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Dietary Influences on Urinary Fluoride over the Course of Pregnancy and at One-Year Postpartum

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Abstract

Dietary factors are known to influence urinary fluoride (UF) levels in nonpregnant people. Maternal UF is used as a biomarker of fluoride exposure; however, dietary influences on UF during pregnancy are unknown. We compared UF levels and assessed the associations between UF and five select dietary influences in pregnancy vs. one-year postpartum: dietary fluoride (F), calcium intake from diet (Ca-diet), calcium intake from supplements (Ca-sup), dietary acid load (AL), and table salt use (TS) in 421 women exposed to fluoridated salt in the Mexican diet. Spot UF (mg/L) was measured by microdiffusion/fluoride-specific electrode and dilution-corrected with specific gravity (SG). Dietary variables were estimated from a validated Food Frequency Questionnaire. Comparisons among UF in pregnancy vs. one-year postpartum were performed with non-parametric tests. Associations between dietary variables and UF were assessed using random effect models (for pregnancy) and linear regression (for one-year postpartum). SG-corrected UF (median, range) during pregnancy (0.77, 0.01–4.73 mg/L) did not significantly differ from one-year postpartum (0.75, 0.15–2.62 mg/L) but did increase every 10 gestational weeks, $\beta = 0.05$ (CI: 0.00–0.10). Different dietary influences on UF were identified at each state. Although Ca-diet and AL were not associated with UF in either state, Ca-sup decreased UF only during pregnancy, $\beta = -0.012$ mg/L (CI: −0.023–0.00). Reporting TS use was associated with 12% increase in UF only at one-year postpartum ($p = 0.026$). These results suggest different dietary influences on UF in the pregnant state, which need consideration when using UF as a biomarker of fluoride exposure.

Keywords Urinary fluoride · Pregnancy · Fluoride exposure · Biomarker

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Introduction

The urinary excretion of fluoride is a biomarker of concurrent fluoride intake and exposure validated for children and nonpregnant adults [1, 2] and traditionally used for the monitoring of water or salt fluoridation programs for the prevention of dental caries [3]. More recently, this biomarker has been used as a proxy for prenatal fluoride exposure in epidemiological studies in women and children, which reported associations between spot urinary fluoride from pregnant women and neurodevelopmental outcomes in children living in communities with implemented water or salt fluoridation programs [4–6]. However, our current knowledge of urinary fluoride in pregnant women as a biomarker of fluoride exposure is based on research mostly conducted in children and nonpregnant adults [1, 2].

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In healthy nonpregnant adults, around 60% of a given amount of absorbed fluoride is excreted through the urinary system [7]. The renal handling of fluoride involves tubular reabsorption mediated by a pH-dependent process; any acute or chronic condition affecting urinary pH increases fluoride's chance to remain circulating in the body or to accelerate its rate of elimination [8]. Other physiological factors that influence the concentration of urinary fluoride in healthy nonpregnant adults include disturbances of the acid–base balance (e.g., chronic respiratory alkalosis) [9] and bone metabolism [10]. Pregnancy is a physiological state of adjustments in the renal system [11], increased bone turnover [12], and chronic respiratory alkalosis [13], which return to nonpregnant levels by about 3 months postpartum [11]. However, although most of the factors that influence the absorption, distribution, and renal excretion of fluoride converge in the pregnant state, it is still unclear whether the urinary fluoride levels found in pregnant women are different to those of nonpregnant women.

Dietary adjustments are common during pregnancy [14], and specific dietary variables that may influence the absorption, distribution, and/or urinary excretion of fluoride in the pregnant state include the acidity of the diet [8] and calcium intake [15]. For instance, the alleviation of common pregnancy symptoms such as nausea and acid reflux includes a decrease in the consumption of precursors of dietary acids [14], which may influence urinary fluoride levels by modifying the urinary pH and the renal handling of fluoride [8, 16]. On the other hand, in pregnant women, the intake of calcium-rich foods is encouraged [17], and the concomitant intake of dietary calcium and fluoride favors the formation of insoluble calcium-fluoride salts, potentially reducing fluoride's intestinal absorption [10]. Another mechanism by which calcium intake may influence urinary fluoride — when ingested at levels lower than adequacy [18] — is the increased mobilization of maternal skeletal mineral stores to the plasma that occurs during the second and third trimesters of pregnancy to meet fetal calcium demands [11]. The release of skeletal stores may also mobilize fluoride and increase its availability in plasma and potentially, its urinary excretion. Despite our knowledge on the dietary and physiological adjustments that occur during pregnancy, whether dietary variables such as the dietary acid load and calcium from dietary sources and supplements influence urinary fluoride levels in pregnant women, remains unknown.

We hypothesized that urinary fluoride levels in pregnancy are different to those of nonpregnant women and that select dietary variables (intake of dietary fluoride and fluoridated salt, dietary calcium intake, calcium intake from supplements, and dietary acid load) influence the concentration of fluoride in urine during pregnancy. Given that calcium intake may affect urinary fluoride levels by influencing fluoride's intestinal absorption and/or bone metabolism, we were especially interested in the influence of calcium from both diet and supplements in the pregnant and nonpregnant states. This information is necessary to identify potential dietary confounders of the association between fluoride exposure and urinary fluoride levels in epidemiology studies and advance our knowledge on fluoride biomarkers, which are important in assessing fluoride exposure in human populations.

The aims of this study were as follows: (1) to compare urinary fluoride during pregnancy vs. one-year postpartum — as a proxy for the nonpregnant state — and (2) to assess the association between urinary fluoride and select dietary variables that may influence urinary fluoride levels, over the course of pregnancy and one-year postpartum.

Materials and Methods

Study Sample and Setting

The source population were women from the Early Life Exposures in Mexico to Environmental Toxicants (ELEMENT) project. ELEMENT comprises three mother–child pregnancy and birth cohorts, initiated in the 1990s to study early life exposures and health outcomes in Mexico City. Mexico has a salt fluoridation program implemented since 1981 [19]; therefore, these women were potentially exposed to fluoridated salt in their diets. Details and demographic characteristics of the entire project are published elsewhere [20]. Briefly, pregnant women who were invited to participate were attending three clinics of the Mexican Institute of Social Security (IMSS), lived in Mexico City, and had the intention to continue living in the city during the next 5 years. Included participants in this study were women in their first trimester (< 14 weeks) who had a healthy singleton pregnancy, no history of hypertension, diabetes, or high-risk pregnancy, intended to breastfeed, and agreed to participate through written informed consent. Participating women were aware that their data were fully anonymized and were invited to attend the research facility up to three time-points during pregnancy and up to one-year postpartum. During each study visit, a trained social worker administered a general demographic questionnaire and a Food Frequency Questionnaire (FFQ) to each participant, and a trained nurse measured weight and height using calibrated instruments. Participants also provided early morning second-void spot urine samples, which were archived in the project's biorepository at − 70 °C until analysis. The Institutional Review Boards of the National Institute of Public Health of Mexico, the University of Michigan, and Indiana University approved all study procedures.

The source population and inclusion criteria for the study sample are summarized in Fig. 1. The source population

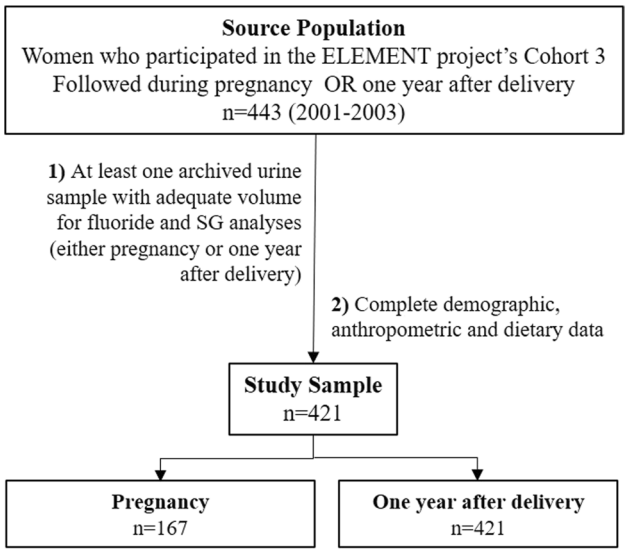

Fig. 1 Flow chart of source population and study sample, drawn from the Early Life Exposures in Mexico to Environmental Toxicants (ELEMENT) project

consisted of participants who were recruited between 2001 and 2003 (ELEMENT Cohort 3) and were followed for up to three visits during pregnancy and up to one-year postpartum ($n = 443$). ELEMENT's Cohort 3 was originally designed as a double-blind randomized clinical trial (RCT) to examine the effects of calcium supplementation on blood lead levels during pregnancy and up to one-year postpartum [21]. Participants who had met the following criteria were included in the study sample: (1) at least one archived urine sample with adequate volume for fluoride and specific gravity analyses (SG), collected at any pregnancy visit or at one-year postpartum, and (2) complete demographic, anthropometric, and dietary data. A total of 421 women with data available one-year postpartum were included in the study sample. From these 421 women, a total of 167 attended visits during pregnancy, and 155 had data for at least one pregnancy visit and one-year postpartum. From the 167 women with available data at any pregnancy visit, 135 attended the study's 1st pregnancy visit; 101 attended the 2nd, and 71 attended the 3rd.

Dietary Variables

All the dietary variables reported in this study were calculated using data from a semiquantitative FFQ that was applied during each study visit, adapted from the Willett semiquantitative FFQ [22]. The questionnaire was validated to estimate dietary intake over the previous month in Mexican women of child-bearing age [23]. It consisted of 104 items and included foods commonly consumed in the 1983 Dietary Survey of the Mexican National Institute of Nutrition [24] and a questionnaire on intake of dietary supplements and multivitamins. Using the reported frequencies and portion sizes from the FFQ, estimates of dietary intake of macro- and micronutrients, minerals, and trace elements from foods and popular dietary supplements and multivitamins were calculated through software developed at the National Institute of Public Health (INSP). The software utilized the INSP-compiled nutrient composition database [25] and a fluoride database specifically developed for the ELEMENT project by analyzing the fluoride content of typical foods and beverages in the Mexican diet. Details on the methodology for the development of the fluoride database can be found in the publication by Cantoral et al. [26]. Briefly, fruits, vegetables, meats, beverages, processed foods, and street foods were bought from different major markets, supermarket chains, and street vendors in Mexico City, and environmental water samples were collected from the household of study participants. Foods that are consumed cooked (meats, rice, pasta, legumes) were boiled using water containing negligible amounts of fluoride and fluoridated salt, following standardized recipes from the National Health and Nutrition Survey. Fluoride analyses of foods and beverages were conducted at the Fluoride Research Laboratory at the Oral Health Research Institute (OHRI), Indiana University School of Dentistry using a modification of the hexamethyldisiloxane (HMDS) method [27] as modified by Martinez-Mier et al. [28], as described in the urine fluoride analyses section below.

Dietary Fluoride Intake Table salt is the major source of dietary fluoride for those living in Mexico City, due to exposure to the implemented salt fluoridation program [19]. The estimates derived from the FFQ included fluoride from beverages, tap and bottled water, and fluoride from salt added to foods during the cooking process [26]. Popular dietary supplements distributed in Mexico do not contain fluoride, and therefore, the estimates are only from diet and do not include supplement use. The use of fluoridated dental products (which can be a source of fluoride intake) was not registered in this population.

Addition of Table Salt to Meals Although the amount of salt that was added to food by participants after cooking is unknown, the FFQ did include a dichotomous question on whether salt was added to meals at the table (yes/no).

Calcium from the RCT An additional source of calcium for about half of the study participants was the calcium supplementation regimen; therefore, the original RCT design of the cohort was leveraged to assess the association between urinary fluoride and calcium supplementation. Women were randomized to receive either the calcium supplement or placebo and were provided with a bottle containing pills

with the assigned treatment at each study visit. Participants were instructed to consume two pills containing 600 mg of calcium carbonate at bedtime (1200 mg calcium/day total). A dichotomous variable on RCT group allocation (supplement/placebo) was used to assess the association between urinary fluoride and calcium supplementation. During each visit, participants returned the treatment bottle to a research assistant, who performed pill counts since the last visit to register adherence to the treatment. This adherence registry was used to estimate a continuous variable of calcium intake (mg/day) from the RCT (% of the required dose ingested per day*1200 mg) [18] and used to assess the association between urinary fluoride and the actual dose of calcium supplement intake in a sensitivity analysis.

Dietary Calcium Intake Estimates of calcium intake included both calcium from foods and beverages, and from other dietary supplements consumed by study participants, reported in the FFQ and were estimated as described in the "Dietary Variables" section.

Dietary Acid Load The excretion of fluoride is influenced by the acidity of the diet [8]. For this study, we estimated the net endogenous acid production (NEAP) — the ratio of the estimated dietary intake of protein (acid precursor) and potassium content (base precursor) used as a proxy of the acid load of the diet in healthy individuals. The dietary acid load was estimated using the equation determined by Frassetto et al. [29]:

$$\mathrm{NEAP} = -10.2 + 54.5 \times (\mathrm{Pro}/\mathrm{K}) \quad (1)$$

where NEAP is the net endogenous acid production (mEq/day), Pro is the daily dietary intake of protein (g/day), and K is the daily intake of potassium (mEq/day) from both diet and supplements.

Urinary Fluoride and Specific Gravity

Urinary fluoride was determined with the HMDS microdiffusion and fluoride-specific electrode method [27], as modified by Martinez-Mier et al. [28]. Each urine sample was thawed and vortexed at room temperature. A 1.0 mL aliquot was reserved for fluoride analyses and a second aliquot (~0.8 mL) was used to measure urinary SG. The 1.0 mL urine aliquot was pipetted into a disposable petri dish. A trap of 0.05 N sodium hydroxide solution was placed in the form of five equal drops on the inside of the lid. After a tight sealing with petroleum jelly and burning a small hole into each lid, HMDS-saturated 3 N sulfuric acid was pipetted and the fluoride was allowed to diffuse overnight at room temperature. The following day, the petri dish was opened, and the solution of diffused fluoride contained in the lid was recovered, buffered to pH 5.2 with acetic acid 0.1 M, and then diluted in deionized water. Most analyses were performed in duplicates (provided the availability of enough urine volume). The concentration of fluoride was then measured using a fluoride ion selective electrode coupled to a pH/ISE meter (Orion™ Fluoride Electrode and Dual Star™ pH-meter, Thermo Scientific, Waltham, MA). A calibration curve using fluoride standards (Orion™ ISE calibration standards) was constructed following the same procedure. Millivolt readings from the samples were recorded and the unknown concentrations of fluoride from urine samples were determined using the equation that explained the relationship between the log of the fluoride concentration of the standards and their corresponding millivolt readings ($R^2 > 0.9$). Testing included a daily standard check using a fluoride standard traceable to NIST (National Institute of Standards and Technology, U.S Department of Commerce) after sample analysis.

The measurement of urinary SG took place in a dark room using a pen refractometer (ATAGO®). The prism head of the refractometer was immersed into the 0.8 mL urine aliquot, and the sample's reading was recorded. SG-corrected fluoride in urine was calculated using a standard equation [30] as modified by Till et al. [31]:

$$\mathrm{UF_{SG}} = \mathrm{UF_i} \times \left(\mathrm{SG_M} - 1\right) / \left(\mathrm{SG_i} - 1\right) \quad (2)$$

where $\mathrm{UF_{SG}}$ is the SG-corrected fluoride concentration (mg/L), $\mathrm{UF_i}$ is the observed fluoride concentration, $\mathrm{SG_i}$ is the SG of the individual urine sample, and $\mathrm{SG_M}$ is the median SG for all available urine samples for either pregnancy or one-year postpartum, depending on the case.

Statistical Analyses

Exploratory data analyses were conducted using univariate and bivariate graphical summaries. Summary statistics used for continuous variables included means, medians, ranges, standard deviations (SD), and interquartile ranges (IQR), whereas categorical variables were summarized with frequencies and percentages. Comparisons were performed using Mann–Whitney, Wilcoxon signed-rank, and chi-squared tests, where appropriate. Given that each physiological state is characterized by unique variables (e.g., gestational age during pregnancy and breastfeeding in the postpartum), two different models to assess the relationship between urinary fluoride and dietary factors were generated: a longitudinal model for pregnancy (with repeated measures at different gestational ages) and a cross-sectional linear regression model for one-year postpartum.

Pregnacy Model To account for time and individual-specific effects during pregnancy, regression models for panel data were generated. Given that individual effects were uncorrelated with the regressors (Hausman test) and the presence of groupwise heteroscedasticity (modified Wald test), pregnancy data were fit to a random effects GLS regression with heteroscedasticity-robust standard errors. The outcome variable was the uncorrected concentration of fluoride in urine (mg/L) and the explanatory variables of interest were dietary fluoride intake (mg/day), reports of adding table salt to meals (yes/no), dietary calcium intake, calcium from RCT (supplement/placebo), acid load of the diet (NEAP), and gestational age (weeks). Based on our review of the literature, covariates that were included as potential confounders were total energy intake, pre-pregnancy body mass index (BMI), and age at the first pregnancy visit. SG was included in the model as a precision covariate, so the significance of other variables could be independent of its effects [32].

One-Year Postpartum Model A cross-sectional OLS linear regression for the association of dietary variables with UF at one-year postpartum was generated. The outcome variable was the natural log-transformed uncorrected urinary fluoride (mg/L) and the explanatory variables of interest were dietary fluoride intake, reports of adding table salt to meals, dietary calcium intake, calcium from RCT (supplement/placebo), and acid load of the diet (NEAP). Covariates included the following: urinary SG, total energy intake, age, time since delivery, breastfeeding (yes/no), and BMI at the one-year postpartum visit. Studentized residuals displayed a normal distribution and were homoscedastic.

For both models (pregnancy, one-year postpartum), sensitivity analyses were performed using the continuous variable of calcium intake (mg/day) from the RCT that was estimated from the treatment's adherence registry, instead of the dichotomous variable (supplement/placebo). All analyses were conducted with STATA v16.0 (StataCorp LP, College Station, TX, USA).

Results

Characteristics of the Study Sample

The characteristics of the study sample are summarized in Table 1. Women with available data during pregnancy ($n = 167$) had a median age (IQR) of 26.7 (7.5). Included women completed 11 to 12 years of education — equivalent to high school diploma — and most had low or very low socioeconomic status (~57%). The majority of women were married or cohabitating with a partner, and most (~65%) had been pregnant at least once before participating in the ELEMENT project. Although most women with data available during pregnancy were classified with a pre-pregnancy BMI in the range of normal, the BMI of women with data available one-year postpartum tended to shift towards the overweight and obese categories, and the difference was statistically significant ($p = 0.002$). Around half of the included women were allocated to the calcium supplementation group, and the other half were allocated to the placebo group of the RCT. The characteristics of the women included vs. excluded during pregnancy and postpartum were similar, although women excluded from the pregnancy analyses for having incomplete data had a higher education level ($p = 0.011$, Supplementary Table 1).

Descriptive Statistics for Key Time-Varying Variables

The measurement of key dietary variables took place up to three times during pregnancy, and once for the one-year postpartum visit, and are summarized in Table 2. Median gestational age for the 1st, 2nd, and 3rd pregnancy visits was 13.1, 25.3, and 33.9 weeks, respectively, and the one-year postpartum visit occurred at a median time of 12.2 months. As expected, total energy intake increased with the progression of gestation and was higher compared to one-year postpartum ($p = 0.000$). The values for dietary intake of fluoride and calcium followed a similar increase over the course of gestation and were statistically significantly higher compared to the one-year postpartum values. In contrast, the acid load of the diet was lower during pregnancy compared to one-year postpartum and the difference was statistically significant ($p = 0.000$). Most women reported not adding table salt to meals, and the use of dietary supplements (different to the RCT supplement) was higher at one-year postpartum compared to pregnancy. Around 30% of women reported to be breastfeeding their children by the time of the one-year postpartum visit.

Urinary Fluoride During Pregnancy and at One-Year Postpartum

Descriptive statistics of the concentration of fluoride from second-void morning spot urine samples at each pregnancy visit and at one-year postpartum are presented in Table 3. Median uncorrected urinary fluoride levels were 0.76, 0.65, and 0.64 mg/L for pregnancy visits 1, 2, and 3, respectively, whereas at the postpartum visit, fluoride levels were at 0.69 mg/L. After correcting for urinary dilution, median fluoride levels at the three pregnancy visits ranged between 0.75 and 0.77 mg/L and median values at the postpartum visit were at 0.75 mg/L. The distributions of fluoride concentrations corrected and uncorrected for the effects of urinary dilution during pregnancy and at one-year postpartum were not significantly different. Median urinary

Table 1 Characteristics of the study sample at pregnancy and one-year postpartum (time-invariant)

	Pregnancy $n=167$	One-year postpartum $n=421$	*p-value*		
	Median	IQR	Median	IQR	
Age* (years)	26.7	7.5	27.8	8.0	–
Completed years of education	12.0	3.0	11.0	3.0	0.163
	Freq	%	Freq	%	
Marital status					
Married/cohabitating	145	87	367	87	0.422
Single/divorced	23	13	54	13	
Socioeconomic status					
High	36	23	44	23	0.920
Middle	30	19	38	20	
Low	62	39	74	39	
Very low	29	18	33	17	
Primigravida					
Yes	57	34	149	35	0.953
No	110	66	272	65	
BMI**					
Underweight	8	5	16	4	0.002
Normal	92	55	179	42	
Overweight	32	19	109	26	
Obese	35	21	117	28	
Calcium supplementation group					
Placebo	79	47	211	50	0.611
Supplemented	88	53	210	50	
Breastfeeding					
Yes	–	–	125	30	–
No	–	–	296	70	

*Age during pregnancy corresponds to the average age at three pregnancy visits

**Pre-pregnancy BMI for pregnancy and BMI at one-year postpartum

Mann–Whitney tests were used for continuous variables and chi-squared tests for categorical variables

SG was higher (urine was more concentrated) at one-year postpartum (1.017) compared to pregnancy (1.015), and the difference was statistically significant ($p=0.002$). Analyses conducted in the subsample of 156 women who had observations for both pregnancy and the one-year postpartum visits were consistent with those of the larger sample (no differences in urinary fluoride in pregnancy vs. postpartum and significantly higher SG at one-year postpartum).

Pregnancy Model

The results of a longitudinal, random effects model for pregnancy are summarized in Table 4. The associations between urinary fluoride and addition of table salt to meals were not significant. Women who were assigned to a calcium supplementation regimen (1200 mg calcium/day) excreted on average 0.12 less mg fluoride/L [95% CI: − 0.23, 0.00], compared to women who were not supplemented with calcium (Table 4). This effect was observed exclusively for calcium from supplements and not for dietary calcium. In sensitivity analyses, when calcium supplementation was included as a continuous variable (calculated by means of pill count at each visit as described in the "Materials and Methods" section), for each 100 mg of calcium supplement intake, supplemented women excreted 0.02 mg/L less fluoride [95% CI: − 0.03, 0.00] compared to non-supplemented women ($p=0.014$). An association between urinary fluoride and dietary acid load was not found. On the other hand, the concentration of fluoride in urine increased 0.05 mg/L [95% CI: 0.00, 0.10] for every 10 weeks of gestation. Figure 2 displays the relationship between gestational age and urinary fluoride as predicted by the multivariate longitudinal model, according to calcium supplementation groups.

One-Year Postpartum Model

Table 5 summarizes the results for the one-year postpartum model. In contrast to what was observed during pregnancy,

Table 2 Key variables measured at different time-points during pregnancy and at one-year postpartum

	Pregnancy			One-year postpartum			*p-value**
	n	Median	IQR	n	Median	IQR	
Gestational age (weeks)							
Visit 1	135	13.1	2.1	–	–	–	–
Visit 2	101	25.3	2.1	–	–	–	–
Visit 3	71	33.9	2.6	–	–	–	–
All observations	307	23.7	14.3	421	0.63	0.37	0.008
Time since delivery (months)	–	–	–	421	12.2	0.8	–
Total energy intake (Kcal/day)							
Visit 1	135	1780.2	847.5	–	–	–	–
Visit 2	101	1790.8	903.5	–	–	–	–
Visit 3	71	1814.7	681.2	–	–	–	–
All observations	307	1792.8	803.0	421	1,613.1	678.4	0.000
Dietary fluoride intake (mg/day)							
Visit 1	135	0.63	0.39	–	–	–	–
Visit 2	101	0.74	0.49	–	–	–	–
Visit 3	71	0.73	0.42	–	–	–	–
All observations	307	0.70	0.43	421	0.63	0.37	0.008
Dietary calcium intake (mg/day)							
Visit 1	135	1021.4	695.5	–	–	–	–
Visit 2	101	1183.0	795.1	–	–	–	–
Visit 3	71	1127.1	582.9	–	–	–	–
All observations	307	1102.7	716.8	421	939.5	616.1	0.000
Acid load (NEAP, mEq/day)							
Visit 1	135	33.2	12.6	–	–	–	–
Visit 2	101	33.9	11.9	–	–	–	–
Visit 3	71	34.2	12.7	–	–	–	–
All observations	307	33.7	12.2	421	36.8	10.7	0.000
	n	Freq	%	n	Freq	%	*p-value**
Adds table salt to meals, yes							
Visit 1	135	35	26	–	–	–	–
Visit 2	101	21	21	–	–	–	–
Visit 3	71	16	23	–	–	–	–
All observations	307	72	23	421	89	21	0.458
Use of other supplements, yes							
Visit 1	135	37	27	–	–	–	–
Visit 2	101	33	33	–	–	–	–
Visit 3	71	26	63	–	–	–	–
All observations	307	96	31	421	173	49	0.007
Breastfeeding, yes							
All observations	–	–	–	421	125	30	–

p-values correspond to comparisons among groups using all available observations for each state. Mann–Whitney tests were used for continuous variables and chi-squared tests for categorical variables

urinary fluoride at one-year postpartum was associated only with the addition of table salt to meals. On average, women who reported adding salt to their meals excreted 12% more fluoride ($p=0.026$) compared to those who did not, controlling for breastfeeding, urinary SG, time since delivery, total energy intake, age, and BMI.

Discussion

In this study, the concentration of urinary fluoride in SG-corrected and uncorrected spot urine was not significantly different in pregnancy vs. one-year postpartum (Table 3). Two reports, comparing spot urine fluoride between samples

Table 3 Urinary fluoride (mg/L) at different visits during pregnancy and at one-year postpartum

	Pregnancy ($n=167$)					One-year postpartum ($n=421$)					*p-value**
	n	Mean	Median	SD	Range	*n*	Mean	Median	SD	Range	
Uncorrected urinary fluoride (mg/L)											
Visit 1	135	0.85	0.76	0.51	0.10–2.35	–	–	–	–	–	–
Visit 2	101	0.80	0.65	0.52	0.09–2.73	–	–	–	–	–	–
Visit 3	71	0.75	0.64	0.53	0.01–2.54	–	–	–	–	–	–
All observations	307	0.81	0.69	0.52	0.01–2.73	421	0.77	0.69	0.49	0.07–2.98	0.415
SG-corrected urinary fluoride (mg/L)											
Visit 1	135	0.82	0.75	0.47	0.08–4.73	–	–	–	–	–	–
Visit 2	101	0.83	0.77	0.39	0.28–2.12	–	–	–	–	–	–
Visit 3	71	0.86	0.77	0.51	0.01–3.02	–	–	–	–	–	–
All observations	307	0.83	0.77	0.46	0.01–4.73	421	0.83	0.75	0.37	0.15–2.62	0.950
SG											
Visit 1	135	1.016	1.017	0.007	1.002–1.035	–	–	–	–	–	–
Visit 2	101	1.014	1.014	0.007	1.002–1.033	–	–	–	–	–	–
Visit 3	71	1.013	1.013	0.005	1.003–1.026	–	–	–	–	–	–
All observations	307	1.015	1.015	0.007	1.002–1.035	421	1.017	1.017	0.008	1.001–1.046	0.002

SD, standard deviation; *SG*, specific gravity

*Mann–Whitney test

Table 4 Associations between dietary factors and uncorrected urinary fluoride during pregnancy ($n=167$; 307 observations)

	Urinary fluoride (mg/L)				
	β	SE	95% CI		*p-value*
Dietary fluoride intake (×100 mg/day)	−0.43	10.85	−21.70	20.84	0.968
Adds table salt to meals					
No	Ref	Ref	Ref	Ref	Ref
Yes	−0.01	0.07	−0.15	0.13	0.856
Calcium from RCT					
Placebo	Ref	Ref	Ref	Ref	Ref
Supplement	−0.12	0.05	−0.23	0.00	0.021
Dietary calcium intake (×1000 mg/day)	−0.04	0.10	−0.22	0.15	0.710
Dietary acid load (×10 mEq/day)	−0.04	0.03	−0.09	0.02	0.208
Gestational age (×10 weeks)	0.05	0.02	0.00	0.10	0.050

coefficients from a longitudinal random effects GLS regression, adjusted for urinary specific gravity, total energy intake and pre-pregnancy BMI and age at first pregnancy visit. *β*, regression coefficient; *SE*, standard error; *CI*, confidence interval

of pregnant and nonpregnant women, concluded that lower concentrations of urinary fluoride during pregnancy vs. nonpregnant controls suggested potential evidence of fetal uptake of fluoride [33, 34]. However, those investigations date back several decades and urinary fluoride was not SG-corrected for dilution effects — which are critical considering the use of spot urine samples — and the challenges that pregnancy presents for water homeostasis and hydration [35]. In fact, in this study, we found that urinary SG was significantly lower during pregnancy (urine was more diluted, Table 3), which can artificially lower the levels of the analyte in the sample and highlights the importance of performing comparisons considering the effects of urine dilution. Our result of lack of significant differences in SG-corrected spot urine fluoride levels between pregnancy and one-year postpartum agrees with two experimental studies that utilized 24-h urine collections [16, 36], which do not require correction for dilution. There were, however, changes in urinary fluoride levels within the gestation period. When gestational age was included as an independent variable in a model to explain urinary fluoride during pregnancy, we observed a 0.05 mg/L increase in urinary fluoride for every 10 weeks of gestation (Table 4). This result is consistent with a recent study conducted in a large sample of pregnant women

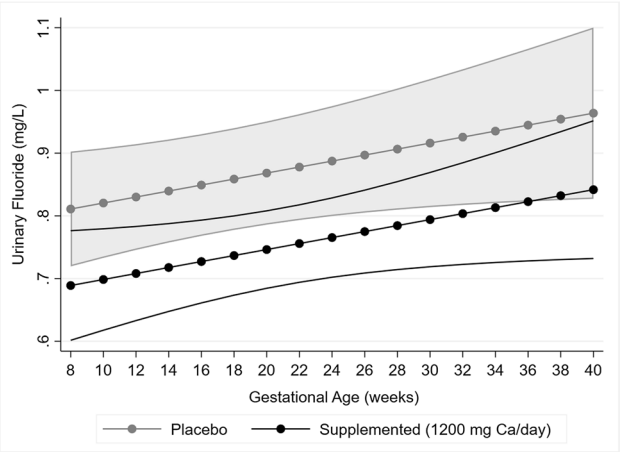

Fig. 2 Predicted urinary fluoride (mg/L) and 95% confidence intervals for non-supplemented (placebo) vs. calcium-supplemented women over the course of gestation, adjusting for urinary specific gravity, total energy intake, pre-pregnancy BMI, and age at first pregnancy visit

($n = 1566$), where maternal urinary fluoride increased from the first to the third trimester [31]. An increase in urinary fluoride over the course of gestation can be explained by the renal-compensated chronic mild respiratory alkalosis experienced during pregnancy, which alkalinizes the urinary pH [13, 37] with a consequent increase in fluoride's excretion rate. Our results differ, however, with a previous study performed in all cohorts from the ELEMENT project which found a tendency for fluoride in urine to decrease over the course of gestation [38]. Methodological differences may explain this discrepancy. In the previous ELEMENT study, the method for the correction of urinary dilution was urinary creatinine, which can be affected by BMI [35, 39], maternal age [40], and gestational age [35]. In the current study, we used urinary SG, which is more appropriate for the correction dilution in spot urine samples when the analyte of interest is subject to renal tubular reabsorption (such as fluoride) [41] and has been reported to be a less variable indicator of hydration status during pregnancy [35]. Collectively, these differences among studies emphasize the influence of dilution-correction methods in the assessment of urinary fluoride in spot urine samples. Changes in urinary fluoride levels have been noted in all studies over the course of gestation, highlighting the importance of including gestational age as a covariate in the analysis of urinary fluoride in pregnant women.

Table 5 Associations between dietary factors and uncorrected urinary fluoride 1 year after postpartum ($n = 421$)

	Log_urinary fluoride (mg/L)	
	Percent change	*p*-value
Fluoride intake (mg/day)	−8.04	0.475
Adds table salt to meals		
No	Ref	Ref
Yes	12.01	0.026
Calcium from RCT		
Placebo	Ref	Ref
Supplement	−1.21	0.768
Dietary calcium intake (× 1000 mg/day)	−10.19	0.159
Dietary acid load (× 10 mEq/day)	−4.51	0.062

coefficients from a cross-sectional OLS linear regression model, adjusted for urinary specific gravity, time since delivery (months), total energy intake, breastfeeding practices, age, and BMI. *β*, regression coefficient; *SE*, standard error; *CI*, confidence interval

Associations between urinary fluoride in second-void spot urine samples and fluoride intake assessed with a FFQ were not found, neither during pregnancy nor one-year postpartum. There is evidence of a positive linear relationship between dietary fluoride intake measured with duplicate-diet approaches and urinary fluoride collected over 24 h in children and nonpregnant adults [1]. However, given that after ingestion, fluoride is rapidly eliminated from the body [10], studies using other dietary assessment methods like questionnaires and recalls over longer periods of time, paired with urine collected over short periods of time (such as the ones that were available for this study), have failed to find significant associations between the dietary intake of fluoride and its urinary excretion [42–44]. Therefore, the question of whether the association between dietary fluoride intake and urinary fluoride excretion differs among pregnant and nonpregnant women remains unanswered, and future studies assessing diet over shorter periods of time paired with 24-h urine samples are encouraged.

As expected for a population exposed to fluoridated salt [19], women who reported adding salt to meals (yes/no) had 12% higher concentration of fluoride in urine compared to those who did not ($p = 0.026$), and this association was observed only at one-year postpartum. The lack of association between reporting addition of table salt to meals and urinary fluoride during pregnancy may be explained by changes in the absorption of fluoride during the pregnant state. It could be hypothesized that the enhancement of vitamin D-mediated intestinal absorption of calcium during pregnancy, which no longer remains in nonpregnant women [45], upregulates the expression of tight-junction proteins that favor the absorption of calcium, in detriment to the intestinal absorption of fluoride [46, 47]. We acknowledge that one of the limitations of this study is the lack of quantitative estimates of table salt intake, and its inclusion would be recommended for future studies in populations exposed to fluoridated salt.

This study was conducted in a population of women who participated in a calcium-supplementation RCT. We found

that women supplemented with calcium (1200 mg/day) had lower urinary fluoride, compared to women assigned to the placebo group. This effect was observed only during pregnancy (not at one-year postpartum) and only with calcium from supplements. A biologically plausible explanation for this finding is that calcium may blunt the increased bone turnover that occurs towards the end of gestation, which mobilizes maternal stores of calcium for fetal transfer [12]. In the cohort of women from which this study's sample was drawn, it was previously demonstrated that calcium administered during pregnancy was associated with reduced bone resorption [18]. Since fluoride is stored in bone, it could be hypothesized that the inhibition of bone resorption associated with calcium supplementation during pregnancy [18] could also inhibit the release of fluoride from bones to plasma. In contrast to what is observed during pregnancy, markers of bone turnover decrease in women who continue lactation one-year postpartum and are not affected by calcium supplementation [48], which would explain why this finding was exclusive to the women who had calcium supplementation during pregnancy.

We also assessed the association between urinary fluoride and the dietary acid load, measured with NEAP estimated from the FFQs [29]. Although acidic diets have shown associations with lower urinary fluoride excretion [8], and in this study, the acid load of the diet had a negative regression coefficient, the association was not significant during pregnancy or at one-year postpartum. The lack of association may be attributed to the use of spot urine samples which usually have weaker correlations with dietary acid load compared to 24-h samples, as previously discussed by Welch et al. [49].

This study had limitations, such as the use of spot urine samples instead of 24-h samples and the lack of quantitative estimates of salt in a population exposed to fluoridated salt. However, it also has several strengths compared to currently available research on this topic including: larger sample sizes, correction for the effects of urinary dilution, repeated measures of fluoride in urine during pregnancy and at one-year postpartum, and the incorporation of regression models controlling for important covariates in each state. Furthermore, we had the ability to test calcium supplementation in a population who participated in a calcium supplementation RCT. Given the increasing number of studies using maternal urinary fluoride as a biomarker of prenatal fluoride exposure and the limited available knowledge on factors influencing urinary fluoride during pregnancy, the conduct of this analysis leveraging already existing data is well-justified.

In summary, these results suggest that dietary influences on urinary fluoride are different in the pregnant vs. the nonpregnant state (one-year postpartum). While urinary fluoride during pregnancy vs. one-year postpartum did not significantly differ, levels did increase as pregnancy progressed. Dietary fluoride intake and dietary acid load measured with 1-month recalls were not associated with urinary fluoride either during pregnancy or at one-year postpartum. Reporting the addition of table salt to meals (yes/no) was not associated with higher urinary fluoride at one-year postpartum but not during pregnancy. Calcium supplementation consumed during the second and third trimesters of pregnancy decreased urinary fluoride during pregnancy but not at one-year postpartum. These results warrant the consideration of the influence of calcium supplementation over the course of pregnancy when using maternal urinary fluoride as a biomarker of fluoride exposure.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12011-021-02799-8.

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Author Contribution Gina A. Castiblanco-Rubio participated in the conception and design of the study, conduction of data analyses, statistical modeling, interpretation of results, and writing of the manuscript. Teresa V. Muñoz-Rocha participated in the management of the ELEMENT database, supervision of data analyses, supervision of statistical modeling, interpretation of data, and edition of the manuscript. Alejandra Cantoral, E. Angeles Martinez-Mier, and Martha M. Téllez-Rojo participated in the conception and design of the study, interpretation of results, and edition of the manuscript. Howard Hu was a co-founder of the ELEMENT cohort and PI of R01ES021446, and Karen Peterson is PI of NIEHS/EPA P01ES022844/RD8354360; both provided oversight of original dietary data collection and participated in interpretation of data and edition of the manuscript. Martha M. Téllez-Rojo and Adriana Mercado-Garcia supervised and coordinated field data collection. Adriana Mercado-Garcia and Adrienne Ettinger participated in the design and implementation of the original cohort study and participated in interpretation of data and edition of the manuscript.

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Data Availability The data that support the findings of this study are available upon reasonable request.

Declarations

Ethics Approval This study was conducted according to the guidelines laid down in the Declaration of Helsinki and the ethics committee of the INSP, Indiana University, Harvard University, and the University of Michigan approved all procedures involving research study participants.

Consent to Participate Written informed consent was obtained from all study participants.

Conflict of Interest The authors declare no competing interests.

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