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Association of maternal urinary fluoride concentrations during pregnancy with size at birth and the potential mediation effect by maternal thyroid hormones: The Swedish NICE birth cohort

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ABSTRACT

Background: Observational studies have indicated that elevated maternal fluoride exposure during pregnancy may impair child neurodevelopment but a potential impact on birth outcomes is understudied.
Objectives: To evaluate the impact of gestational fluoride exposure on birth outcomes (birth size and gestational age at birth) and to assess the potential mediating role of maternal thyroid hormones.
Methods: We studied 583 mother-child dyads in the NICE cohort in northern Sweden. Maternal fluoride exposure was assessed by measuring urinary concentrations at late pregnancy (median: 29th gestational week) using an ion selective electrode. Plasma levels of free and total thyroxine (fT4, tT4) and triiodothyronine (fT3, tT3), and thyroid stimulating hormone (TSH) were measured with electrochemiluminescence immunoassays. The infant's weight, length, head circumference, and gestational age at birth were extracted from hospital records.
Results: Median urinary fluoride concentration was 0.71 mg/L (5th-95th percentile 0.31–1.9 mg/L; specific gravity adjusted). In multivariable-adjusted regression models, every 1 mg/L increase of maternal urinary fluoride was associated with a mean increase in birth weight by 84 g (95%CI: 30, 138), length by 0.41 cm (95% CI: 0.18, 0.65), head circumference by 0.3 cm (95%CI: 0.1, 0.4), and with increased odds of being born large for gestational age (OR = 1.39, 95%CI: 1.03, 1.89). Every 1 mg/L increase of maternal urinary fluoride was also associated with a mean increase of the plasma fT3:fT4 ratio (B = 0.007, 95%CI: 0.000, 0.014), but not with the hormones or TSH. In mediation analyses, the maternal fT3:fT4 ratio did not explain the urinary fluoride-birth size relationships.
Discussion: Gestational urinary fluoride concentrations were associated with increased size at birth and even with increased odds of being born large for gestational age. The fluoride-related associations with increased size at birth were not explained by changes in maternal thyroid hormone levels.

1. Introduction

Fluoride has clear benefits for dental health, but is otherwise not known to be required for any body function (Featherstone, 1999). Exposure to fluoride occurs via fluoride-enriched dental care products, via naturally high levels of fluoride in the drinking water in some geographical areas, or, in other regions, via fluoride supplementation of drinking water, salt, or milk (O Mullane et al., 2016). The content of

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fluoride in food is usually low, except for tea and marine fish, and foods prepared with fluoride-rich water (European Food Safety Authority, 2013). It has been estimated that 80% or more of ingested fluoride is absorbed passively in the gastrointestinal tract, half of which is fairly rapidly excreted via the urine, while half is retained, mainly in bones and teeth (Buzalaf and Whitford, 2011). High-level fluoride exposure may alter the structure of the mineralized tissues causing dental fluorosis, a condition affecting tooth enamel, or even skeletal fluorosis, a metabolic bone disease (Chachra et al., 2008). The World Health Organization recommends a maximum fluoride concentration in drinking water of 1.5 mg/L to prevent fluorosis (World Health Organization, 2011).

Fluoride crosses the placenta (Malhotra et al., 1993; Monterrat-Carret et al., 1996; Sastry et al., 2010), and exposure to elevated fluoride concentrations during pregnancy has recently been associated with lower child intelligence in Canada (Green et al., 2019) and Mexico (Bashash et al., 2017) and an increased risk of attention-deficit-hyperactivity-disorder symptoms in Mexico (Bashash et al., 2018). However, studies regarding the effects of prenatal fluoride exposure on infant size or gestational age at birth are limited and inconclusive (Aghaei et al., 2015; Goodman et al., 2021; Mohanty et al., 2011). To our knowledge, there is only one prospective study on the impact of fluoride exposure on size at birth, conducted in the MIREC cohort in Canada, which found no significant association (Goodman et al., 2021). The underlying mechanism of early-life fluoride toxicity is unclear. The U.S. National Research Council has suggested that fluoride may affect the endocrine system, and in particular, the function of the thyroid gland (Carton and Park, 2006). Exposure to fluoride appears to suppress thyroid hormones (Carton and Park, 2006), and observational studies in school-aged children and adults have found associations between elevated fluoride exposure via drinking water and increased odds of being hypothyroid (Kheradpisheh et al., 2018; Peckham et al., 2015; Singh et al., 2014) or increased levels of thyroid stimulating hormone (TSH) among iodine-deficient individuals (Malin et al., 2018). Thyroid hormones are strong determinants of fetal growth and development (Forhead and Fowden, 2014), however, the impact of fluoride exposure on thyroid hormone levels in pregnant women has so far not been evaluated.

In the present study, we aimed to evaluate the impact of maternal fluoride exposure, assessed through urinary concentrations, on infant size (weight, length, head circumference) and gestational age at birth in a birth cohort in northern Sweden, where natural fluoride concentrations in the drinking water vary greatly due to geological properties (Aggeborn and Öhman, 2021; Aneblom et al., 2005). We, also, aimed to assess if the fluoride exposure was associated with thyroid hormone levels in pregnancy and if so, whether the latter mediates any identified association between maternal fluoride exposure and the birth outcomes. A secondary aim was to identify dietary sources, other than drinking water, of fluoride exposure in this population.

2. Methods

2.1. Study cohort

The birth cohort NICE (Nutritional impact on the Immunological maturation during Childhood in relation to the Environment) consists of families recruited in 2015–2018, in the southern and eastern parts of Region Norrbotten, in northern Sweden (Barman et al., 2018). Information concerning the study was provided to the families at their first prenatal visit to the local maternity clinic in gestational week 10–12. Those accepting to participate signed informed consent at the routine ultrasound visit (gestational week 18–19). The inclusion criteria for participation in the NICE cohort included residing in Norrbotten region, planning to give birth at the Sunderby Hospital, and being able to communicate in written and spoken Swedish.

In total, 655 pregnancies were enrolled in the study (Fig. 1). After excluding families already participating with a previous birth, participant consent withdrawal, twin pregnancies, miscarriages, and stillbirths, 629 mother-infant pairs remained, from whom, urine volume to measure fluoride concentrations was available from 591 pregnant women (participants with available exposure data). We had available data on birth weight for 581 infants, on birth length for 548 infants, on

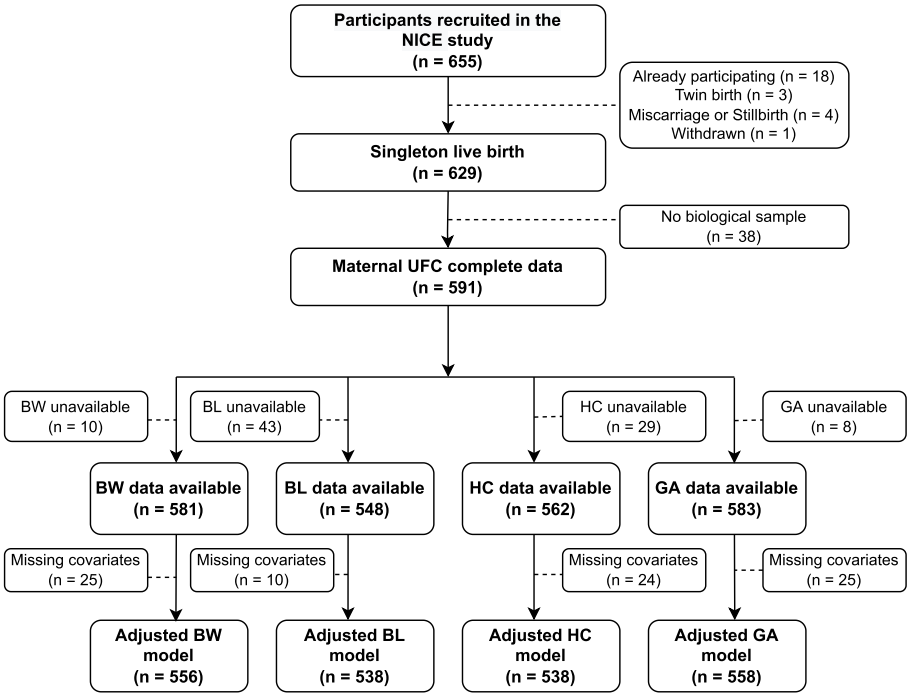

Fig. 1. Flowchart of the mother-child pairs included in the present study Abbreviations: NICE: Nutritional Impact on the Immunological Maturation during Childhood in relation to the Environment; UFC: urinary fluoride concentration; GA: gestational age; BW: birth weight; BL: birth length; BHC: birth head circumference.

head circumference at birth for 562 infants, and on gestational age at birth for 583 infants. Cases which lacked covariate data were excluded (season of sampling: 0.5%, maternal education: 1%, maternal smoking: 0.3%, maternal BMI: 2.5%, gestational age at birth: 0.2%). This left 556 mother-child dyads in the analyses of birth weight, 538 in the analyses of birth length and head circumference, and 558 in the analyses of gestational age at birth (corresponding to 91%–94% of the participants with available exposure data).

This study was conducted in accordance with the ethical standards of the Helsinki declaration, and it has been approved by the Regional Ethical Review Board in Umeå, Sweden. Written and verbal information had been given to the participating parents before they provided written consent.

2.2. Sample collection

Blood and spot urine samples were collected at a visit to the local maternity health clinics in gestational week 29 on average (SD: 1.5; range: 24–36) (Barman et al., 2018; GustinK Barman et al., 2021). Maternal urine samples were also collected at 4 months postpartum (n = 486). Blood samples were collected in 10-mL EDTA tubes (Becton Dickinson, Plymouth, UK). Urine samples were collected in routine collection cups and transferred to 24-mL polyethylene bottles. The samples were stored at 4 °C until transported to the laboratory at the Sunderby hospital on the same or following workday. Blood samples were centrifuged for 5 min at 2400 rpm (Hettich Rotina 420, Hettich Lab Technology, Tuttlingen, Germany), after which the plasma fraction was aliquoted in polypropylene tubes (Micronic, Nordic Biolabs AB, Sweden) and stored at −80 °C.

2.3. Exposure assessment

Fluoride exposure was based on urine concentration, a recognized marker of ongoing exposure (Till et al., 2018). Although the excretion of ingested fluoride in urine is rapid (half-life in the circulation about 5 h), the frequent intake of drinking water, water-based beverages, and food prepared with water leads to fairly constant exposure, and thus, relatively constant urinary concentrations (Ekstrand and Ehrnebo, 1983; Idowu et al., 2019; Whitford, 1994). Urinary fluoride concentrations were measured at Karolinska Institutet, Stockholm, using a fluoride specific electrode (Orion 9609BNWP and Orion Star A214 pH/ISE meter; Thermo Fisher Scientific, Waltham, MA USA) according to the user manual. Aliquots of 0.5 mL urine were diluted 1:1 with a total ionic strength adjustment buffer (Tisab II, Thermo Fisher Scientific) and then vortexed. The external calibration curve ranged between 0.07 and 11 mg/L, and was prepared daily through serial dilution from a 100 mg/L fluoride standard solution (Thermo Fisher Scientific) in deionized water with adjustment buffer (50% Tisab II). Urinary fluoride concentrations were determined by comparing the mV readings with the mV of the standard curve. The limit of detection was 0.05 mg/L [mean blank value (n = 14) plus two times the standard deviation] (Thomas et al., 2016). Only two urine samples had fluoride concentrations below the detection limit and their concentrations were kept as measured (Helsel, 1990). To monitor stability across each run, a standard solution (0.90–2.2 mg/L) was measured every second hour, showing an average drift of 3 ± 2%. The accuracy was monitored by measuring two reference materials, fluoride in water (Product number: QC3162; Sigma-Aldrich, USA; certified fluoride value: 0.420 mg/L) and Seronorm Trace Elements Urine L-2 (LOT: 1011645; Sero AS, Norway; recommended analytical fluoride value: 4 mg/L), before the first and after the last urine sample in each run. The obtained mean fluoride concentration was 0.48 ± 0.04 mg/L (n = 28, recovery 114%) and 3.8 ± 0.2 mg/L (n = 28, recovery 94%), respectively. In addition, an in-house control (a urine sample) was also measured before and after each run (n = 28 runs; mean ± SD fluoride concentration 0.70 ± 0.04 mg/L), showing an inter-day coefficient of variation (CV%) of 6%.

To compensate for the variation in urine dilution, the fluoride concentration in each urine sample was adjusted to the mean specific gravity (mean = 1.017), measured using a digital refractometer (EUROMEX RD712, Clinical Refractometer, Holland). The following formula was applied: adjusted urinary fluoride concentration = unadjusted urinary fluoride concentration × (specific gravity$_{mean}$ − 1)/(specific gravity$_{sample}$ − 1), as reported previously (Nermell et al., 2008).

2.4. Dietary intake assessment

A semi-quantitative Food Frequency Questionnaire (FFQ; “Meal-Q″) was e-mailed to the pregnant women around gestational week 34 to collect information on their diet (Christensen et al., 2013, 2014; Nermell et al., 2008; Stråvik et al., 2019). The FFQ included 102–174 questions on the intake frequency (times per day or week) and the quantity of the consumed food items during the previous month. The exact number of questions in Meal-Q depends on the responses to specific questions and the follow-up questions for different items. Information about portion size was presented in pictures with portions of carbohydrates (pasta, rice, and potato), protein (meat, fish, and vegetarian), and vegetables. The intake, in grams per day, was estimated based on both the frequency of the intake and the reported portion size. Beverage consumption was recorded as the frequency of drinking a predefined volume of each beverage. Intake of the specific food items (e.g., game meat) was grouped to create the “total intake” of the food groups (e.g., total meat intake).

2.5. Birth outcomes’ assessment

Information regarding birth weight (g), birth length (cm), head circumference at birth (cm), and gestational age at birth (days; for IVF fetuses gestational age was determined based on the second trimester ultrasound) was obtained from the hospital records at the Sunderby hospital. The ponderal index was calculated as weight (grams) * 100/length (cm)^3. Small for gestational age and large for gestational age at birth were classified based on two different cut-off points and the mean birth weight derived from ultrasound-based growth curves (Maršál et al., 1996). The cut-off points to classify infants in the small-for-gestational-age category were weight below 2 SDs or weight below the 10th percentile for the gestational age according to sex-specific growth curves derived from ultrasound measurements in a Scandinavian population (Maršál et al., 1996). The respective cut-off points for large-for-gestational-age classification were weights above 2 SDs from the mean birth weight and weights above the 90th percentile for corresponding gestational age (Maršál et al., 1996). Preterm delivery was defined as a birth delivered prior to gestational week 37.

2.6. Assessment of thyroid hormones and TSH

Maternal thyroid hormones were previously measured and the data for the cohort have been reported in detail elsewhere (GustinK Barman et al., 2021). In brief, the plasma concentrations of thyroid-stimulating hormone (TSH) were analyzed at an accredited laboratory (Department of Clinical Chemistry at the University Hospital of Malmö, Sweden) using a single-step sandwich method with electrochemiluminescence immunoassays (ECLIA; Roche Cobas, Roche Diagnostics, Solna, Sweden), while free and total thyroxine (fT4, tT4) and free and total triiodothyronine (fT3, tT3) were analyzed via automated ECLIA (Roche Cobas, Roche Diagnostics, Solna, Sweden), consisting of a two-step immunometric-competitive technique followed by chemiluminescent emission measurement. The ratios of fT4 to tT4, fT3 to tT3, and fT3 to fT4 were calculated. As population and trimester-specific reference ranges for maternal hormone concentrations are lacking, we used an upper TSH reference-limit of 4.0 mIU/L as an indication of potential hypothyroidism (Alexander et al., 2017). Seven per cent of the participating mothers had a diagnosis related to thyroid

dysfunction (n = 41). Mothers with a thyroid dysfunction diagnosis were excluded from all the analyses regarding associations with thyroid hormones.

2.7. Covariate data collection

Information on maternal age (years), parity (number of previous deliveries), education (elementary school, high school, university), pre-pregnancy smoking (never, sometimes, daily), pre-pregnancy alcohol consumption (never, sometimes, or daily), previously diagnosed maternal thyroid dysfunction (yes/no), and infant sex (male/female) was obtained from the hospital records. Data on maternal weight and length were obtained at the maternity clinic in the first trimester and maternal body mass index (BMI) was calculated as weight (kg) divided by squared height (m^2). Pre-pregnancy maternal smoking was used (ever, never) instead of smoking in pregnancy, since the latter was available only for a sub-set of the women (n = 447). Alcohol consumption during pregnancy was obtained from the hospital records and no pregnant woman consumed any alcohol during pregnancy in accordance with recommendations. Information on the source of drinking water at residency (municipality/private well) was obtained through a questionnaire concerning environmental factors (Barman et al., 2018). Season of sampling was defined as spring (March, April, May); summer (June, July, August); fall (September, October, November), and winter (December, January, February). As iodine is essential for thyroid hormone production (Werner et al., 2005), we also measured the maternal urinary iodine concentration (UIC), a valid biomarker of recent iodine intake on a population level (World Health Organization, 2013), in the same spot urine sample as fluoride. The measurements of UIC have been described in detail elsewhere (GustinK Barman et al., 2021). The UICs were adjusted for specific gravity to compensate for variation in urine dilution. We used the population cut-off UIC values suggested by the World Health Organization for classification of iodine intake during pregnancy to divide the participants into two categories (UIC <150 μg/L and UIC ≥150 μg/L) (World Health Organization, 2013).

2.8. Statistical analyses

Statistical analyses were performed using Stata/IC 13.0 (StataCorp, TX, USA) and R© version 3.6.2 (R Foundation for Statistical Computing, Vienna, Austria). A *P-value* of less than 0.05 was considered as statistically significant but robustness and consistency of the results were also considered in the interpretation of the obtained results.

Correlations between maternal urinary fluoride concentrations and maternal dietary data (g/day of each food item or food group) were explored using unsupervised hierarchical cluster analysis and visualized in a heat map using R version 3.6.2. In the heat map, the inserted dietary variables were automatically clustered based on the correlations between them and were placed accordingly (correlated variables placed close to each other in connected clusters). The direction of the correlations between urinary fluoride concentrations and dietary data is coded by color (red indicates positive correlations and blue indicates negative correlations) and the magnitude is visualized by color shade (darker shades indicate higher magnitude). Correlations between maternal urinary fluoride concentrations and dietary data were also stratified by the season of sampling.

Associations between maternal urinary fluoride concentrations, birth outcomes (weight, length, head circumference, and gestational age), and potential covariates were initially explored using Spearman rank test, Mann-Whitney *U* test, or Kruskal-Wallis-test (combined with Dunn's test for post-hoc pairwise comparisons), depending on the type of data. A directed acyclic graph (Textor et al., 2011) was constructed based on previous knowledge and the observed associations in the data, using DAGitty version 3.0, to identify potential confounders (Figure S1). Infant's sex (male, female) and gestational age at birth (days) were selected as a priori adjustment factors. Thereafter, the analyses of maternal urinary fluoride and birth outcomes were adjusted for maternal age (years), parity (primiparous and multiparous), maternal BMI in early pregnancy (kg/m^2) or height (cm; in the analyses of birth length), maternal education (no university degree, university degree), maternal smoking status before pregnancy (ever, never), and season of sampling (spring, fall, winter, summer). Linearity of the associations between the maternal urinary fluoride concentrations with birth-size outcomes and gestational age at birth was examined using generalized additive models (Fig. 2) and was evaluated both visually and based on the *P*-gain (>0.1), defined as the difference in normalized deviance between the generalized additive model and the linear model. As we did not observe any indication of non-linearity in the models (Fig. 2), linear regression models were performed to explore the multivariable-adjusted associations of maternal urinary fluoride concentrations with birth weight, length, head circumference, and gestational age. The associations of maternal urinary fluoride and the categorical outcomes (small or large for gestational age and preterm delivery) were assessed with multivariable-adjusted logistic regression models. Since previous studies have indicated that the infant's sex may modify the association of fluoride exposure with other developmental outcomes (Green et al., 2019; Liu et al., 2019), we added a multiplicative interaction term in the main models and stratified the models by infant sex. Sensitivity analyses were conducted excluding women with reported diagnosed thyroid dysfunction (n = 41), infants born preterm (n = 28), or families having a private well at their residence. We also repeated the main models excluding maternal urinary fluoride concentrations above the 99th percentile (n = 6; corresponding to 3.9 mg/L) to evaluate whether the associations were impacted by extreme fluoride concentrations, and with additional adjustment for the time of urine sample collection.

Pregnant women with diagnoses related to thyroid dysfunction (n = 41) were excluded from all the analyses concerning thyroid hormone concentrations, as done in previous relevant analyses (GustinK Barman et al., 2021; Gustin et al., submitted). Associations of maternal urinary fluoride concentrations with maternal thyroid function biomarkers (TSH, tT4, fT4, tT3, fT3, fT4:tT4, fT3:tT3, fT3:fT4) were initially explored with Spearman's rank correlation test. A separate directed acyclic graph was constructed to select confounders for the models of maternal urinary fluoride concentrations with maternal thyroid hormones and TSH (Figure S2; minimal suggested adjustment set: maternal age, parity, maternal BMI in early pregnancy, maternal education, maternal smoking before pregnancy, and season of sampling). TSH concentrations were $\log_2$-transformed to improve the distribution (right skewed). We used generalized additive models to examine the linearity of the associations between maternal urinary fluoride concentrations and thyroid hormones or TSH (Figure S3). The generalized additive models did not suggest deviance from linearity. Therefore, the associations were evaluated using multivariable-adjusted linear regression models. As an association between urinary fluoride concentrations and altered thyroid hormones has previously been identified in iodine deficient individuals in a Canadian study (Malin et al., 2018), we examined the role of maternal UIC in the associations between maternal urinary fluoride concentrations and thyroid hormones' levels or TSH by inserting a multiplicative term in the models (exposure * UIC categories [150 μg/L and ≥150 μg/L]) and stratifying the analyses by these categories. Mediation analysis ("*paramed*" – Stata module for causal mediation analysis using parametric regression models) was performed with parametric regression models between maternal urinary fluoride concentrations, fT3:tT3, and birth size variables, to calculate a model for the mediator conditional on exposure and covariates, and a model for the outcome conditional on exposure, the mediator, and covariates (Valeri and VanderWeele, 2013).

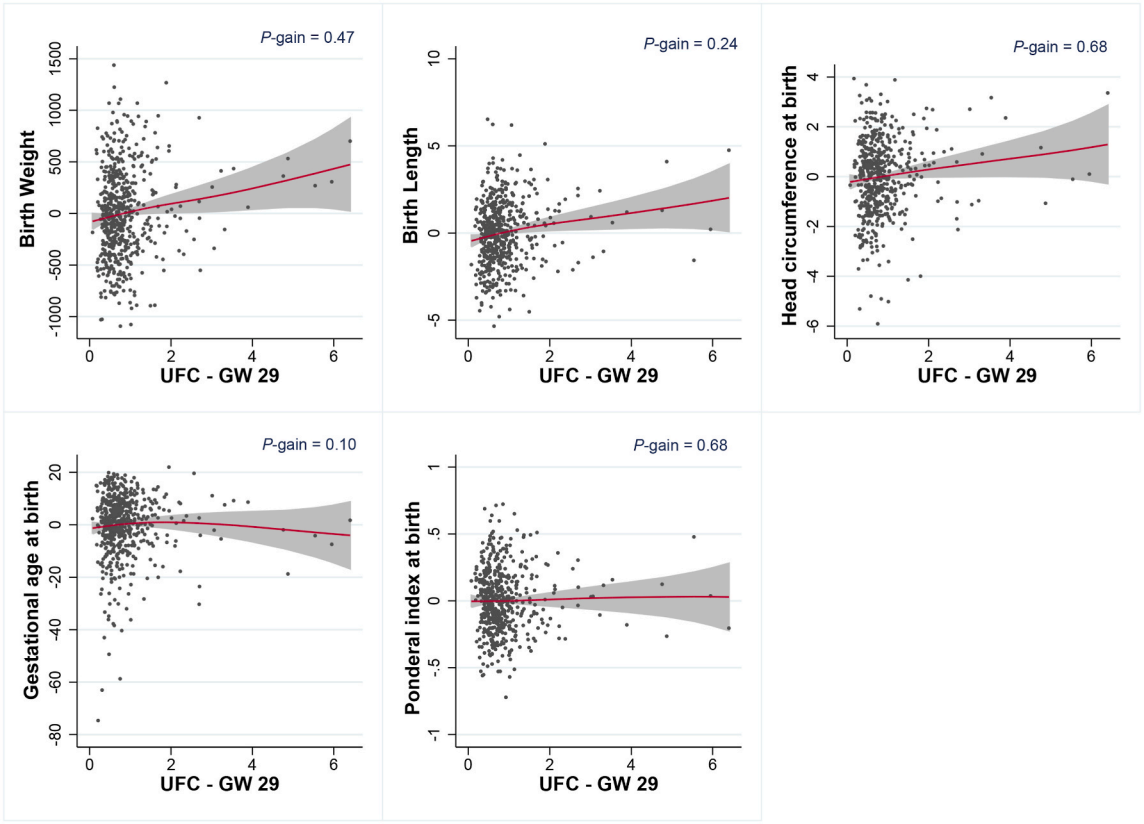

Fig. 2. Generalized additive models (GAMs) of maternal urinary fluoride concentration (UFC) at gestational week 29 (GW 29) adjusted for specific gravity with birth outcomes (gestational age, weight, length, head circumference, and the ponderal index at birth); GAMs are adjusted for maternal age (years), maternal parity (primiparous/multiparous), maternal body mass index (BMI; kg/m^2) or maternal height in the analyses of child birth length (cm), maternal education (university degree/no university degree), maternal smoking status before pregnancy (ever/never), gestational age at birth (days; not included in the GAM of gestational age at birth), child sex (male/female), and season of urine sample collection (spring/summer/autumn/winter).

3. Results

3.1. Participants' background characteristics and urinary fluoride concentrations

The background characteristics of the 558 mother-infant pairs included in the study are presented in Table 1. Five per cent of the pregnant women delivered their children prior to gestational week 37 (preterm births). Fifty-five percent of the infants were girls. The mean birth length was 50.0 cm (SD = 2.1) for girls and 50.7 cm (SD = 2.4) for boys, the mean head circumference was 34.6 cm (SD = 1.4) for girls and 35.3 cm (SD = 1.6) for boys, and the mean birth weight was 3530 g (SD = 503) for girls and 3640 g (SD = 621) for boys. Seven percent of the participating girls (n = 26) and 10% of the participating boys were born small for gestational age (n = 22), while 12% of the girls (n = 38) and 16% of the boys (n = 39) were born large for gestational age (10th and 90th percentile cut-offs, respectively).

The distribution characteristics of urinary fluoride concentrations at gestational week 29 and at 4th month postpartum are presented in Table 2. The median urinary fluoride concentration, adjusted to the mean specific gravity of 1.017, at gestational week 29 was 0.70 mg/L (5th-95th percentile: 0.31–1.9), with a maximum value of 6.4 mg/L. Concentrations were higher in women with a private well at their residence (median = 0.94 mg/L; n = 34; P = 0.013) than in women with access to municipality water (median = 0.70 mg/L; n = 388). Women with a university degree had slightly higher urinary fluoride concentrations (median = 0.73 mg/L; n = 389) than those without a university degree (median = 0.68; n = 169; $P < 0.021$). Also, urinary fluoride concentrations differed by the season of sampling (chi^2 = 9.2; P=0.024); in post-hoc pairwise comparisons the women's urinary fluoride concentrations were higher in fall (median = 0.78 mg/L; $P = 0.002$) and winter (median = 0.73 mg/L; $P < 0.05$) than in spring (median = 0.66 mg/L), and higher in fall (median = 0.78 mg/L) than in summer (median = 0.69 mg/L; $P < 0.05$). The mothers' urinary fluoride concentrations at gestational week 29 were moderately correlated with their urinary fluoride concentrations at 4 months postpartum (rho = 0.5, P-value <0.001).

The unsupervised clustered dietary analyses (Fig. 3) showed that maternal urinary fluoride concentrations were positively correlated with tea intake (rho = 0.19, $P < 0.001$). The correlation was most evident in winter samples (rho = 0.24, $P = 0.005$), followed by the correlation in fall samples (rho = 0.19, $P = 0.037$) and then summer samples (rho = 0.16, $P = 0.049$; spring: rho = 0.12, $P = 0.119$). Moreover, the correlation of urinary fluoride concentrations was stronger with black tea (rho = 0.17, $P < 0.000$) than with green tea (rho = 0.11, $P = 0.013$). Urinary fluoride concentrations were also weakly positively correlated with fruit intake (rho = 0.12, $P = 0.004$), which clustered together with water and water-based beverages.

3.2. Maternal urinary fluoride concentrations and birth outcomes

In the bivariate correlations, maternal urinary fluoride concentrations during pregnancy were positively correlated, although weakly, with birth weight (rho = 0.11; $P < 0.05$) and birth length (rho = 0.13; $P < 0.05$), while there was no correlation with head circumference (rho = 0.07; $P = 0.08$) or gestational age at birth (rho = 0.03; $P = 0.48$).

Table 1
Characteristics of the mothers-infant pairs included in the study.

	n	Mean (±SD) or %	Median (range)
Maternal characteristics			
Age (years)	558	30.6 (4.7)	30 (19, 45)
Weight in early pregnancy (kg)	558	71.0 (14.1)	68.0 (43.4, 137.0)
Height (cm)	558	167.0 (6.1)	167 (149, 184)
BMI in early pregnancy (kg/m^2)	558	25.4 (4.9)	24.4 (16.6, 50.4)
Parity (%)	558		
Primiparous	263	47	–
Multiparous	295	53	–
Maternal education (university; %)	558		
No	169	30	–
Yes	389	70	–
Pre-pregnancy smoking status (%)	558		
Non-smoking	523	94	–
Smoking	35	6	–
Drinking water source at residence	422		
Municipal	388	92	–
Private well	34	8	–
Season of sampling	558		
Spring	165	30	–
Summer	146	26	–
Autumn	115	21	–
Winter	132	24	–
Thyroid dysfunction (%)	558		
No	517	93	–
Yes	41	7	–
Infant characteristics			
Gestational age at birth (weeks; %)	558	39.9 (1.7)	40.1 (29.0, 42.7)
Preterm delivery (<37 GWs)	558		
Full term delivery	530	95	–
Preterm delivery	28	5	–
Sex (%)	558		
Female	308	55	–
Male	250	45	–
Birth weight (g)	556	3579 (561)	3560 (1110, 5165)
Birth length (cm)	538	50.30 (2.25)	50 (42, 58)
Birth head circumference	538	34.90 (1.60)	35 (29.4, 39)
Ponderal index	524	2.8 (0.2)	2.8 (2.1, 3.6)
Small for gestational age (SGA; <10th percentile)	556		
No	508	91	–
Yes	48	9	–
Small for gestational age (SGA; <2 SD)	556		
No	547	98	–
Yes	9	2	–
Large for gestational age (LGA; >90th percentile)	556		
No	479	86	–
Yes	77	14	–
Large for gestational age (LGA; >2 SD)	556		
No	535	96	–
Yes	21	4	–

Abbreviations: BMI: body mass index; UFC: urinary fluoride concentration; GW: gestational week; SG: specific gravity; SD: standard deviation.

In multivariable-adjusted linear regression models (Table 3), each 1 mg/L increase in maternal urinary fluoride concentration was associated with a mean increase of 84 g in birth weight (95%CI: 30, 138; $P = 0.003$), of 0.41 cm in birth length (95%CI: 0.18, 0.65; $P = 0.001$), and of 0.3 cm in head circumference (95%CI: 0.1, 0.4; $P = 0.001$). There was no association with gestational age at birth or the ponderal index (birth weight x 100/length^3). We did not observe any indication of an interaction between maternal urinary fluoride concentrations and infant sex in relation to birth size or gestational age at birth (Table 3). Stratification by infant sex showed stronger associations of maternal urinary fluoride concentrations with birth size measures in girls than in boys, however, the confidence intervals were wide and overlapping (Table S1). In multivariable-adjusted logistic regression models

Table 2
Distribution characteristics of urinary fluoride concentrations at gestational week 29 and at 4 months postpartum.

	N	Mean (±SD)	Median (IQR)	Range	5th - 95th percentile
UFC [a] (mg/L; GW 29)	558	0.86 (0.67)	0.71 (0.48)	0.07–6.4	0.31–1.9
UFC [a] (mg/L; 4 months postpartum)	463	0.89 (0.54)	0.77 (0.57)	0.14–4.8	0.32–1.9

Abbreviations: UFC: urinary fluoride concentrations; GW: gestational week; SD: standard deviation; IQR: interquartile range.
[a] Urinary fluoride concentrations are adjusted for specific gravity.

(Table 4), each 1 mg/L increase in maternal urinary fluoride concentration was associated with 39% increase in the odds (95%CI: 1.03, 1.89; $P = 0.034$) of being born large for gestational age. No association was evident between maternal urinary fluoride concentration and the odds of being born small for gestational age, or of a preterm delivery.

In sensitivity analysis, the estimates were very similar to the respective estimates reported in the main models when we excluded preterm deliveries (n = 27), women with a reported diagnosed thyroid dysfunction (n = 41), or women with access to drinking water from a private well (n = 34). Also, the estimates were not markedly impacted when extreme urinary fluoride concentrations were excluded from the analyses (>99th percentile; n = 6) or when the models were additionally adjusted for the timing of urine sample collection (n = 488 to 520 depending on the outcome).

3.3. Maternal urinary fluoride concentrations and thyroid hormones

In bivariate analyses, maternal urinary fluoride concentrations were weakly correlated with tT3 levels during pregnancy (rho = 0.09; $P < 0.05$; Table S3). In the multivariable-adjusted linear regression models (Table 5), we did not find any association with thyroid hormones or TSH but each 1 mg/L increase in maternal urinary fluoride was associated with a mean increase in the fT3:fT4 ratio of 0.007 (95%CI: 0.000, 0.014; $P = 0.047$), which corresponds to a 0.1SD increase of fT3:fT4 ratio. We did not observe any significant interaction between maternal urinary fluoride concentrations and UICs in relation to thyroid hormone levels (Table S4). Stratification of the models by maternal UICs (<150 μg/L & ≥150 μg/L) indicated different direction of the associations of urinary fluoride concentrations with fT4, tT4, and TSH in the two categories, but none of the models was statistically significant. The associations of maternal urinary fluoride concentrations with tT3 and fT3 did not differ between women in the two UIC categories. The association of urinary fluoride concentrations and fT3:fT4 ratio was statistically significant in mothers with UIC below 150 μg/L, but not in mothers with concentrations equal to or above 150 μg/L.

As maternal fT3:fT4 was the only thyroid-related biomarker associated with maternal urinary fluoride concentrations (Table 5), and it was also associated with birth weight and length as shown elsewhere (Gustin et al., submitted), the fT3:fT4 ratio was evaluated as a possible mediator of these associations. The mediation analysis (Table 6) indicated that maternal fT3:fT4 ratio did not explain the observed association of maternal urinary fluoride with infant birth weight and length [(weight - indirect effect: B = 3 g, 95%CI: 1, 11) and (length - indirect effect: B = 0.02 cm, 95%CI: 0.00, 0.06), corresponding to less than 5% of the total-effect estimate on weight and length].

4. Discussion

To our knowledge, this is the first prospective cohort study to report a positive association between maternal urinary fluoride concentrations during pregnancy and size at birth, which was evident with weight, length, and head circumference. We did not find any evidence of sex

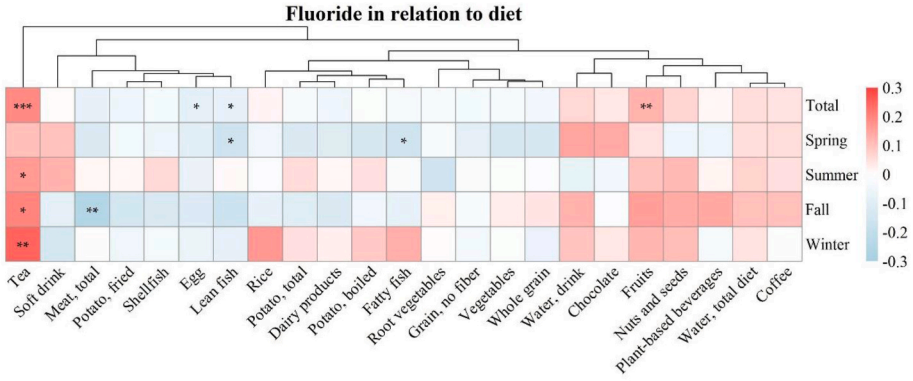

Fig. 3. Heat map of clustered bivariate correlations between maternal urinary fluoride concentrations and maternal diet during pregnancy based on a Food Frequency Questionnaire. The associations are based on Spearman rank correlation test; the direction of the correlations is indicated by color (red indicates positive and blue indicates negative correlations); the magnitude of the correlations is denoted by the color shade (deeper shades depict stronger correlations and lighter weaker) and statistical significance is presented by asterisks (*P < 0.05; **P < 0.01; ***P < 0.001). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 3
Multivariable-adjusted linear regression models of maternal urinary fluoride concentrations at gestational week 29 with the birth outcomes (gestational age, weight, length, head circumference, and the ponderal index at birth).

	n	Maternal UFC at gestational week 29 [a]				
		Crude models		Adjusted models [b]		Interaction by infant sex [c]
		B (95% CI)	*P -value*	B (95% CI)	*P -value*	*P*-value
Birth weight (g)	556	93 (24, 162)	0.009	**84 (30, 138)**	0.003	0.894
Birth length (cm)	538	0.39 (0.11, 0.67)	0.006	**0.41 (0.18, 0.65)**	0.001	0.183
Head circumference at birth (cm)	538	0.3 (0.1, 0.5)	0.009	**0.3 (0.1, 0.4)**	0.001	0.309
Gestational age at birth (days)	558	0.2 (−1.3, 1.7)	0.809	0.1 (−1.4, 1.7)	0.865	0.230
Ponderal index [d]	524	0.00 (−0.03, 0.03)	0.951	0.01 (−0.02, 0.04)	0.638	0.444

Abbreviations: UFC: urinary fluoride concentration; CI: confidence interval.
[a] Urinary fluoride concentrations are adjusted for specific gravity.
[b] Estimates are adjusted for maternal age (continuous; years), parity (categorical; primiparous/multiparous), maternal body mass index in early pregnancy (BMI; continuous; kg/m^2) or maternal height in the analyses of length at birth (continuous; cm), maternal education (categorical; no university degree/university degree), maternal smoking status before pregnancy (categorical; ever/never), gestational age at birth (continuous; days; not included in the analyses of gestational age at birth), child sex (categorical; male/female), and season of urine sample collection (categorical; spring/summer/autumn/winter).
[c] *P*-for-interaction values are derived from the adjusted models with an addition of a multiplicative term (exposure * infant sex).
[d] Ponderal index = weight (grams) * 100/[length (cm)]^3.

Table 4
Multivariable-adjusted logistic regression models of maternal urinary fluoride concentrations at gestational week 29 with the odds of a preterm delivery and of being born small or large for gestational age.

	Maternal UFC (mg/L) at gestational week 29 [a]			
	Controls, n	Cases, n	OR (95% CI) [b]	*P -value*
SGA (<10th percentile)	508	48	0.71 (0.38, 1.33)	0.287
SGA (<2SD)	547	9	1.23 (0.55, 2.76)	0.612
LGA (>90th percentile)	479	77	**1.39 (1.03, 1.89)**	0.034
LGA (>2SD)	535	21	1.27 (0.74, 2.19)	0.387
Preterm birth [c]	530	28	0.80 (0.38, 1.66)	0.543

Abbreviations: UFC: urinary fluoride concentration; SGA: small for gestational age; LGA: large for gestational age.
[a] Urinary fluoride concentrations are adjusted for specific gravity.
[b] Odds ratios are adjusted for maternal age (continuous; years), parity (categorical; primiparous/multiparous), maternal body mass index in early pregnancy (BMI; continuous; kg/m^2), maternal education (categorical; no university degree/university degree), maternal smoking status before pregnancy (categorical; ever/never), gestational age at birth (continuous; days; not included in the analyses of preterm birth), child sex (categorical; male/female), and season of urine sample collection (categorical; spring/summer/autumn/winter).
[c] Preterm birth was defined as a delivery prior to the 37th gestational week.

differences in the associations. Maternal urinary fluoride concentrations were also associated with increased odds of being born large for gestational age. In cross-sectional analyses, the urinary fluoride concentrations were weakly positively associated with the plasma fT3:fT4 ratio, but not with any other thyroid biomarker. The observed associations between maternal urinary fluoride concentrations and size at birth were not explained by changes in the maternal thyroid hormone levels.

There is a limited number of studies evaluating the impact of fluoride exposure on child anthropometry at birth and their results have been inconsistent. An ecological study conducted in regions of the geographical fluoride belt in Iran reported positive correlations of fluoride concentrations in drinking water in 35 villages [median (range): 1.6 mg/L (0.3, 3.5)] with weight and length at birth (Aghaei et al., 2015; Derakhshani et al., 2014). Conversely, a cross-sectional study in a region of India with elevated water fluoride concentrations [drinking water: median (range) = 2.2 (0.9–5.2) mg/L; water used for cooking and household chores: median (range) = 5.8 (3.6–16) mg/L] reported that concentrations of fluoride in maternal serum and in cord blood serum were inversely correlated with birth weight and gestational age at birth (Mohanty et al., 2011). However, both studies merely conducted correlational analysis and did not adjust for any potential confounders. A recent Canadian study (MIREC cohort), conducted in regions applying water fluoridation, is the only prospective study which has evaluated multivariable-adjusted associations of maternal fluoride exposure during pregnancy with birth outcomes (Goodman et al., 2021). In accordance with our finding, they did not find any association of maternal urinary fluoride with gestational age at birth. Also, they reported a positive association of maternal urinary fluoride with birth weight in the crude models, which was attenuated in the multivariable-adjusted models (Goodman et al., 2021). However, the exposure levels were slightly lower than in our study [urinary fluoride concentration in the MIREC study: median (range) = 0.5 mg/L (0.05–3.3 mg/L) and in the NICE study: median (range) = 0.70 mg/L (0.07–6.4 mg/L)] and the magnitude of the association with birth weight was only slightly smaller than the respective in our study (55 g increase in the MIREC study and

Table 5
Descriptive statistics of maternal thyroid biomarkers and multivariable-adjusted linear regression models of maternal urinary fluoride concentration with thyroid biomarkers at gestational week 29.

	Maternal UFC (mg/L) at gestational week 29 [a]				
	n	Mean (±SD)	Median (range)	B (95% CI) [b]	*P*-value
Thyroid Stimulating Hormone (TSH; mIU/L, log_2)	516	1.8 (1.2)	1.6 (0.07, 20.2)	0.02 (−0.09, 0.12)	0.741
free Thyroxine (fT4; pmol/L)	516	12.3 (1.6)	12.3 (7.9, 17.5)	−0.13 (−0.34, 0.09)	0.243
free Triiodothyronine (fT3; pmol/L)	516	4.1 (0.44)	4.1 (2.9, 5.6)	0.03 (−0.02, 0.09)	0.224
total Thyroxine (tT4; nmol/L)	516	124 (19.6)	122 (71.4, 193)	−0.03 (−2.63, 2.58)	0.984
total Triiodothyronine (tT3; nmol/L)	516	2.6 (0.44)	2.6 (1.5, 4.5)	0.05 (−0.01, 0.10)	0.083
fT4:tT4	516	0.10 (0.01)	0.10 (0.06, 0.14)	−0.001 (−0.003, 0.000)	0.100
fT3:tT3	516	1.60 (0.18)	1.59 (1.12, 2.18)	−0.018 (−0.041, 0.004)	0.115
fT3:fT4	516	0.34 (0.06)	0.33 (0.20, 0.55)	**0.007 (0.000, 0.014)**	0.047

Abbreviations: UFC: urinary fluoride concentration; CI: confidence interval.
[a] Urinary fluoride concentrations are adjusted for specific gravity.
[b] Estimates are adjusted for maternal age (continuous; years), maternal body mass index (BMI; continuous; kg/m^2), maternal education (categorical; no university degree/university degree), maternal smoking status before pregnancy (categorical; ever/never), parity (categorical; primiparous/multiparous), and the season of urine samples collection (categorical; spring/summer/autumn/winter.

Table 6
Mediation effects investigating whether maternal ratio of free triiodothyronine concentration to total triiodothyronine concentrations mediate the association between maternal urinary fluoride concentration and infant weight and length at birth.

Exposure	Outcome	Mediator	Total effect [α]	Direct effect [α]	Indirect effect [α]
			B (95%CI)	B (95%CI)	B (95%CI)
UFC[a]	Birth weight	fT3:fT4	**56 (10, 98)**	**53 (9, 95)**	3 (−1, 11)
UFC[a]	Birth length	fT3:fT4	**0.32 (0.10, 0.54)**	**0.31 (0.10, 0.53)**	0.02 (−0.00, 0.06)

Abbreviations: UFC: urinary fluoride concentration; free triiodothyronine: fT3; total triiodothyronine: tT3.
b Estimates are adjusted for maternal age (continuous; years), maternal body mass index (BMI; continuous; kg/m2) or maternal height in the analyses of birth length (continuous; cm), maternal education (categorical; no university degree/university degree), maternal smoking status before pregnancy (categorical; ever/never), parity (categorical; primiparous/multiparous), gestational age at birth (continuous; days), child sex (categorical; male/female) and the season of urine samples collection (categorical; spring/summer/autumn/winter).
[a] Urinary fluoride concentrations are adjusted for specific gravity.

84 g increase in the NICE study, per 1 mg/L increase in urinary fluoride). To note, the focus of the MIREC study was small for gestational age while the association with large for gestational age was not evaluated (Goodman et al., 2021).

Our results suggest that maternal urinary fluoride concentrations during pregnancy were associated with increased odds of being born large for gestational age. We controlled for multiple well-established determinants of increased size at birth (i.e., maternal obesity, multiparity, advanced gestational age, higher maternal education, male infant sex, and maternal non-smoking status), however, we did not have information on the mothers' size at birth. When we used the "above 2SD" cut-off of birth weight to define large for gestational age, the association did not reach significance, probably due to the fewer cases which resulted in limited power. However, the estimate was similar in size and direction (27% increase) to the estimate of the model based on the "above 10th percentile" cut-off of birth weight. To our knowledge, no other study has identified such an association. Given the potential impact of macrosomia on both the mother and the newborn (i.e., increased risk for caesarean delivery, postpartum hemorrhage, vaginal lacerations, increased rate of admissions to the neonatal intensive care unit) further studies are obviously warranted. The birth size in our population is consistent with nationwide data from the Swedish population (mean birth weight = 3494 g and 14% of the newborns with weight above 4000 g at birth) (Socialstyrelsen, 2020). Moreover, we have previously shown that pregnancy outcomes, including infant birth weight and gestational age at birth, do not differ between our participants and nonparticipating pregnant women in Norrbotten County (N = 4976) (Ögge et al., 2022). Although Sweden has a low obesity prevalence in the general population compared to most Western countries (especially among pregnant women; ≈14%) (Socialstyrelsen, 2020), an increasing rate of large size at birth, with a potential link to later obesity, has been reported (Cnattingius et al., 2012; Sundquist et al., 2004; Surkan et al., 2004).

The distribution of the maternal urinary fluoride concentrations (range: 0.07, 6.4 mg/L) indicates a wide variation of fluoride exposure in the studied women residing in the southern and eastern parts of Region Norrbotten in northern Sweden. This is supported by previously reported wide variations of fluoride concentrations in drinking water from both public water supplies (several plants reporting >1 mg/L) (Aggeborn and Öhman, 2021) and private wells (several wells with >4 mg/L) (Aneblom et al., 2005) in the study area. However, these reports covered fairly few water sources and the overlap of these data with the water sources of the NICE families is not known. In total 8% of the study participants reported having water from a private well at their residence. However, having access to municipality water instead of a private well did not attenuate the associations with birth size in this study. Urinary fluoride concentrations were not correlated with the food intake data, apart from a weak positive correlation with tea, which may be the result of the additional water consumption and of the tea intake itself (Waugh, 2019). Black, followed by green, tea is known to contain more fluoride than other food items (Malinowska et al., 2008). Indeed, the correlation between urinary fluoride concentrations and reported tea consumption was stronger for black tea than for green tea. Notably, the urinary fluoride concentration was more strongly correlated with hot tea consumption in winter and fall than in summer and spring, probably due to the seasonal variation in the consumption of tea with more frequent intake during the cold seasons (ice tea consumption is not common in Sweden). Furthermore, the median urinary fluoride concentration was higher in winter and fall than in summer and spring, following a similar pattern as with tea intake in our population. It is noteworthy that in addition to several general factors that have been shown to or are expected to affect fluoride metabolism and urinary excretion (i.e., renal clearance rate, skeletal uptake rate, pH of the gastric contents, amount and form of consumed fluoride, diet), urinary fluoride concentrations during gestation may also be influenced by an increased release of fluoride into the circulation due to the high bone turnover in pregnancy (Åkesson et al., 2004; Black et al., 2000; Gulson et al., 1998).

Several experimental findings have reported suppressed thyroid hormones in fluoride exposed animals (Basha et al., 2011; Bobek et al., 1976; Jianjie et al., 2016; Zhan et al., 2006), and findings from a Canadian observational study suggested that exposure to fluoride may reduce thyroid hormone levels in individuals who have moderate-to-severe iodine deficiency (Malin et al., 2018). Contrary to the findings of the aforementioned studies (Basha et al., 2011; Bobek

et al., 1976; Jianjie et al., 2016; Malin et al., 2018; Zhan et al., 2006), we did not find any clear associations between the urinary fluoride concentrations and thyroid hormone levels, except for a weak positive association with plasma fT3:fT4 ratio. Also, we did not find any interaction between the urinary concentrations of fluoride and iodine in relation to the thyroid hormone levels, conversely to the reported findings of the previous Canadian study, despite that the mothers in the present study had low iodine intake during pregnancy (GustinK Barman et al., 2021; Stråvik et al., 2021). On the other hand, a statistically significant increase in the fT3:fT4 ratio in women with UICs below 150 μg/L that was not evident in women with concentrations equal or above 150 μg/L was found, which may suggest a possible increased vulnerability of women with low iodine intake. Nevertheless, maternal thyroid hormones did not explain the identified associations of maternal urinary fluoride concentrations with infant size at birth (weight and length). The mechanisms involved in an effect of gestational fluoride exposure on size at birth are unknown. Maternal hyperglycemia may be implicated as fluoride is known to inhibit insulin synthesis causing elevated blood glucose levels (Lin et al., 1976; Menoyo et al., 2008), and the delivery of hyperglycemic blood to the fetus leads to excessive glucose storage as glycogen and fat (Kamana et al., 2015; Yan and Yang, 2014). Secondly, an effect of fluoride on maternal lipid metabolism can be hypothesized as several experimental animal studies have shown that fluoride treatment affects lipid metabolism (Chiba et al., 2015; Miltonprabu and Thangapandiyan, 2015; Pereira et al., 2016; Sun et al., 2014; Umarani et al., 2015; YuZhou et al., 2021). Alterations in maternal lipid profiles have been linked to fetal macrosomia regardless of the glucose tolerance status of the mothers (Rao and Ping, 2021).

The main strengths of this study include the prospective design and the assessment of individual fluoride exposure using urinary concentrations, which are considered to be the most suitable biomarker for contemporary fluoride exposure at a population level (World Health Organization, 2014). However, the present study also has several limitations. Foremost, only one spot urine sample collected in late pregnancy was used to assess urinary fluoride concentrations, which may have resulted in exposure misclassification due to late-pregnancy physiology, although the concentrations were adjusted for specific gravity to account for variation in urine dilution, and also due to participant behavior prior to sampling (e.g., consumption of fluoride-rich water or food) as fluoride has a very short half-life in the body. Still, we found that the mothers gestational urinary fluoride concentrations were fairly well correlated with their urinary fluoride concentrations at 4 months postpartum; and thus, most likely, they do reflect the ongoing fluoride exposure in the present population. Available data on a thyroid dysfunction diagnosis and of thyroid hormone measurements during pregnancy gave the opportunity to control for a possible impact of maternal thyroid disease on the reported findings, and to examine whether a thyroid-disruption effect of fluoride would explain the observed associations with birth size. However, thyroid hormones were, also, only measured once in late pregnancy, and therefore, we cannot exclude a possible thyroid disruption in early pregnancy. Moreover, the exposure and the mediator were measured in samples collected at the same timepoint; while, in order to assume causal inference, the exposure should be measured prior to the mediator. However, since the main source of fluoride exposure is drinking water, we expect that exposure is fairly constant over time, which is supported by the correlation between the two separate sampling time points presented above. Moreover, the power of the iodine-related analyses was fairly low, since measurements of urinary iodine concentrations were not available for all study participants and they are prone to short-term variations. We had no information on gestational diabetes, and we did not measure maternal insulin levels or plasma lipids, which limited the interpretation of potential underlying modes of action. Lastly, although we have adjusted our analyses for multiple potential confounders and our associations were robust, we cannot exclude the possibility that residual confounding has affected the reported findings.

5. Conclusion

This is the first prospective study suggesting that gestational fluoride exposure may increase size at birth and even increase the risk of being born large for gestational age. We did not find any association of maternal urinary fluoride concentrations with TSH or thyroid hormones in late pregnancy, except for a weak positive association with the fT3:fT4 ratio, and maternal thyroid hormones did not explain the observed associations between gestational urinary fluoride concentrations and infant size at birth. Considering the ubiquity of fluoride in drinking water and that being born large for gestational age is linked with perinatal complications, additional prospective cohort studies are warranted to confirm our findings and to elucidate the mechanisms by which fluoride may affect intrauterine growth. Furthermore, we believe that our findings highlight the need for further research on the possible adverse effects of early-life fluoride exposure on child development and health.

Credit author statement

Mariza Kampouri: Data analysis, Data visualization, Investigation, Writing – original draft, Writing – review & editing. **Klara Gustin**: Writing – review & editing. **Mia Stråvik**: Data visualization, Writing – review & editing. **Malin Barman**: Writing – review & editing. **Michael Levi**: Writing – review & editing. **Vasiliki Daraki**: Writing – review & editing. **Bo Jacobsson**: Writing – review & editing. **Anna Sandin**: Writing – review & editing. **Ann-Sofie Sandberg**: Writing – review & editing. **Agnes E. Wold**: Writing – review & editing. **Marie Vahter**: Conceptualization, Supervision, Investigation, Methodology, Writing – review & editing. **Maria Kippler**: Conceptualization, Supervision, Investigation, Methodology, Funding acquisition, Project administration, Writing – review & editing.

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.

Data availability

The data that has been used is confidential.

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Appendix A. Supplementary data

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