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Prenatal exposure to tap water containing nitrate and the risk of small-for-gestational-age: A nationwide register-based study of Danish births, 1991–2015

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Abstract

Background: Prenatal nitrate exposure from household tap water has been associated with increased risk of fetal growth restriction, preterm birth, birth defects, and childhood cancer. We aim to examine the association between maternal consumption of drinking-water nitrate during pregnancy and small-for-gestational-age (SGA) in a nationwide study of Danish-born children, as only one prior study has examined this association.

Methods: We linked individual-level household estimates of nitrate in tap water and birth registry data to all live singleton Danish births during 1991–2015 from Danish-born parents where the mother resided in Denmark throughout the pregnancy. Exposure was both binned into four categories and modeled as an ln-transformed continuous variable. SGA was defined as the bottom 10% of births by birth weight per sex and gestational week. Multiple logistic regression models

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2023.107883.

with generalized estimating equations were used to account for siblings born to the same mother while controlling for relevant confounders.

Results: In the cohort of 1,078,892 births, the median pregnancy nitrate exposure was 1.9 mg/L nitrate. Compared to the reference group (≤2 mg/L), we found an increased risk of SGA in the second category (>2–5 mg/L) (OR = 1.04, 95% CI: 1.03–1.06) and third category (>5–25 mg/L) (OR = 1.02, 95% CI: 1.00–1.04) but not in the highest (>25 mg/L). There was strong (p = 0.002) evidence of an increase in SGA with nitrate in the model with continuous exposure (OR = 1.02, 95% CI: 1.01–1.04 per 10-fold increase in nitrate). Results were robust when restricting to households with nitrate levels at or below the current Danish and European Union regulatory drinking water standard (50 mg/L nitrate).

Conclusions: Our findings suggest that exposure from nitrate in household tap water, even below current regulatory standards, may increase risk of SGA, raising concerns of whether current allowable nitrate levels in drinking water protect children from SGA.

Keywords

Nitrate; Drinking water; Tap water; Small for gestational age (SGA); Severe small for gestational age; Logistic regression

1. Introduction

Nitrate is commonly found in aquifers around the world, e.g. US (Spalding and Exner, 1993), Europe (Sutton et al., 2011), globally (Shukla and Saxena, 2018), mainly due to agricultural leaching of nitrogen (N). Nitrate is extremely water soluble and may leach into groundwater depending on the level of nitrogen leaching from the soil zone and on hydrogeological and geochemical conditions in the subsurface. Nitrate is stable in aquifers with oxic groundwater, which may remain contaminated for decades before discharging to surface waters (Hansen et al., 2017). This is particularly a concern in Denmark, where all drinking water is sourced from groundwater, and many other parts of the world with intensive agriculture and vulnerable aquifers that rely on groundwater as main drinking water source (Burow et al., 2010; Hansen et al., 2017; Nolan et al., 1997). Since the 1980s, nitrate levels in public supplies have been decreasing: in 1980, approximately 70% of the publicly supplied consumers received drinking water with <10 mg/L, whereas by 2012, this number had increased to 94% (Schullehner and Hansen, 2014).

There are several plausible mechanisms by which in utero exposure to nitrate may cause fetal growth restriction. First, nitrate is converted to nitrite in the gastrointestinal tract which oxidates hemoglobin to methemoglobin reducing the oxygen carrying capacity of the blood. Fetuses are particularly vulnerable to the effects of nitrite because they have low levels of MetHb reductase (cytochrome b5 reductase), which converts methemoglobin back to hemoglobin (Gruener et al., 1973; Lukens, 1987). Higher levels of MetHb have been associated with maternal anemia as a pregnancy complication (Tabacova et al., 1997), and anemia has been associated with increased risk of low birthweight (Figueiredo et al., 2018), which is also a marker of fetal growth restriction. Nitrate is also known to interfere

with thyroid function (Aschebrook-Kilfoy et al., 2013). Subclinical hypothyroidism has been associated with fetal growth restriction (Tong et al., 2016).

Current regulatory limits for nitrate in public drinking water supplies (EU: 50 mg/L nitrate; US: 44 mg/L nitrate) were set to protect infants from methemoglobinemia, not from adverse birth outcomes, such as small-for-gestational-age (SGA). Nitrate in drinking water has also been associated with other adverse birth outcomes, including markers of fetal growth restriction (Coffman et al., 2021), birth defects (Blaisdell et al., 2019; Stayner et al., 2022), preterm birth (Coffman et al., 2022; Sherris et al., 2021; Stayner et al., 2017), and some childhood cancers (Stayner et al., 2021) at nitrate concentrations lower than the existing regulatory standard.

SGA is often classified as the lowest 10% of birth weights per sex per gestational week, while severe cases of SGA are often classified as the lowest 5% of births. SGA infants are possibly at an increased risk of ischemic heart disease, hypertension and stroke in adulthood, which may be caused by abnormal vascular development (Balest, 2021). Further complications of SGA may include perinatal asphyxia, meconium aspiration, polycythemia, hypoglycemia, and hypothermia (Balest, 2021).

Despite the importance of SGA and the ubiquity of nitrate contaminated tap water, to the best of our knowledge, only one study has been published on the potential relationship between nitrate in drinking water and SGA. In a cohort study of 11,446 births (642 cases) in western France, Migeot et al. (2013) found an association between second trimester nitrate exposure from drinking water and elevated risk for SGA among children without quantifiable exposure to atrazine [adjusted odds ratio for second tertile of exposure versus the first (tertile limits: 14.13 mg/L and 26.99 mg/L): 1.74; 95% confidence interval (CI): 1.10, 2.75]. There is a clear need for more studies with a large population and well-characterized individual-level estimates of exposures with few co-contaminants and well-defined outcomes.

The aim of this study is to meet this need by utilizing Danish national registry data to examine the association between nitrate in drinking water and the risk of SGA in a large nationwide population.

2. Methods

2.1. Study design and population

We examined SGA by linking data on birth outcomes from the *Danish Medical Birth Registry* (DMBR) (Bliddal et al., 2018; Knudsen and Olsen, 1998) with estimates of household levels of nitrate in drinking water that were developed from the Danish national drinking water monitoring database, *Jupiter* (Hansen and Pjetursson, 2011). We restricted our population to singleton live-born babies born in Denmark during the years of 1991–2015 from Danish-born parents where the mother resided in Denmark for the entirety of the pregnancy. Children with foreign born parents were excluded in order to reduce the potential for confounding by lifestyle factors such as dietary intake of nitrate and nitrite. The main analyses were further restricted to those with information on nitrate from a public

waterworks for each day of the pregnancy and with complete covariate information. We considered births from 1991 because the most reliable estimates of nitrate levels in Danish drinking water are available from 1990 onwards (Schullehner and Hansen, 2014). Data from the different registries in Denmark were linked using an encrypted, unique personal identification number assigned to each resident in Denmark (Pedersen, 2011).

2.2. Exposure assessment

The methodology for the estimation of household levels of nitrate in drinking water has been described and validated in previous publications (Schullehner et al., 2017a, 2017b). In short, extensive data of nitrate concentrations in public water systems are available for the past 35 years from *Jupiter* (Schullehner, 2022). Most of the public water systems in Denmark have reported at least one nitrate measurement per year since the 1990′s (Schullehner et al., 2017b). Prior analyses of waterworks samples have demonstrated that nitrate is relatively stable over a one-year period and that levels at homes are highly correlated with those measured at the waterworks. This is because there is negligible removal or transformation of nitrate after the treatment at the waterworks or in the drinking water distribution system (Schullehner et al., 2017b).

When a water supply area had more than one waterworks supplying the area, average nitrate concentrations were calculated using annual drinking water production volume of each waterworks as weights. For the years without measurements, nitrate averages were imputed using measurements from within a three-year window of a given year (which was deemed sufficient owing to the stability of nitrate within this window) (Schullehner et al., 2017b). Nitrate in household drinking water utilized in analyses was aggregated to yearly averages. Imputation ensures that the smallest waterworks are not excluded from analyses and was only necessary in 84,316 (8%) of the study population (mean of imputed measures: 5.4 mg/L, SD: 9.1, and mean of non-imputed measures: 4.1 mg/L, SD: 7.0) where most measurements used for imputation were sampled from within a 1-year period.

Using a national well water registry, all Danish addresses were classified as to whether they are supplied by a public waterworks (97%) or a private well (3%). Since 2017, private well users have not been required by law to test or submit nitrate data to *Jupiter*, although some users do. Even before 2017, data from private wells were submitted less frequently than those from a public supply and often have higher levels of nitrate compared to public waterworks (Schullehner and Hansen, 2014). In main analyses we excluded those supplied by private wells at any point during the pregnancy, as nitrate concentrations are only available for approximately 53% of the private wells in Denmark (Schullehner and Hansen, 2014) but included them in a sensitivity analysis.

Complete residential address histories for each mother–child dyad have been obtained for the entire Danish population from the *Danish Civil Registration System* (Pedersen, 2011; Pedersen et al., 2006). Reports of nitrate concentrations from drinking water facilities were linked to the maternal address(es) via the Danish personal identification number to determine daily drinking water nitrate concentrations. Pregnancy exposures were calculated as averages weighted by number of days at the residence(s). Trimester specific estimates were similarly computed and were highly correlated with the pregnancy exposures (r =

0.98–0.99) since the nitrate data are yearly averages. Thus, the results using trimester exposures showed no meaningful changes (results not shown).

2.3. Outcome assessment

Birth weight and gestational week were obtained from the DMBR, which contains information from all home and hospital births in Denmark. Only those born with recorded birth weights ≥ 125 g and gestational ages from 20 to 44 weeks were included in analyses to minimize entry errors (Alexander et al., 1996). The 99th percentile of birth weight in the study population is 4800 g. Gestational week was assessed by date of last menses and occasionally corrected by ultrasounds after they were implemented at Danish hospitals in 2004. We only had access to the corrected gestational week data. Birth weight was used to create indicator variables for SGA, defined as the bottom 10% of births per sex per gestational week, and for severe SGA, as the bottom 5%. Since we had access to the entirety of births in Denmark, using an external reference growth chart was deemed unnecessary and might have introduced error.

2.4. Covariates

Data on potential confounders were obtained from the DMBR and the *Integrated Database for Longitudinal Labour Market Research* (Petersson et al., 2011). Based on prior work (Coffman et al., 2021), continuous covariates were modeled as restricted cubic splines with two knots (Brenner and Blettner, 1997). Covariates that were included in the main analyses were: sex of the child, year of birth (spline), birth order (1, 2, ≥ 3), maternal age (spline), maternal smoking during pregnancy (yes, no), maternal educational attainment (less than high school, high school, higher than high school), maternal income normalized for inflation to 2013 levels using the Consumer Price Index (The World Bank, 2019) (spline), and maternal employment status (employed, unemployed, not in the workforce). All socioeconomic status (SES) variables are from two years prior to birth.

Additional confounders explored in sensitivity analyses included season of birth (four levels), urbanicity (five levels), paternal age (spline), paternal SES markers [i.e., education, income (spline), employment status], pre-pregnancy maternal body height (spline) and weight (spline). Maternal pre-pregnancy height and weight were only recorded from 2003 onwards.

2.5. Statistical analyses

Multiple logistic regression was used to assess the association between nitrate in maternal household drinking water and the odds of SGA by estimating odds ratios (OR) with 95% confidence intervals (CI) based on two-sided hypothesis testing. We used complete case analysis with generalized estimating equations and with robust standard errors to account for the non-independence of births from the same mother. A multilevel model that takes geographical clustering into account was performed in a sensitivity analysis.

Nitrate concentrations were modeled as categorical variables or as ln-transformed continuous variables since the ln-transformation improved fit compared to the untransformed, continuous model. Results from the continuous models were scaled to

correspond to 10 mg/L compared to 1 mg/L to ease interpretation. Three cut-points for the categorical analyses were defined a priori by taking into consideration the trade-off between biological meaningfulness of nitrate cut-points and whether they balance the population, given the skewed distribution of nitrate. The referent category was defined as any weighted average ≤2 mg/L nitrate, the mid cut-point was set to 5 mg/L while the highest category included only weighted averages >25 mg/L nitrate, which is the EU action level for nitrate in drinking water and one half of the current European Union (EU) standard. Trend tests were performed by converting the categorical nitrate variable into a continuous variable with the values 1, 2, 3 and 4 thereby modeling the change in log-odds for every increase in nitrate category. Lastly, five different splines models were investigated to find a model that fits the data in order to investigate any possible non-linear relationship between exposure and risk of SGA. A spline model was chosen where nitrate was represented as a cubic spline with three knots. Non-linearity was tested through a likelihood ratio test, and the models were without GEE as likelihood ratio tests are not possible with GEE.

All statistical analyses were conducted using R 4.0.4 (R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria).

2.6. Sensitivity analyses

We conducted several sensitivity analyses. The first of which examined the association between nitrate in drinking water and severe SGA. In the second analysis we restricted to births with estimates of pregnancy nitrate at or below the EU standard of 50 mg/L nitrate to examine if the current standard is sufficient for preventing SGA due to nitrate from household tap water (a reduction of 1,986 births).

In a third set of sensitivity analyses we included variables for maternal pre-pregnancy height and weight; variables that were only available from 2003 onwards. We compared the results from these models to those from our main models on the same population subset with available height and weight variables (a reduction of 605,276 births).

In the fourth sensitivity analysis we examined the impact of including private well water data in our analyses. In this analysis, we included those born to mothers, who at some point during pregnancy, were living in households supplied by a private water source with a known nitrate exposure (an addition of 47,186 births).

The fifth set of sensitivity analyses included potential *a priori* confounders of interest added into the main model individually to assess their influence on the association. These include season of birth, urbanicity, and paternal age, income, education, and employment status.

The sixth sensitivity analysis compared the main model results to restricting the population to firstborn children (a reduction of 601,113 births), as it is well-established that firstborn children are often smaller than children of other birth orders (Kiserud et al., 2018). Firstborn children are thus a risk factor for SGA.

A seventh sensitivity analysis was performed to address the potential effect on the results from geographical clustering as mothers that reside in the same area have similar exposure

to nitrate from drinking water. We used a multilevel model with the same covariates as in the main model and with the regions of Denmark as grouping variable.

In the eighth sensitivity analysis we investigated whether preterm births (gestational age <37 weeks), which are present in the study population, may be driving the results as there is considerable overlap between SGA and preterm birth status, and since previous work found an increasing exposure–response relationship between nitrate in drinking water and moderately preterm birth. In this sensitivity analysis we excluded all children born preterm (a reduction of 51,167 births).

Interactions between urbanicity, maternal income, paternal income, time and ln-transformed pregnancy nitrate were examined, again without GEE as we used likelihood ratio tests. To ease interpretability of the potential interactions, maternal and paternal income were untransformed, continuous variables in the full and reduced models, where they were previously represented as cubic splines.

2.7. Ethical considerations

In keeping with Danish legislation, the Danish Data Protection Agency, the Danish Health Data Authority, and Statistics Denmark approved this registry-based study. In accordance with Danish legislation, informed consent was not necessary. This study has also been approved by the University of Illinois at Chicago's Institutional Review Board.

3. Results

3.1. Main analyses

A total of 1,078,892 births met the inclusion criteria for the main analyses (i.e., were live-born singletons from 1991 to 2015 with a recorded birth weight and gestational age, born to Danish-born parents where the mother resided at a Danish address during pregnancy supplied by a public waterworks with a linkable nitrate value) (Fig. S1). In the cohort, the median nitrate exposure, averaged over the entire pregnancy, was 1.9 mg/L nitrate (interquartile range (IQR): 1.00–3.4 mg/L) (Fig. 1), and 39,919 experienced drinking water with >25 mg/L nitrate (Table 1). Strong associations between the covariates and nitrate categories and between covariates and SGA status were observed ($p < 0.01$), except for child's sex and nitrate categories ($p < 0.06$) based on X^2 tests (Table 1 and S1). SGA infants were more likely to have nitrate exposures >2 mg/L, to be first born, to be born in the earlier years of the cohort, born to young mothers, to mothers who smoked, and to mothers who only had a primary school education (Table S1).

We observed an increase in odds of SGA in all nitrate categories compared to the referent category (≤2 mg/L nitrate), except in the highest category which was comprised of only 3.7% of the study population. The largest effect size was estimated in the second nitrate category (>2–5 mg/L nitrate) with OR = 1.04 (95% CI = 1.03, 1.06). Furthermore, a positive and significant trend was observed across categories ($p = 0.005$) (Table 2). A positive and statistically significant ($p = 0.002$) exposure–response was observed in the continuous model with ln-transformed pregnancy nitrate. Based on this model, the OR for 10 mg/L compared to 1 mg/L nitrate (or equivalently for every 10-fold increase in nitrate) was 1.02 (95%

CI: 1.01–1.04) (Table 2). Investigating the possible non-linear relationship between nitrate exposure and the OR of SGA conveyed no additional information than that based on the categorical model (Fig. 2). The likelihood ratio test showed a better fit for the spline model than for the linear model ($p < 0.001$), confirming the non-linear relationship that is seen in the categorical model. Examples of underfitted, fitted and overfitted spline models can be seen in Fig. S2.

3.2. Sensitivity analyses

Sensitivity analyses showed similar results in magnitude and direction as the main analysis. In examining severe SGA, we observed point estimates in the continuous and categorical model similar to the SGA results with somewhat wider confidence intervals, e.g., OR = 1.02 (95% CI: 1.00–1.04) in the continuous model. We found a borderline significant ($p = 0.07$) and positive, linear trend (Table 2). When the analysis was restricted to those who had average nitrate levels in water at or below the current EU standard, results were robust compared to the main analysis (Table 2).

Though results were weakened when restricting to individuals with available height and weight compared to the main model, the addition of maternal height and pre-pregnancy weight as confounders showed similar estimates compared to the model without maternal height and weight on the same restricted sample population. These results were also slightly different than those in the main model in that there was no linear trend (Table S2). Estimates were robust when adding in the population of those connected to private well water to the main analysis population (Table S3) and with the addition of each variable considered *a priori* as a potential confounder (Table S4). The analyses restricted to firstborn children resulted in effect size estimates of the same magnitude as those from the main model, again with somewhat wider confidence intervals (Table S5). Results did not change in any meaningful way when running a multilevel model that accounted for the potential correlation due to the regions of Denmark (Table S6). Restricting the study population to term births did not attenuate effect size estimates (Table S7). Table S8 shows the results from the interaction tests between ln-transformed nitrate and urbanicity, maternal and paternal income and time. Only the interaction between ln-transformed pregnancy nitrate and urbanicity was significant at level 0.05. On investigating this association, we found an increased risk of SGA in the suburb of the Capital and in provincial towns, though not in the Capital, provincial cities and rural areas (Table 3).

4. Discussion

4.1. Main findings

This is the first population-based cohort study to consider the potential impact from exposure to nitrate in drinking water during pregnancy on the risk of SGA. It is the second study overall to examine this potential association.

Using individual-level data from Denmark, we observed an increased risk for SGA and severe SGA at nitrate exposure levels that were below current EU regulatory limits (EU: 50 mg/L nitrate; US: 44 mg/L nitrate), particularly at low levels (≤25 mg/L nitrate). Overall, the

continuous models and tests for trend indicated an increasing risk with exposure. In models using categorical estimates of nitrate exposure, the risk of SGA was also elevated with the highest risk at the low levels of > 2–5 mg/L nitrate compared to the referent (≤2 mg/L); a pattern that was also seen in the spline model. Though this may seem contradictory given the trend tests and the continuous models, a possible reason for this could be the distribution of the study population into the nitrate categories with far more births in the lowest two categories (≤2 mg/L and > 2–5 mg/L nitrate). The highest nitrate category (>25 mg/L in the main model) contained a sample size of only 39,919 births (cases = 4,078). Live-birth bias (Liew et al., 2015) could also be influencing results, as our study design included live-births only. A recent study using a similar design as ours reported an association between nitrate in drinking water during the first trimester and the risk of pregnancy loss before gestational week 22 in models using continuous exposure but not in models using categorical variables (Ebdrup et al., 2022). This study provides some support for the presence of live-birth bias in our study, although it is also subject to a potential survival bias since it did not include spontaneous abortions.

We observed weakened results in the model that included maternal height and weight as well as in the restricted main model, indicating that the weakened effect was because the sample size was reduced rather than because of the control for height and weight.

A higher frequency of firstborn children was seen among SGA cases than among non-cases in this study (Table S1). The estimated effect sizes among firstborns were similar to those estimated in the full cohort, and firstborn children are therefore not modifying the results from the main model.

4.2. Comparison with other studies

Only one research group has published literature regarding this association (Migeot et al., 2013), and it was complicated by co-exposure to atrazine. These researchers did, however, see an increased odds of SGA during the second trimester in the second tertile of nitrate exposure compared to the first tertile when atrazine metabolites were not detected (OR: 1.74; 95% CI: 1.1–2.8) (tertile limits: 14.13 mg/L and 26.99 mg/L). In their study, average pregnancy nitrate was 22.91 ± 9.48 mg/L among women unexposed to atrazine, which is considerably higher than in our study. Nitrate and atrazine have been detected together in some drinking water supplies in the US and may react to form *N*-nitrosoatrazine, which is more toxic than either of the components individually, potentially inducing malformations during embryonic development (Joshi et al., 2013). Denmark formally banned atrazine use in 1994, but atrazine and its metabolites may still be occasionally detected in public water supplies (Skaarup et al., 2022; Thorling et al., 2021; Voutchkova et al., 2021).

Similar to our results, Migeot et al. (2013) did not observe a monotonic increase in risk of SGA and nitrate exposure in categorical analyses. Like their study, we also observed the highest category of exposure with a weakened effect compared to the second.

The findings of the present study are also consistent with other studies that examine the association between nitrate from drinking water and other markers of fetal growth restriction, such as birth weight, body length (Coffman et al., 2021), very low birth weight

(Stayner et al., 2017), preterm birth (Coffman et al., 2022; Sherris et al., 2021), and intrauterine growth restriction (Bukowski et al., 2001).

4.3. Design considerations

Our study, while larger and more comprehensive than Migeot et al. (2013), was still unable to account for differences in individual's diets, vitamin C supplementation (Super et al., 1981; Ward et al., 2005), nitrosatable drug use (Blender et al., 2004), maternal microbiome (Ward et al., 2018) or other possible co-exposures because these data were not available for our study. While drinking water in Denmark typically complies with guideline levels, we cannot exclude confounding by other contaminants that may co-occur with nitrate. In general, nitrate-containing groundwater mainly occurs in the upper approximately 50 m of Danish aquifers where the groundwater quality is also influenced by other anthropogenic compounds. Nitrite is commonly found in the anoxic nitrate reducing parts of aquifers due to denitrification and incomplete reduction of nitrate. However, at the waterworks, aeration and sand filtration of abstracted groundwater secure that nitrite is nitrified to nitrate to meet drinking water standards before distribution to consumers (Schullehner et al., 2017b).

We were also unable to quantify the amount of water a woman consumed and assumed equivalent consumption for all pregnancies. There is the possibility of exposure misclassification with consumption of bottled water or water from other sources than the home tap. However, with only 19.2 L consumption of pre-packaged bottled water per person per year in Denmark (Union of European Soft Drinks Associations (UNESDA), 2018), the potential for misclassification from this source remains minimal. Although it is considered to be rare, it is also possible that even though a residence lies within the boundary of a public waterworks, the home may be supplied by an unregistered private well. Furthermore, 22% of water supply areas have more than one waterworks supplying the area (Schullehner and Hansen, 2014). We used maps of waterworks, engineering records and weighting to account for these supply areas, but there is the possibility of residual misclassification.

The limitations of SGA as a marker of fetal growth restrictions have been noted by Wilcox et al. (Wilcox, 2001; Wilcox et al., 2021). SGA has substantial overlap with preterm births (<2,500 g at term), which muddles the interpretation of this measure as a marker of fetal growth restrictions. He suggests that the best measure of fetal growth restriction is the mean birth weight among full term births (Wilcox, 2001). We have in another paper examined this outcome based on the same population and found strong evidence of a decrease in birth weight with prenatal exposure to nitrate (Coffman et al., 2021). In this study, we also observed a decrease in body length with exposure to nitrate but not for head circumference. Together, we believe the results from our studies provide strong evidence that nitrate is a risk factor for fetal growth restriction.

4.4. Strengths

A major strength of this study is the detail, quality and size of the individual-level measures of exposures, outcomes, and covariates over 25 years of collection. We were able to use records from >1 million births, making this the largest study examining the relationship between nitrates and SGA to date.

Another strength of this study is the location. Denmark is a relatively economically and culturally homogenous population in which all individuals have free access to high-quality prenatal health care. Therefore, it is far less likely that there will be appreciable confounding by socioeconomic factors as opposed to the US (Schaider et al., 2019) where there are substantial inequalities in income and access to health care. Furthermore, Danes predominantly drink tap water originating from groundwater, which is not chlorinated (Evlampidou et al., 2020). The individual-level exposures have been used previously and are known to be reliable and accurate (Schullehner et al., 2017b).

5. Conclusions

Our study is the largest conducted to date on the association between nitrate levels in drinking water and SGA and used comprehensive individual-level data from residential and birth registries for the entire Danish population. We found an elevated risk of SGA in the second and third categories of exposure compared to the referent group, but estimates were near the null at the highest level of exposure. The decreasing effect estimates observed in the categorical model may be due to live-birth bias.

The large sample size of our study made it possible to detect relatively small increases in risk of SGA in our study. The risk for 10 mg/L nitrate in drinking water relative to 1 mg/L was predicted to increase the risk of SGA by only 2% (OR = 1.02, 95% CI = 1.01, 1.04) based on our continuous model. By comparison, being a cigarette smoker was found to increase the risk of SGA by 135% (OR = 2.35, 95% CI = 2.32, 2.39) in our model. Nonetheless a 2% increase in risk of such a serious outcome at levels below current regulatory standards is still of public health concern.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data availability

The authors do not have permission to share data.

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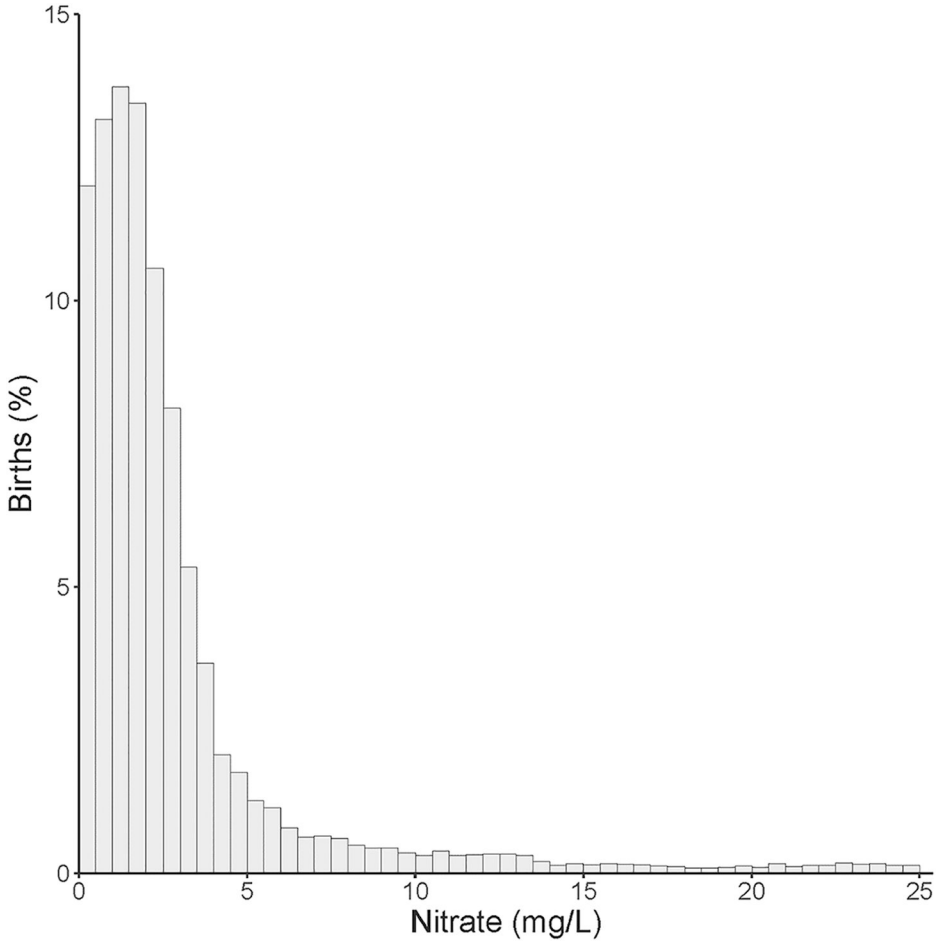

Fig. 1.

The distribution of pregnancy average nitrate shown for those in the main analysis population with exposures ≤ 25 mg/L, corresponding to 1,038,973 individuals.

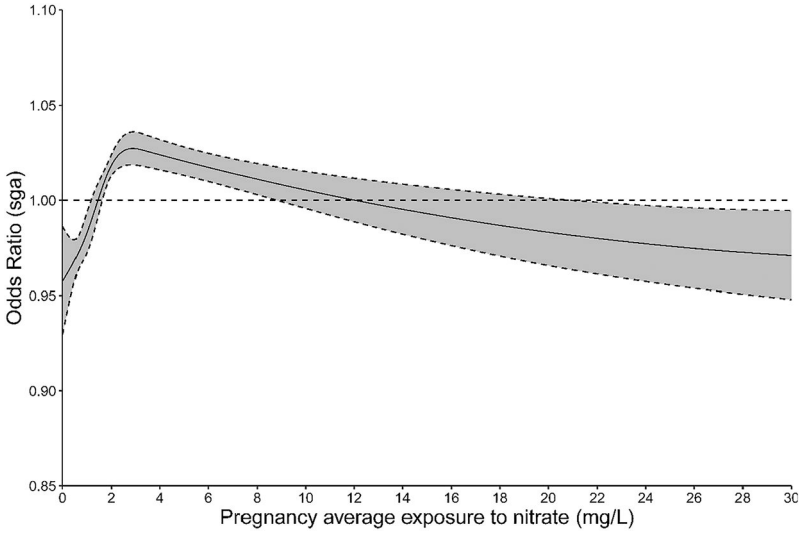

Fig. 2.

The spline model with exposure represented as a cubic spline with three knots performed using logistic regression with glm and the same covariates as in the main model (sex, calendar year, birth order, and maternal age, smoking, education, income, and employment status).

Table 1

Characteristics of the study population by nitrate in home drinking water during pregnancy.

	Estimated pregnancy nitrate (mg/L) in household drinking water			
Characteristic	**≤ 2**	**> 2–5**	**> 5–25**	**> 25**
Total[a], *n (%)*	564,623 (52)	340,101 (32)	134,249 (12)	39,919 (4)
Small-for-gestational-age[b], *n* (%)				
No	509,233 (53)	303,609 (31)	119,770 (12)	35,841 (4)
Yes	55,390 (50)	36,492 (33)	14,479 (13)	4,078 (4)
Sex, *n* (%)				
Female	275,231 (52)	165,094 (32)	65,236 (12)	19,229 (4)
Male	289,392 (52)	175,007 (32)	69,013 (13)	20,690 (4)
Birth order, *n* (%)				
1	245,996 (52)	158,649 (33)	55,812 (12)	17,322 (4)
2	219,235 (53)	129,844 (31)	52,790 (13)	15,157 (4)
≥ 3	99,392 (54)	51,608 (28)	25,647 (14)	7,440 (4)
Urbanicity, *n* (%)				
Capital	59,154 (39)	89,236 (58)	4,797 (3)	19 (0)
Suburb of capital	55,160 (41)	61,804 (46)	18,387 (14)	176 (0)
Provincial city[c]	92,112 (67)	11,850 (9)	15,758 (12)	16,930 (12)
Provincial town[d]	160,091 (52)	97,897 (32)	44,104 (14)	6,872 (2)
Rural areas[e]	198,106 (57)	79,314 (23)	51,203 (15)	15,922 (5)
Region *n* (%)				
North Jutland	24,986 (22)	19,422 (17)	38,160 (34)	29,323 (26)
Middle Jutland	204,736 (79)	23,476 (9)	26,334 (10)	5,628 (2)
Southern Jutland	158,363 (69)	42,990 (19)	24,862 (11)	2,620 (1)
Capital Area	129,366 (39)	166,965 (51)	31,153 (10)	1,070 (0)
Zealand	47,172 (32)	87,248 (58)	13,740 (9)	1,278 (1)
Year of birth, *n* (%)				
1991–1995	104,350 (46)	71,840 (31)	42,624 (19)	10,465 (5)
1996–2000	119,293 (53)	66,929 (30)	29,799 (13)	7,807 (3)

Characteristic	Estimated pregnancy nitrate (mg/L) in household drinking water			
	≤ 2	> 2–5	> 5–25	> 25
2001–2005	116,462 (53)	70,050 (32)	25,831 (12)	8,691 (4)
2006–2010	118,386 (55)	69,749 (32)	20,640 (10)	7,240 (3)
2011–2015	106,132 (56)	61,533 (33)	15,355 (8)	5,716 (3)
Season of birth, *n* (%)				
January–March	135,464 (52)	82,386 (32)	33,357 (13)	9,857 (4)
April–June	142,620 (52)	85,791 (31)	34,498 (13)	10,022 (4)
July–September	151,651 (52)	91,839 (32)	35,557 (12)	10,556 (4)
October–December	134,888 (53)	80,085 (31)	30,837 (12)	9,484 (4)
Maternal age (years), *n* (%)				
< 25	70,213 (48)	46,951 (32)	22,118 (15)	5,944 (4)
25–29	205,876 (52)	119,205 (30)	51,649 (13)	16,155 (4)
30–34	200,376 (54)	117,650 (31)	42,821 (11)	12,798 (3)
≥ 35	88,158 (53)	56,295 (34)	17,661 (11)	5,022 (3)
Maternal smoking, *n* (%)				
No	454,577 (53)	266,443 (31)	99,394 (12)	30,883 (4)
Yes	110,046 (48)	73,658 (32)	34,855 (15)	9,036 (4)
Maternal height[f] (cm, quartiles), *n* (%)				
< 165	64,571 (55)	37,770 (32)	11,414 (10)	4,059 (3)
165–169	65,687 (56)	37,227 (32)	11,069 (9)	4,083 (4)
169–173	63,379 (56)	36,108 (32)	10,273 (9)	3,724 (3)
> 173	69,897 (56)	39,356 (32)	10,879 (9)	4,120 (3)
Missing	301,089 (50)	189,640 (31)	90,614 (15)	23,933 (4)
Maternal weigh[f] (kg, quartiles), *n* (%)				
< 59	58,520 (55)	35,826 (34)	8,669 (8)	3,408 (3)
59–66	68,991 (55)	40,838 (33)	10,539 (8)	3,949 (3)
66–76	68,219 (56)	38,594 (32)	11,305 (9)	4,389 (4)
> 76	67,804 (56)	35,203 (29)	13,122 (11)	4,240 (4)
Missing	301,089 (50)	189,640 (31)	90,614 (15)	23,933 (4)
Maternal education[g], *n* (%)				

Characteristic	Estimated pregnancy nitrate (mg/L) in household drinking water			
	≤ 2	> 2–5	> 5–25	> 25
Primary school	119,474 (49)	78,362 (32)	37,462 (15)	10,131 (4)
High school	264,620 (53)	152,280 (30)	65,561 (13)	19,250 (4)
Higher education	180,529 (54)	109,459 (33)	31,226 (9)	10,538 (3)
Maternal employment status[g], *n* (%)				
Employed	461,178 (53)	276,553 (32)	106,675 (12)	31,514 (4)
Unemployed	30,940 (48)	20,124 (31)	10,408 (16)	3,103 (5)
Not seeking work	72,505 (52)	43,424 (31)	17,166 (12)	5,302 (4)
Maternal income[h] (DKK, quartiles), *n* (%)				
< 177,800	139,891 (52)	85,337 (32)	34,134 (13)	10,361 (4)
177,800–248,000	141,791 (53)	76,999 (29)	39,086 (14)	11,847 (4)
248,000–313,800	142,824 (53)	80,920 (30)	35,599 (13)	10,380 (4)
> 313,800	140,117 (52)	96,845 (36)	25,430 (9)	7,331 (3)
Paternal age (years), *n* (%)				
< 25	34,465 (47)	24,296 (33)	11,054 (15)	2,830 (4)
25–29	152,569 (52)	90,280 (31)	39,872 (14)	12,225 (4)
30–34	211,786 (53)	122,686 (31)	47,923 (12)	14,629 (4)
≥ 35	165,803 (53)	102,839 (33)	35,400 (11)	10,235 (3)
Paternal education[g], *n* (%)				
Primary school	114,621 (49)	73,988 (32)	34,593 (15)	9,375 (4)
High school	290,995 (53)	167,314 (30)	73,263 (13)	21,469 (4)
Higher education	153,455 (54)	94,530 (34)	24,854 (9)	8,736 (3)
Missing	5,552 (47)	4,269 (36)	1,539 (13)	339 (3)
Paternal employment status[g], *n* (%)				
Employed	509,550 (53)	302,376 (31)	120,175 (12)	35,572 (4)
Unemployed	19,667 (46)	14,366 (34)	6,528 (15)	1,843 (4)
Not seeking work	33,736 (52)	21,983 (34)	7,212 (11)	2,444 (4)
Missing	1,670 (49)	1,376 (40)	334 (10)	60 (2)
Paternal income[h] (DKK, quartiles), *n* (%)				
< 259,600	137,138 (51)	88,491 (33)	33,470 (12)	10,481 (4)

Characteristic	Estimated pregnancy nitrate (mg/L) in household drinking water			
	≤ 2	> 2–5	> 5–25	> 25
259,600–339,300	141,073 (52)	78,614 (29)	38,373 (14)	11,520 (4)
339,300–428,700	143,388 (53)	81,661 (30)	34,479 (13)	10,051 (4)
> 428,700	142,731 (53)	91,108 (34)	27,883 (10)	7,860 (3)
Missing	293 (51)	227 (40)	44 (8)	7 (1)

Note: All X^2 tests for independence between categorical nitrate and above covariates were rejected at $p < 0.001$, except for sex ($p = 0.06$).

[a.] The study population: singleton live births in Denmark from January 1, 1991 to December 31, 2015 to Danish-born parents with a nitrate estimate from a public waterworks supplying drinking water to a Danish maternal address for each day of the pregnancy, with a birth weight measurement, a gestational age and with nonmissing covariates in the main model (main model covariates: sex, calendar year, birth order, and maternal age, smoking, education, income, and employment status).

[b.] When the birth weight is less than or equal to the lowest decile, calculated within same sex and gestational age infants.

[c.] Municipalities having a town with >100,000 inhabitants.

[d.] Municipalities having a town with 10,000–100,000 inhabitants.

[e.] Municipalities in Denmark where the largest town has <10,000 inhabitants.

[f.] Available from 2003 onward only. Quartiles are calculated among those with a recorded maternal height and weight.

[g.] As reported two years before birth.

[h.] As reported two years prior to birth, standardized to 2013 values and rounded off to the nearest hundred.

Table 2

Odds ratios for severe small-for-gestational-age within the main analysis population and for small-for-gestational-age among those with nitrate measurements at or below the current EU standard (50 mg/L nitrate) compared to the unrestricted main model results.

			SGA (main results)			Severe SGA		≤ 50 mg/L nitrate			
Nitrate (mg/L)	**Total (*n*)**	**Cases (*n*)**	**OR (95% CI)**	***p* value**	**Cases (*n*)**	**OR (95% CI)**	***p* value**	**Total (*n*)**	**Cases (*n*)**	**OR (95% CI)**	***p* value**
≤ 2	564,623	55,390	Ref (1.00)		27,819	Ref (1.00)		564,623	55,390	Ref (1.00)	
> 2–5	340,101	36,492	1.04 (1.03, 1.06)	< 0.001	18,590	1.05 (1.03, 1.07)	< 0.001	340,101	36,492	1.04 (1.03, 1.06)	< 0.001
> 5–25	134,249	14,479	1.02 (1.00, 1.04)	0.04	7,419	1.02 (0.99, 1.05)	0.23	134,249	14,479	1.02 (1.00, 1.04)	0.04
> 25	39,919	4,078	1.00 (0.96, 1.03)	0.92	2,061	0.99 (0.94, 1.04)	0.71	37,933	3,837	0.99 (0.96, 1.03)	0.78
Trend	1,078,892	110,439	1.01 (1.00, 1.02)	0.005	55,889	1.01 (1.00, 1.02)	0.07	1,076,906	110,198	1.01 (1.00, 1.02)	0.01
Continuous[a] (10 mg/L vs. 1 mg/L)	1,078,892	110,439	1.02 (1.01, 1.04)	0.002	55,889	1.02 (1.00, 1.04)	0.01	1,076,906	110,198	1.02 (1.01, 1.04)	0.002

Abbreviations: SGA = small for gestational age; OR = odds ratio; CI = confidence interval; Ref = referent group.

Models were fitted using logistic regression with generalized estimating equations to control for the non-independence of births from the same mother and were controlled for sex, calendar year, birth order, and maternal age, smoking, education, income, and employment status.

[a]. All continuous nitrate exposures were ln-transformed, ln(x) for × = nitrate, and OR (95% CI) estimates are shown for drinking water containing 10 mg/L nitrate compared to 1 mg/L nitrate.

Table 3

The association between nitrate exposure and small-for-gestational-age by level of urbanicity.

Urbanicity	Total (*n*)	OR (95% CI)	*p*-value[d]
			< 0.001
Capital	153,206	1.04 (0.94, 1.16)	
Suburb of Capital	135,527	1.08 (1.01, 1.14)	
Provincial city[a]	136,650	0.99 (0.96, 1.02)	
Provincial town[b]	308,964	1.07 (1.05, 1.10)	
Rural areas[c]	344,545	1.01 (0.99, 1.03)	

Abbreviations: OR = odds ratio; CI = confidence interval; GLM = generalized linear models.

Note: The models were fitted using logistic regression with GLM and did thus not account for non-independence of births from the same mother. The reduced model was controlled for sex, calendar year, birth order, and maternal age, smoking, education, income, and employment and urbanicity, and the full model adjusted for an additional interaction term between ln-transformed nitrate and urbanicity.

Exposure was ln-transformed, ln(x) for x = nitrate, and OR (95% CI) estimates are shown for drinking water containing 10 mg/L compared to 1 mg/L.

[a.] Municipalities having a town with >100,000 inhabitants.

[b.] Municipalities having a town with 10,000–100,000 inhabitants.

[c.] Municipalities in Denmark where the largest town has <10,000 inhabitants.

[d.] The p-value is from the likelihood ratio test comparing the models with and without the interaction term.