Dietary Fluoride Intake Over the Course of Pregnancy in Mexican Women

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Ethical Standards Disclosure: 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. Written informed consent was obtained from all study participants.

Abstract

Objective: To estimate dietary fluoride intake (F^-) over the course of pregnancy and the overall adjusted difference in dietary F^- intake by pregnancy stages and levels of compliance with dietary recommendations.

Design: Secondary data analysis from a longitudinal pregnancy cohort study in a population exposed to fluoridated salt. Women were followed during the early, middle and late stages of their pregnancy (n=568). The dietary intake of recommended prenatal nutrients according to Mexican dietary guidelines and F^- intake (mg/day) were estimated with a validated Food Frequency Questionnaire. Data were summarized with descriptive statistics. Levels of F- intake were compared to the USA's IOM Adequate Intake (AI) of 3 mg/day for pregnancy. Adjusted differences in F^- intake by pregnancy stages and levels of compliance with recommendations were estimated using random effects models.

Setting: Mexico City.

Participants: Women participating in the Early Life Exposures in Mexico to ENvironmental Toxicants (ELEMENT) project, from 2001 to 2003.

Results: Median dietary F^- intake (IQR) throughout pregnancy ranged from 0.64 (0.38) in the early-, to 0.70 (0.42) in the middle-, and 0.72 (0.44) mg/day in the late-stage (0.01 mg F^-/kg/day). Corresponding adjusted intakes of F^- [95% CI] were 0.72 [0.70-0.74], 0.76 [0.74-0.77], and 0.80 [0.78-0.82] mg/day. Women who were moderately and highly compliant with Mexican dietary recommendations ingested, on average, 0.04 and 0.14 mg F^-/day more than non-compliant women ($p<0.005$).

Conclusions: Dietary F^- intake was below current AI, was greater with the progression of pregnancy and in women who were moderately and highly compliant with dietary recommendations.

Keywords: fluoride, dietary exposure, prenatal exposure, pregnancy

Introduction

Fluoride (F^-) is present in small amounts in the soil, water, plants, and animals, therefore it is naturally present as a trace element in the diet[(1)]. An extensive body of evidence has proven a clear reduction in dental caries prevalence in communities exposed to F^-, and it has been added to the diet via water or salt for human consumption as part of community fluoridation programs to prevent dental caries[(2)]. Like other nutrients and trace elements in the diet, F^- has both beneficial and detrimental effects: with low exposures, it prevents and controls dental caries, while higher exposures can lead to hard-tissue changes such as dental fluorosis[(3)]. Given that the risk for developing dental fluorosis is present only during critical periods of tooth development, dietary F^- intake has been extensively and traditionally monitored in children[(4)], but rarely in other age groups. There is, however, emerging evidence on potential adverse effects of prenatal F^- exposure[(5)]. Associations between F^- concentration in urine during pregnancy and poor neurodevelopmental outcomes in the offspring have been reported; not only in populations with endemic fluorosis and exposed to high levels of F^-[(6)], but also in populations exposed to low levels, such as the ones considered optimal for community water and salt fluoridation programs[(7–9)]. This newly emerging evidence on the potential side effects of prenatal F^- exposure suggests monitoring of F^- intake in other susceptible groups, such as pregnant women, may be warranted.

In 1997 and based on data collected in children and nonpregnant adults, the USA's Institute of Medicine (USA-IOM) recommended an Adequate Intake (AI) for F^- of 3 mg/day (0.05 mg/kg/day). Using a pre-pregnancy body weight for women >19 yrs of ~61 kg as a reference, the recommendation for both pregnant and non-pregnant women was also set as 3 mg F^-/day[(10)]. Given the existing knowledge-gap on F^- intake in the context of the dietary and physiological changes of pregnancy, there is a need for observational studies of F^- intake in populations of pregnant women. For instance, pregnant women are encouraged to increase the dietary intake of foods and supplements containing nutrients that are beneficial for maternal and fetal health, such as calcium, iron and folate[(10)]. In contrast, the dietary intake of F^- is not particularly encouraged or discouraged during pregnancy and has no reported benefits for fetal health. In a previous investigation on the concentration of F^- in foods and beverages available in Mexico City, we found small amounts of F^- in Mexican dietary staples (cereals, legumes and animal products)[11]. These foods are rich sources of folate, calcium, iron and protein[12] and their frequent consumption may increase the total daily dietary intake of F^-. We hypothesized that Mexican pregnant women, attempting to meet dietary recommendations, increase their dietary intake of F^-. Understanding how F^- intake changes over the course of pregnancy and, whether meeting the requirements of key beneficial nutrients also increases the dietary intake of F^-, can serve the purpose of informing future dietary recommendations for pregnancy.

The objectives of this study were to estimate dietary F^- intake over the course of pregnancy and the overall adjusted difference of dietary F- intake by pregnancy stage and levels of compliance with Mexican dietary recommendations.

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Methods

Study sample and setting

The study population was pregnant women participating in the Early Life Exposures in Mexico to ENvironmental Toxicants (ELEMENT) project, which has been described previously(13). ELEMENT comprises three mother-child pregnancy and birth cohorts, initiated in the 1990s to study early life exposures and health outcomes in Mexico City. In this analysis we included pregnant women from Cohort 3, who were recruited between 2001 and 2003 (N=670). Pregnant women attending three clinics of the Mexican Institute of Social Security (IMSS) were invited to participate, but only those with gestational age <14 weeks, a healthy singleton pregnancy, no history of systemic diseases (hypertension, diabetes) and intention to stay in Mexico City who agreed to participate through informed consent were included and followed thereafter. Sociodemographic questionnaires included several questions on variables such as age, educational attainment, parity, marital status, and self-reported weight before pregnancy. Details and demographics of the entire project can be found in the ELEMENT project's profile publication(13). The project has a dedicated research facility next to Mexico City's ABC Medical Center and participating women were invited to attend the facility at the early-, middle- and late-stages of their pregnancy(13). 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(14). Women were randomized to receive either the calcium supplement (1200 mg calcium/day) or placebo. During each pregnancy visit, each woman was interviewed by a social worker, who performed anthropometry (weight and height) using calibrated instruments, applied a general demographic questionnaire and a Food Frequency Questionnaire (FFQ). Only women from Cohort 3 with FFQ and all variables of interest available in the database were included for the analyses (N=568). All participants who were included had data available for at least two pregnancy stages; and 511 had data for all three.

Measurement of F^- in foods, water and other beverages

Mexico City has naturally-occurring water fluoride levels <0.7 ppm[15] and does not have community water fluoridation as used in other countries. Instead, since 1981 a salt fluoridation program was implemented as a method to deliver F^- for caries prevention (250 ppm of F-/kg) in regions with water fluoride levels <0.7 ppm[16,17]. Therefore, the main sources of dietary F^- intake in Mexico City are foods with intrinsic F^- content and those containing fluoridated salt added either during the cooking process and/or at the table before consumption. A database was developed specifically for the ELEMENT project by analyzing the F^- content of typical foods and beverages in the Mexican diet[11]. Details on the methodology for the collection of food and beverages and the analysis of F^- can be found in the publication by Cantoral et. al[11]. Briefly, fruits and vegetables were bought from 3 different major markets in Mexico City. Meat products, processed foods, juices, beverages and industrialized foods were bought from 4 large supermarket chains. Natural juices were purchased from street vendors, flavored waters (a traditional Mexican beverage prepared with water, fruit and sugar) were bought from ice cream parlors and dairy was purchased in creameries. To measure the concentration of F^- in water drank by participating women, a trained research assistant visited the household of study participants of the ELEMENT project (n=552) and collected water samples (~5 mL). To standardize the concentration of F^- in the water and salt in foods that are consumed cooked (meats, rice, pasta, legumes), these were boiled using water containing negligible amounts of F^- (<0.01 mg/L) and fluoridated salt (250 ppm of F-/kg), following standardized recipes from the National Health and Nutrition Survey. Traditional foods (e.g tamales and corn-based foods) were purchased cooked from street vendors. Fluoride analyses 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[18] as modified by Martinez-Mier et al[19]. Participants reported the intake of mainly bottled and tap water, which had mean F^- levels ±SD of 0.16±0.13 ppm and 0.14±0.09 ppm, respectively. These two average values were the ones included in the database for FFQ-derived estimations of F^- intake from water.

Estimation of dietary intake of F^-, macro-and micronutrients

Dietary F^- intake (mg F^-/day) was assessed through a semiquantitative questionnaire consisting of 104 items, adapted from the Willett semi-quantitative FFQ[20] to include foods and beverages commonly consumed in the 1983 Dietary Survey of the Mexican National Institute of Nutrition[21], and validated to estimate dietary intake over the previous month in Mexican women of child-bearing age (15-44 years)[22]. The FFQ was applied by a trained social worker at each study visit once per trimester, using visual and measuring aids (spoons, cups) for the identification of foods and portion sizes. Estimates of dietary intake of macro- and micronutrients (in µg or mg/day) were calculated through software developed at the National Institute of Public Health (INSP) using methods for the analysis of dietary data from the Mexican National Health and Nutrition Survey[23]. To generate estimates of daily dietary intake, the software utilizes the following data: 1) the average content of nutrients and trace elements (including F^-) of each food/beverage item reported the INSP-compiled nutrient composition database[24] and the ELEMENT F^- database; and 2) the reported frequencies and portion sizes of foods and beverages from the FFQ. Further details on the development, validation and calculations derived from the FFQ are available in the publication by Hernandez-Avila et al[22]. Although the FFQ did not include quantitative estimations of table salt intake, it did include a dichotomous question on whether table salt was added to foods right before eating them (yes/no), which we used for this secondary data analysis. Given that this cohort was originally designed as a calcium RCT and women may had chosen to ingest other dietary supplements as well, a variable with group-assignment to the RCT (placebo/supplemented) and another one specifying intake of other dietary supplements (yes/no) was available to control for potential confounding.

Assessment of compliance with dietary recommendations

Dietary F^- intake in Mexican women is neither contraindicated nor encouraged in Mexican pregnancy dietary recommendations. The Mexican Official Norms regulating health services for pregnant women, that were current for the cohort, highly encouraged an increase in the dietary intake of key nutrients[12] in order to meet the Adequate Intake (AI) or Estimated Average Requirement (EAR) as recommended by the Dietary Reference Intakes (DRIs) by the USA-IOM[10]. Recommendations on dietary intake during pregnancy were made according to pre-pregnancy BMI and pregnancy stage[25]. The four key nutrients encouraged in the dietary recommendations were calcium (AI: 1000 mg/day), iron (EAR: 22 mg/day), folate (EAR:520 µg/day) and protein (EAR: 0.88 g/kg/day). Intake was recommended from various dietary sources for all nutrients[26]; and in the case of calcium, folate and iron, the recommendation included intake from both diet and supplements. The dietary intake of each nutrient was estimated only from the intake reported in the FFQ. Since one of the objectives of this study was focused on the relationship between compliance with nutrients from dietary sources and dietary F^- intake, supplement sources were excluded from the calculation.

Women were classified as compliant with an individual nutrient if their estimated daily dietary intake was equal to or above the AI or EAR for that particular nutrient during pregnancy. Overall compliance at each stage of pregnancy was categorized according to individual-nutrient compliance, as follows: *noncompliance* if noncompliant with all the nutrients (0/4); *moderate compliance* if compliant with one or two nutrients (1/4 or 2/4); and *high compliance* if compliant with three or all the four nutrients (3/4 or 4/4).

Covariates

The selection of covariates was based on our knowledge of factors that may influence dietary F^- intake and bivariate analyses. These included: pregnancy stage, compliance with dietary recommendations, group allocation in the calcium supplementation RCT, intake of other supplements, addition of salt at the table, total daily energy intake, pre-pregnancy BMI and educational attainment.

Data analyses

Study participants were stratified by pregnancy stage. Differences in key dietary variables across pregnancy stages were assessed with Friedman test (for continuous, non-normally distributed repeated measures variables) or chi-square tests (for categorical variables). Differences in dietary F^- intake between the RCT allocation groups and according to table salt use were tested with the non-parametric Mann-Whitney test. To estimate the overall adjusted difference in dietary F^- intake by pregnancy stage (early, middle, late) and levels of compliance with Mexican dietary recommendations (none, moderate, high), we fitted multivariate regression models for panel data using the *xtreg* command in STATA. Panel data models were chosen because they examine both individual- and time-specific effects to deal with unobserved heterogeneity or individual effects. Regression diagnostics revealed significant differences in the individual- and time-specific variance components (panel effect; Breusch-Pagan LM test $p<0.05$) and no correlation between individual effects and the regressors (Hausman test, $p> 0.05$); therefore, one-way random effects GLS regressions provided the best fit. After the model's estimates were obtained, adjusted predictions for dietary F^- intake were calculated for each pregnancy stage using the *margins* command. The association between dietary F^- intake and individual intake of nutrients (calcium, iron, folate, protein) was assessed following the same approach. All analyses were conducted with STATA v16.0 (StataCorp LP, College Station, TX, USA).

Results

Characteristics of the sample and dietary F^- intake by pregnancy stage

Table 1 summarizes and compares the characteristics of women included in the analytical sample (N=568) and those who were excluded (N=102). Overall, the median age of the study participants at the time of recruitment was 26.4 years and the median gestational age for women who completed FFQs at the early, middle or late stages of pregnancy was 13.6, 25.4 and 34.3 weeks, respectively. Median weight ranged between 60.5 - 69.4 kg during the three pregnancy stages and most women (57.7%) had a pre-pregnancy BMI categorized as normal according to the guidelines that were current at the time of data collection[14]. Most women (64.3%) had been pregnant more than once; and their highest educational level was secondary school (67.8%). Finally, in the analytical sample, 289 women were allocated to the treatment group of the calcium supplementation RCT, whereas 279 were allocated to the placebo group. The characteristics of women who were excluded were not significantly different from those who were included.

Detailed descriptive statistics of dietary F^- intake by pregnancy stages and for all available observations are provided in **Table 2.** This sample of pregnant women living in Mexico City had a median dietary F^- intake of 0.69 mg/day, ranging between a minimum of 0.11 and a maximum of 3.73 mg/day (for a total of 1649 observations at all pregnancy stages).

Bivariate statistics for key dietary variables by pregnancy stages

Table 3 summarizes bivariate statistics of key dietary variables by pregnancy stages. We found variability in both dietary F^- intake and total energy intake over the course of pregnancy. Women had a median dietary F^- intake (IQR) that ranged from 0.64 (0.38) in the early stage to 0.72 (0.44) mg F^-/day in the late stage and the tendency to increase was statistically significant. Considering that the median weight across pregnancy was 65 kg, this range of median F^- intake throughout pregnancy corresponds to 0.01 mg/kg/day. Estimates of dietary F^- intake between those who reported adding salt at the table to their meals and those who did not, were not significantly different (0.67 mg/day IQR 0.47 vs 0.70 mg/day, IQR 0.40, respectively) (data not shown in Table 3). In contrast to dietary F^-, median total energy intake significantly decreased during the middle stage of pregnancy. There were also variations in overall compliance and individual-nutrient compliance across pregnancy stages. Compliance with both calcium and iron were associated with pregnancy stage. For calcium, compliance rose from 51.2% in the early, 58.6% in the middle and, 62.0% in the late stages of pregnancy (p<0.001), whereas for iron it went from 6.0% in the early-, 7.1% in the middle- and, 5.5% in the late-stage −although for the latter, no statistically significant association was found. In contrast, compliance with protein was associated (p<0.001) with pregnancy stage (68.7, 62.6 and 56.8% for the early, middle and late stages of pregnancy, respectively). Compliance with folate intake from dietary sources experienced a slight increase towards the middle stage, followed by a decrease towards the end of gestation (5.8, 6.3 and 3.8% for the early, middle and late stages of pregnancy, respectively), although this change was not associated with pregnancy stage (p=0.151). Over the course of pregnancy, women were mostly moderately compliant with dietary recommendations (p=0.412). Only a slight increase in the proportion of women who were noncompliant towards the end of pregnancy was observed (not statistically significant). Towards the end of pregnancy, the proportion of women reporting adding table salt to meals was lower (30.3% in early- vs. 22.6% in late-pregnancy; p=0.012); whereas the proportion of women reporting the use of dietary supplements was higher (23.4% in early- vs. 37.9% in late-pregnancy; p<0.001) (**Table 3**). Median F^- intake from dietary sources for women allocated to the calcium supplementation group in the RCT was significantly higher than that of women allocated to the placebo group (overall median 0.71 vs 0.67, p=0.03; data not shown in Table 3) therefore we also included RCT allocation group as a covariate in the models.

Adjusted dietary F^- intake by pregnancy stages and compliance with dietary recommendations.

After adjustment for covariates, the association between dietary F^- intake and pregnancy stages was significant (p<0.001, **Table 4**). Compared to the early stage, women in the middle and late stages of their pregnancy ingested on average, 0.04 and 0.08 more mg F^-/day, respectively. The adjusted predictions of dietary F^- intake from foods and beverages during pregnancy therefore increase from 0.72 [CI: 0.70-0.74] in the early-stage, 0.76 [CI: 0.74-0.77] in the middle-stage, to 0.80 [CI: 0.78-0.82] in the late stage (**Figure 1**). Women who reported intake of dietary supplements other than the calcium supplement provided for the RCT supplementation group ingested, on average, 0.03 less mg F^-/day, compared to women who did not take other supplements (p=0.027). Furthermore, compared to non-compliant women, those who were moderately and highly compliant with dietary recommendations ingested on average, 0.04 and 0.14 more mg F^-/day, respectively (**Table 4**). The covariates included in the model were total energy intake, allocation group in the calcium supplementation RCT, intake of other supplements and pre-pregnancy BMI.

In order to understand which nutrients were associated with changes in dietary F^- intake, we also assessed the association between compliance with individual nutrients and dietary F^- intake. Compared to women who did not meet calcium and iron recommendations, those who were compliant with calcium and iron recommendations ingested on average, 0.12 and 0.18 more mg F^-/day, respectively (p<0.001). In contrast, women who were compliant with folate recommendations ingested on average, 0.12 less mg F^-/day, compared to those who did not meet recommendations (p=0.009) (**Table 5**). Compliance with protein intake recommendations was not associated with dietary F^- intake.

Discussion

Median dietary F^- intake in this sample of pregnant women living in Mexico City was 0.69 (min - max: 0.11 – 3.73) mg/day, or 0.01 mg/kg/day. To date, only two other studies have reported dietary F^- intake during pregnancy in large samples of pregnant women. In Canada, F^- intake from beverages was assessed in 162 pregnant women living in communities with access to fluoridated water, with a reported intake of (mean ± SD) 0.93 ± 0.43 mg/day[9]. Another study, conducted in Spain in 575 pregnant women also living in a community with access to fluoridated water, reported a median F^- intake from beverages of 0.02 mg/kg/day (min - max: 0.005 – 0.043)[27]. While the median dietary F^- intake reported in our study is lower than in the other study populations, there are differences that limit the ability to make direct comparisons between these three studies. First, the study samples were drawn from populations exposed to different vehicles for community fluoridation. In Mexico City, community-wide fluoridation of salt is used, while in the Canadian and Spanish studies, women had access to fluoridated drinking water. Second, the studies from Canada and Spain only report F^- intake from beverages, while we report F^- intake from both foods and beverages, including bottled and tap water. Nonetheless all three study populations found dietary F^- intakes in pregnancy below the USA-IOM's AI recommendation of from all sources.

Our report has several strengths in the area of F^- exposure assessment, including repeated measures reported over the course of pregnancy, use of a validated instrument of dietary assessment and, a F^- database specific to the population under study. Limitations of this study include those inherent to FFQ-derived estimates and secondary data analyses. First, low F^- water (<0.01 mg/L) and fluoridated salt with standard F^- levels (250 ppm/kg)[16] were used to prepare foods that are consumed cooked. There may be variation in the natural levels of fluoride in water and even among and within brands of salt[15], with the potential to add uncertainty to the estimates. And second, we lack quantitative estimates of salt added after cooking. We believe, however, that as fluoride levels in Mexico City are low (<0.7 ppm)[15] and levels of F- in salt are regulated by the government[16,17], the lack of a quantitative estimate of F^- in salt after cooking is the main source of uncertainty in the present study, which most likely underestimates the true dietary F^- intake. To control for potential differences that could be explained by the habit of adding salt at the table, we used a dichotomous question on the practice of adding table salt to meals after the cooking process (yes/no) that was available in the FFQ. This variable allowed us to make a rough calculation of what total F^- intake would look like in women who add salt to their meals. Using a quantitative report of added table salt by Mexican non-pregnant women aged 23-50 years in the State of Mexico (5.4 g of salt/day)[28] and assuming all salt gets ingested at a concentration of 250 ppm F^-, women in our study who reported adding table salt to their meals (median dietary F^- intake of 0.67 mg/day) would be ingesting about 1.31 mg F^-/day (0.02 mg F^-/kg/day) −approximately double the amount of those who reported not adding extra salt. This rough calculation, however, should be interpreted with caution, as quantitative estimations of salt intake after cooking were not made in the sample under study. We recommend for future studies in populations of pregnant women exposed to fluoridated salt, the inclusion of quantitative measures of table salt intake. Furthermore, the median dietary intake found for this population (0.69 mg/day) constitutes the contribution of only intrinsic F^- in foods and F^- from salt added to foods during the cooking process. To provide a broader perspective of fluoride exposure, future research should also consider the contribution of other sources of F^-, such as occupational exposures and the unintentional intake of fluoridated oral hygiene products.

Dietary F^- intake in this sample of pregnant women increased with the progression of pregnancy, suggesting that dietary F^- intake does change during gestation − as would be expected given increased consumption by pregnant women to meet the nutrient demands of the growing fetus. We were interested in testing whether the increase observed in F^- intake throughout pregnancy remained after controlling for covariates that explain dietary F^- intake such us total energy intake, the practice of addition of salt at the table, and compliance with dietary recommendations. We chose these variables because most nutrients, including F^-, have a positive linear relationship with total energy intake[29]; pregnant women increase their total energy intake towards the end of gestation responding to an increased resting metabolic rate[30]; and there is tendency to change dietary behaviors with the progression of pregnancy[31]. In fact, controlling for total energy intake, women ingested more F^- in the middle and late stage of pregnancy compared to the early stage, plus 0.14 mg F/day (~0.2 mg/day total) if they were compliant with pregnancy dietary recommendations. The biological significance of an increase of ~0.2 mg/day of F^- intake during the third trimester of pregnancy (~7% of the current recommendation) is, however, still unknown and should be considered for future research. Studies on the association between maternal dietary F^- intake and health outcomes in the offspring are needed to investigate the biological significance of the current levels of intake. Only one study has reported that a 1-mg increase in maternal F^- intake from beverages was associated with a 3.7 decrease in intelligence scores among boys and girls[9]; which would be of public health significance for about 10% of the women in the present study (90th percentile, **Table 1**). Further research on the effect of lower prenatal F^- exposure levels on neurodevelopment −such as the median dietary intake found in this study (0.69 mg/day), is needed to inform future dietary F^- intake recommendations for pregnant women.

We were interested in understanding whether Mexican women, attempting to meet dietary recommendations, increase their dietary intake of F^-. We found and association between women who were compliant with recommendations for both calcium and iron and increased F^- intake levels (**Table 5**). This observation could be explained by the frequent consumption of calcium- and iron-rich foods with low-to-moderate amounts of fluoride, which can lead to an overall increase in F^- intake. In Mexico, foods rich in both calcium and iron with a moderate content of F^- include milk, corn-based products, and legumes[11]. We also found that women who reported to consume dietary supplements had lower dietary F^- intakes. A plausible explanation for this negative association is that individuals who proactively take supplements tend to eat lower amounts of nutrient-dense foods[32]. Therefore, it is possible that pregnant women in this sample who reported supplement intake, relied on supplementation to meet their dietary goals instead of choosing more nutrient-dense foods. Sociodemographic factors have also been reported to influence dietary F^- intake[33], however, although we found no association, we cannot entirely tease out their effect on dietary intakes of this study's population given that it included a relatively homogeneous group of women attending the clinics of the Social Security System in Mexico (IMSS) that serves a low-to-middle income population.

Within the limitations of this study, we conclude that the levels of dietary F^- intake were below the current AI, were greater towards the end of gestation and in women who were moderately and highly compliant with Mexican dietary recommendations. Given the mounting evidence of potential adverse effects[7–9] and multiple sources of exposure to F^-, additional assessment and monitoring of dietary intakes and exposures from other community sources, especially in vulnerable populations such as pregnant women and children, should be considered in future dietary recommendations for F^- intake.

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Table 1. Characteristics of women with complete data for all covariates of interest that were included in the analytical sample (included) and women who were excluded because they had incomplete data.

Continuous	All*	Included			Excluded			*p-value*
		n	Median	IQR	n	Median	IQR	
Age at study recruitment (years)	670	568	26.4	8.0	102	27.1	8.1	0.840
Gestational age (weeks)								
Early pregnancy	635	568	13.6	2.3	67	13.4	2.0	0.775
Middle pregnancy	557	554	25.4	2.3	3	24.7	2.3	0.503
Late pregnancy	528	527	34.3	2.5	1	33.7	0.0	0.726
Weight (kg)								
Early pregnancy	668	568	60.5	13.5	100	61.75	15.5	0.798
Middle pregnancy	555	554	65.5	13.1	1	77	0.0	0.462
Late pregnancy	528	527	69.4	13.5	1	58.2	0.0	0.163

Categorical	All*	Included			Excluded			*p-value*
		n	Freq	%	n	Freq	%	
Pre-pregnancy BMI								
Underweight	670	568	35	6.2	102	15	14.7	0.082
Normal			328	57.7		50	49.0	
Overweight			102	18.0		20	19.7	
Obese			103	18.1		17	16.6	
Educational Attainment								
None	670	568	1	0.2	102	0	0.0	0.707
Elementary			129	22.7		21	20.6	
Middle School			385	67.8		68	66.7	
Highschool			53	9.3		13	12.7	
Marital Status								
Married	670	568	387	68.1	102	73	71.6	0.451
Common-law marriage			111	19.5		22	21.6	
Single			69	12.2		7	6.8	
Divorced			1	0.2		0	0.0	
First pregnancy, yes	670	568	203	35.7	102	34	32.1	0.640
Assigned to Ca supl., yes†	670	568	289	50.8	102	45	44.1	0.208

Note: Comparisons between included and excluded women were performed with Mann-Whitney tests for continuous variables and Chi squared tests for categorical variables
*Total number of women with data available for each variable
†Number of women assigned to the calcium supplementation group in the Randomized Clinical Trial

Table 2. Dietary fluoride intake (mg/day) by pregnancy stages. The "All stages" category represents data for all observations available during pregnancy.

Pregnancy stage	N	Mean	SD	Min	10%	25%	50%	75%	90%	Max
Early	568	0.72	0.35	0.11	0.35	0.49	0.64	0.87	1.18	2.05
Middle	554	0.76	0.36	0.14	0.38	0.52	0.70	0.94	1.20	3.73
Late	527	0.79	0.39	0.11	0.40	0.52	0.72	0.96	1.24	3.08
All stages	1649	0.76	0.37	0.11	0.38	0.51	0.69	0.93	1.21	3.73

Table 3. Bivariate statistics for key dietary variables by pregnancy stages.

	Pregnancy Stage						
	Early (n=568)		Middle (n=554)		Late (n=527)		
	Median	IQR	Median	IQR	Median	IQR	*p-value**
Fluoride intake (unadjusted, mg/day)	0.64	0.38	0.70	0.42	0.72	0.44	<0.001
Total Energy Intake (Kcal)	1811.5	802.5	1785.7	828.2	1802.2	706.0	<0.001
	%		%		%		*p-value*†
Compliance with individual nutrients (% above AI or EAR)							
Calcium (AI: 1000 mg/day)	51.2		58.6		62.0		<0.001
Iron (EAR: 22 mg/day)	6.0		7.1		5.5		0.571
Folate (EAR: 520 µg/day)	5.8		6.3		3.8		0.151
Protein (EAR: 0.88 g/kg/day)	68.7		62.6		56.8		<0.001
Compliance‡							
None	25.4		27.1		30.4		
Moderate	66.9		64.7		62.7		0.412
High	7.8		8.1		6.8		
Adds salt at the table, yes	30.3		28.6		22.6		0.012
Use of other supplements, yes	23.4		32.0		38.0		<0.001

IQR: Interquartile range
* Friedman test (n=511)
† Chi-square test
‡ *None:* noncompliant with all of the nutrients (0/4); *moderate:* compliant with one or two nutrients (1/4 or 2/4); and *high:* compliant with three or all the four nutrients (3/4 or 4/4).

Table 4. Adjusted associations between daily fluoride intake (mg/day), pregnancy stage and compliance with dietary recommendations*

n=568	β*	SE	95% CI		p-value
Pregnancy Stage					
Early	Ref	Ref	Ref	Ref	Ref
Middle	0.04	0.01	0.01	0.06	<0.001
Late	0.08	0.01	0.06	0.10	<0.001
Compliance †					
None	Ref	Ref	Ref	Ref	Ref
Moderate	0.04	0.02	0.01	0.08	0.004
High	0.14	0.03	0.08	0.20	<0.001
Intake of other supplements‡					
Yes	-0.03	0.01	-0.05	0.00	0.027
Adds salt at the table					
Yes	-0.01	0.01	-0.04	0.01	0.289

***** Estimates from a one-way random effects GLS regression model adjusted for total energy intake, allocation group in the calcium supplementation RCT, intake of other supplements and pre-pregnancy BMI.

† *None:* noncompliant with all of the nutrients (0/4); *moderate:* compliant with one or two nutrients (1/4 or 2/4); and *high:* compliant with three or all the four nutrients (3/4 or 4/4).

‡ Supplements other than the one provided for the calcium supplementation RCT

Table 5. Adjusted associations between daily fluoride intake and compliance with individual key nutrients*

n=568	β*	SE	95% CI		p-value
Calcium					
Non compliant	Ref	Ref	Ref	Ref	Ref
Compliant	0.12	0.01	0.09	0.14	<0.001
Iron					
Non compliant	Ref	Ref	Ref	Ref	Ref
Compliant	0.18	0.27	0.13	0.24	<0.001
Folate					
Non compliant	Ref	Ref	Ref	Ref	Ref
Compliant	-0.12	0.03	-0.18	-0.064	<0.001
Protein					
Non compliant	Ref	Ref	Ref	Ref	Ref
Compliant	-0.02	0.02	-0.05	0.01	0.247

***** Estimates from a one-way random effects GLS regression model adjusted for pregnancy stage, allocation group in the calcium supplementation RCT, intake of other supplements, total energy intake, addition of salt at the table (yes/no), pre-pregnancy BMI and educational attainment.

Figure legends

Figure 1. Estimated fluoride intake (95% CI) by pregnancy stage*

***** Estimates from a one-way random effects GLS regression model adjusted for total energy intake, allocation group in the calcium supplementation RCT, intake of other supplements and pre-pregnancy BMI.