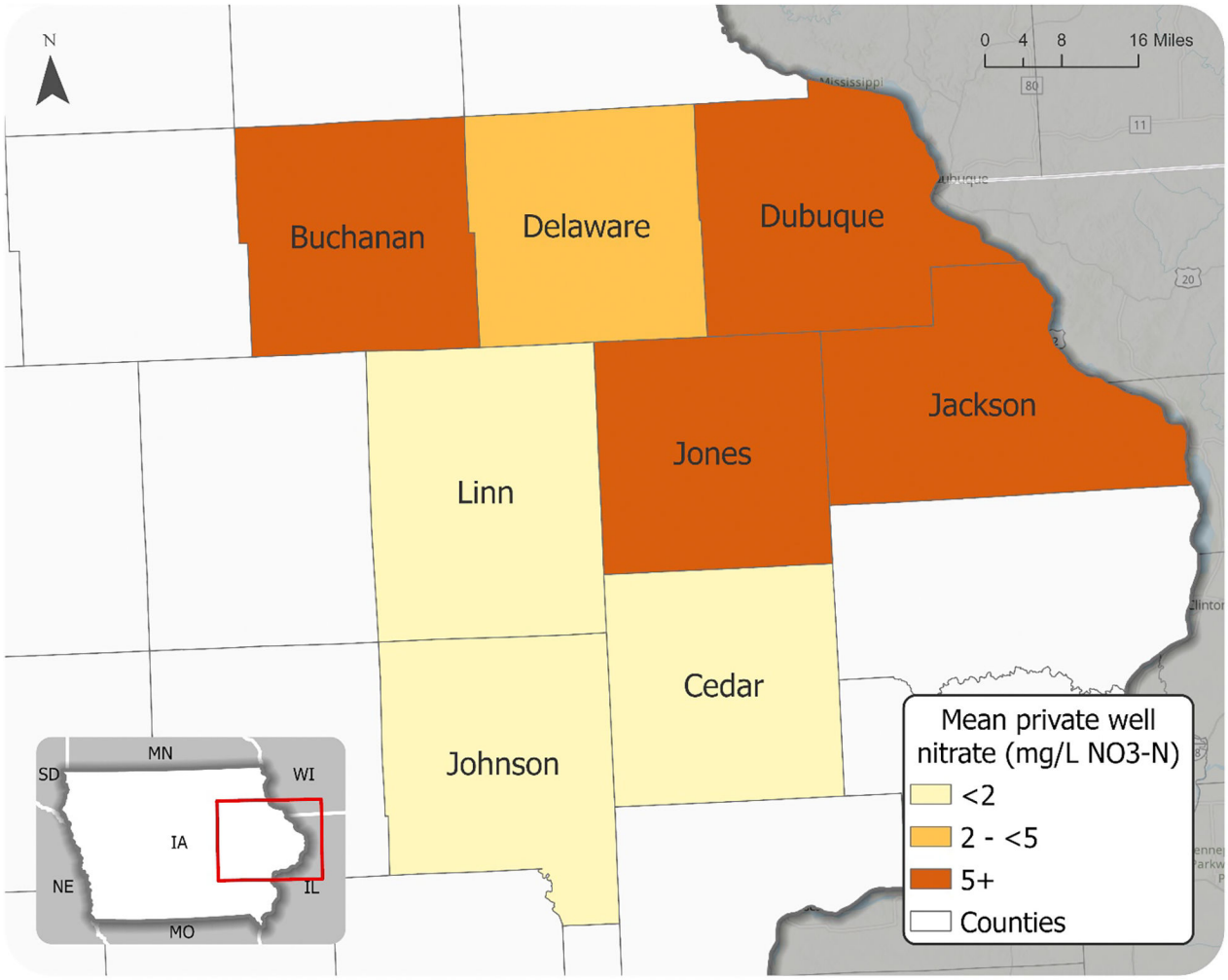

Figure 1:

Mean concentrations of nitrate (mg/L NO_3-N) in tap water from private wells for 47 eastern Iowa well study participants living in 8 Iowa counties

Table 1.

Demographic, well characteristics, and nitrate intakes among farmers using private wells (N=47) in eastern Iowa.

Characteristic	N (%)
Age (years), mean ± standard deviation	64.2 ± 6.3
Body mass index (BMI; kg/m^2)	
<18.5 (underweight)	0 (0)
18.5 to < 25 (healthy weight)	11 (23.4)
25 to <30 (overweight)	20 (42.6)
30+ (obese)	16 (34.0)
Smoking status	
Current	1 (2.1)
Former	8 (17.0)
Never	38 (80.9)
Well water treatment	
Reverse osmosis	4 (8.5)
Charcoal filter only	6 (12.8)
No treatment	37 (78.7)
Well water nitrate concentrations[a] (mg/L NO_3-N)	
<0.04 (detection limit)	15 (31.9)
0.1 to <10	17 (36.2)
>=10 to 29.5	15 (31.9)
Well depth in feet	
< 100	17 (36.2)
100 – 150	12 (25.3)
> 150	18 (38.3)
Crop distance from well	
< 100 yards (300 feet)	36 (76.6)
101–199 yards (301– 599 feet)	9 (19.2)
200–299 yards (600– 899 feet)	2 (4.3)
Water intake (L/day), median (IQR)	
24-hours	1.0 (0.6–1.4)
Usual (last 12-months)	1.2 (1.1–1.7)
Nitrate intake in past 24-hrs (mg NO_3), median (IQR)	
Diet	30.4 (17.9–88.1)
Water	0.9 (0.3–37.5)
Total	63.9 (23.0–157.6)

IQR = interquartile range

[a]Nitrate concentrations in water samples are reported in mg/L NO3-N to compare to the U.S. Environmental Protection Agency regulatory limit.

Table 2.

Distributions of percentage of nitrate (NO_3) intake from diet and drinking water sources for farmers in eastern Iowa (N=47)

	Percentage of total nitrate intake (mg/day NO_3)				
Nitrate source	**Minimum**	**25th percentile**	**Median**	**75th percentile**	**Maximum**
24-hour intakes					
Diet	10.7%	57.4%	97.3%	99.5%	100%
Water	0.01%	0.5%	2.7%	42.6%	89.4%
Usual water intake (last 12 months)					
Diet	9.0%	41.5%	93.2%	98.9%	100%
Water	0.02%	1.1%	6.8%	58.5%	91.0%

Table 3.

Distribution of urinary nitrate concentrations from first morning void samples for farmers using private wells as their drinking water source in eastern Iowa.

		Urinary nitrate (mg/g creatinine)						
	N	**Minimum**	**10th**	**25th**	**Median**	**75th**	**95th**	**Maximum**
All Participants	47	19.2	26.9	36.6	51.9	67.3	128.7	175.8
Well depth (feet):								
< 100	17	27.9	31.3	42.0	58.8	69.1	128.7	128.7
100 – 150	12	26.9	39.8	40.9	49.6	72.3	175.8	175.8
> 150	18	19.2	21.5	28.0	40.1	63.3	132.9	132.9
Tap water concentration (NO_3-N mg/L):								
<0.04	15	19.2	21.5	28.0	38.9	58.8	128.7	128.7
>=0.04 to <10	17	21.6	26.9	41.6	59.3	69.1	118.0	118.0
>=10	15	27.9	39.8	43.6	61.8	79.1	175.8	175.8

Table 4.

Percent increase in urinary nitrate concentrations (mg NO_3/ml) from multivariate models for one natural log (Ln) increase in 24-hour and usual nitrate intakes among farmers in eastern Iowa (N=47).

Nitrate Source Ln(mg NO3)	**Exp(β)[1]**	**95% CI**	**R^2**
24-hour intakes:			
Diet	1.13	0.99–1.28	0.47
Water	1.07	1.02–1.12	0.52
Total Nitrate (24-hr water and diet)	1.19	1.06–1.34	0.53
24-hour intakes (mutually adjusted)			
Diet	1.11	0.98–1.28	0.55
Water	1.06	1.01–1.12	
Usual intake:			
Water (mg/day last 12 mos)	1.08	1.03–1.13	0.54
Total Nitrate (usual water and 24-hr diet)	1.19	1.05–1.36	0.52

[1]Models adjusted for urinary creatinine and age as continuous variables.