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# Longitudinal associations between early-life fluoride exposures and cardiometabolic outcomes in school-aged children

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ABSTRACT

*Background/Aim:* Fluoride is a natural mineral present in food, water, and dental products, constituting ubiquitous long-term exposure in early childhood and across the lifespan. Experimental evidence shows fluoride-induced lipid disturbances with potential implications for cardiometabolic health. However, epidemiological studies are scarce. For the first time, we evaluated associations between repeated fluoride measures and cardiometabolic outcomes in children.

*Methods:* We studied ~ 500 Mexican children from the Programming Research in Obesity, Growth, Environment and Social Stressors (PROGRESS) cohort with measurements on urinary fluoride at age 4, and dietary fluoride at ages 4, 6, and 8 years approximately. We used covariate-adjusted linear mixed-effects and linear regression models to assess fluoride associations with multiple cardiometabolic outcomes (ages 4–8): lipids (total cholesterol, HDL, LDL, and triglycerides), glucose, HbA1c, adipokines (leptin and adiponectin), body fat, and age- and sex-specific z-scores of body mass index (zBMI), waist circumference, and blood pressure.

*Results:* Dietary fluoride intake at age 4 was associated with annual increases in triglycerides [β per-fluoride-doubling = 2.02 (95 % CI: 0.37, 3.69)], cholesterol [β = 1.46 (95 % CI: 0.52, 2.39)], HDL [β = 0.39 (95 % CI: 0.02, 0.76)], LDL [β = 0.87 (95 % CI: 0.02, 1.71)], and HbA1c [β = 0.76 (95 % CI: 0.28, 1.24)], and decreased leptin [β = -3.58 (95 % CI: −6.34, −0.75)] between the ages 4 and 8. In cross-sectional analyses at age 8, higher tertiles of fluoride exposure were associated with increases in zBMI, triglycerides, glucose, and leptin ($p$-tertile trend $< 0.05$). Stronger associations were observed in boys at year 8 and in girls prior to year 8 ($p$-sex interaction $< 0.05$). Fewer but consistent associations were observed for urinary fluoride at age 4, indicating increased annual changes in HDL and HbA1c with higher fluoride levels.

*Conclusion:* Dietary fluoride exposures in early- and mid-childhood were associated with adverse cardiometabolic outcomes in school-aged children. Further research is needed to elucidate whether these associations persist at later ages.

## 1. Introduction

Fluoride, the ionic form of the common element fluorine, is frequently used as an additive to prevent tooth decay and promote bone formation (NIH, 2023), particularly during childhood. While fluoride is present in dental products, it is commonly found in fluoridated municipal drinking water, at trace levels in natural water sources, as well as in food and beverages. In Mexico, while municipal water is not fluoridated, there is wide availability of naturally occurring fluoride in drinking water (Armienta and Segovia, 2008; Farías et al., 2021), and exposure via foods containing elevated levels of fluoridated salt (Cantoral et al., 2019). More specifically, salt fluoridation is required in Mexican municipalities when naturally occurring fluoride does not exceed certain limits (0.7 ppm) (Secretaría de Salud, 1994; Gobierno de México, 2023).

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Fluoride measured in urine and plasma is short-lived with an approximate half-life of 5 h in humans (Carlson et al., 1960; Ekstrand and Ehrnebo, 1983). Fluoride intake is widely ubiquitous as it can be ingested daily unintendedly via foods, drinking water, and dental products constituting a continuous long-term exposure. Even though fluoride may be an effective prophylactic against caries, particularly in children (USDHHS, 2015), increased fluoride exposures may also contribute to long-term deleterious effects, such as fluorosis (Holt et al., 1994; Tavener et al., 2006; Wong et al., 2010), DNA methylation changes (Wang et al., 2021), or neurological effects (Ding and Yanhui-Gao, 2011; Xu et al., 2020; Yu et al., 2018; Wang et al., 2007; Malin and Till, 2015; Bashash et al., 2017; Choi et al., 2012), as shown in previous cross-sectional studies, prospective studies, and clinical trials (Holt et al., 1994; Tavener et al., 2006; Wong et al., 2010; Wang et al., 2021; Ding and YanhuiGao, 2011; Xu et al., 2020; Yu et al., 2018; Wang et al., 2007; Malin and Till, 2015; Bashash et al., 2017; Choi et al., 2012). Similar effects have also been observed in experimental studies in animals, and, to a lesser extent, in epidemiological studies in adults, including fluorosis (Everett, 2011), bone fractures (Li et al., 2001), carcinogenicity (Bucher et al., 1991), high blood pressure (Yousefi et al., 2018), liver function (Lu et al., 2017; Wang et al., 2019; Shanthakumari et al., 2004; Guo et al., 2003; Chattopadhyay et al., 2011; de Camargo and Merzel, 1980; Chen et al., 2013), insulin and glucose metabolism impairment (Itai et al., 2021; Trivedi et al., 1993), or endocrine sex-steroid and thyroid hormone disruption (Basha et al., 2011; Shoback and McGhee, 1988; Chen et al., 1988; Puranik et al., 2015; Skórka-Majewicz et al., 2020; Thippeswamy et al., 2021; Kumar et al., 2018; Kheradpisheh et al., 2018; Ortiz-Pérez et al., 2003; Ma et al., 2017; Zhou et al., 2013).

Experimental research shows that fluoride promotes lipid disturbances (Wang et al., 2000; Ma et al., 2012; Oncü et al., 2007; Hassan and Yousef, 2009; Miltonprabu and Thangapandiyan, 2015), suggesting adverse cardiometabolic health effects in humans. However, epidemiological studies are scarce and are mostly cross-sectional. Furthermore, the cardiometabolic effects stemming from fluoride exposure are understudied in children and the few studies available have shown inconclusive results (Liu et al., 2020; Liu et al., 2019; Malin et al., 2019; Xiong et al., 2007; Wang et al., 2020; Bai et al., 2020). Studies in children have focused on obesity (Liu et al., 2020; Liu et al., 2019), liver function (Malin et al., 2019; Xiong et al., 2007), lipids (Liu et al., 2020), glucose (Liu et al., 2020), and hormones (Wang et al., 2020; Bai et al., 2020) primarily. While the evidence is limited in children, findings from these cross-sectional studies indicated potential deleterious health effects including fluoride associations with alterations in liver function (Malin et al., 2019; Xiong et al., 2007), thyroid, and sex hormones (Wang et al., 2020; Bai et al., 2020), Furthermore, results for other cardiometabolic health markers have been inconsistent; for instance, fluoride was associated with increased BMI (Liu et al., 2019), glucose, and overall cardiometabolic risk (Liu et al., 2020), whereas no associations were observed between plasma fluoride and lipids in children (Liu et al., 2020). Because longitudinal studies in children with repeated fluoride exposure measurements across sensitive developmental periods and assessing cardiometabolic outcomes are lacking, the long-term effects of fluoride exposure on cardiometabolic outcomes such as circulating levels of lipids or anthropometric measures other than BMI largely remain understudied.

To date, there is a lack of consensus on an optimal fluoride intake quantity (Warren et al., 2009), and further research is needed especially in children during sensitive developmental periods, given the potential negative health effects raised by prior experimental research (Miltonprabu and Thangapandiyan, 2015; Shoback and McGhee, 1988; Chen et al., 1988; Puranik et al., 2015; Wang et al., 2000; Ma et al., 2012; Oncü et al., 2007).

Therefore, we hypothesized in this study that fluoride exposure is associated with adverse cardiometabolic outcomes in children. In this longitudinal cohort, we examined both cross-sectional and prospective associations of fluoride exposure on cardiometabolic health in children living in Mexico City.

## 2. Materials and methods

### 2.1. Study design and population

We conducted a prospective cohort study to evaluate associations between repeated fluoride measures and cardiometabolic outcomes in Mexican children from the population-based Programming Research in Obesity, Growth, Environment and Social Stressors (PROGRESS) cohort. The PROGRESS cohort enrolled a total of 948 mother-newborn pairs between 2007 and 2011 that have been periodically followed up to date. All children and their respective mothers were affiliated with the Mexican Social Security System (*Instituto Mexicano del Seguro Social* or IMSS). Women were considered for inclusion if they were pregnant, had $\geq$ 18 years of age, were at 12–20 weeks of gestation at enrollment, lived within a designated geographic area, were planning to remain in close geographic proximity to the study for at least three years, and were exempt from chronic diseases. Medical exclusion criteria in mothers were history of infertility, heart or renal disease, diabetes, psychosis or use of anti-epilepsy drugs, use of steroids, consumption of one or more alcoholic drinks per day, or drug addiction at recruitment. Children were followed up consistently after birth, every 2 years ever since. In this study, we refer to ages 4, 6, and 8 as the follow-up visits at approximately 4, 6, and 8 years of age. At all examinations, information on socio-demographic, lifestyle, and environmental factors was collected through validated questionnaires, along with biological samples and health outcomes measured from the children and their mothers using standardized protocols. In the present study, we included approximately 500 children with at least one fluoride and cardiometabolic outcome measure available. All women enrolled in the study provided signed consent in Spanish. This study was approved by the Institutional Review Boards of the Icahn School of Medicine at Mount Sinai, and the National Institute of Public Health in Mexico *(Instituto Nacional de Salud Pública)*.

### 2.2. Fluoride assessment

A 101-item validated food frequency questionnaire (FFQ) was administered to PROGRESS mothers in order to assess dietary patterns of their children over the last 7 days and was repeated at ages 4, 6, and 8 as a proxy of continuous exposure to fluoride specific to diet (Green et al., 2020). Dietary-derived fluoride levels were calculated for 591 children at age 4, 593 at age 6, and 567 at age 8 (**Figure S1**), from estimated total food and beverage fluoride intake levels (in mg/day) using FFQs and based on standard levels of fluoride contents in 182 foods and beverages from Mexico City (Cantoral et al., 2019). PROGRESS FFQs captured frequency (number of days and times per day) and serving size of each food and beverage item. Then, the frequency and the serving size were multiplied by each other to obtain a total intake per week and was divided by 7 to calculate the final average dietary fluoride intake per day. To estimate the fluoride content in foods and beverages, we used an FFQ (Denova-Gutiérrez et al., 2016) validated with a 7-day recall in the Mexican population that adhered to the pattern of foods that are consumed most commonly in Mexico and in which preparations of foods already comprised added salt in concordance with standardized recipes from the Mexican National Health and Nutrition Survey or Encuesta Nacional de Salud y Nutrición (ENSANUT). This methodology allowed us to account for sources of variation stemming from amounts of salt used when cooking foods. Furthermore, in order to account for the variability due to the type of water used to prepare food when characterizing these tables, we used for samples prepared in the laboratory the average fluoride levels in different types of pre-cooked foods (those with high content of salt), as well as in home-cooked foods using a common brand of bottled water used in this population (i.e. Evian water, with a negligible amount of fluoride content of around 0.04 mg/L) (Cantoral

et al., 2019) to not obfuscate the fluoride content in foods. Furthermore, the FFQ assessed the frequency and quantity of drinking water (number of 240 mL-glasses per day). We calculated fluoride intake via drinking water by multiplying the FFQ amounts with the average level of fluoride (0.14 mcg/mL) measured across different water types most commonly consumed in Mexico, including tap water, bottled water, filtered water, street water, and well water (Cantoral et al., 2019).

Urinary fluoride (CUF in μg/mL) was measured in 583 non-fasting children using a spot urine sample collected at follow-up year 4 (**Figure S1**) (Green et al., 2020). Spot urine samples were collected approximately during the morning hours. Urinary excretion is the major pathway eliminating fluoride from the body (Idowu et al., 2019; Rugg-Gunn et al., 2011; WHO, 2014). Therefore, urinary fluoride concentration is a proxy of recent exposure, fluoride intake from multiple sources, including diet, and/or also representative of potentially non-adsorbed exposure (Zohouri et al., 2006; Gillespie et al., 2022; Liu et al., 2011). CUF concentrations were analyzed at the Oral Health Research Institute at Indiana University using a modification of the hexamethyldisoloxane (HMDS; Sigma Chemical Co.) microdiffusion procedure (Martínez-Mier et al., 2009; Martínez-Mier et al., 2011), as detailed previously (Green et al., 2020). To account for variation in urine dilution, CUF concentrations were standardized for specific gravity (SG) (Green et al., 2020; Hauser et al., 2004) as follows: $CUF_{SG}$ (mg/L) = CUF*i* * (SG*m* − 1)/(SG*i* − 1) where $CUF_{SG}$ (mg/L) is the SG standardized fluoride concentration, CUF*i* is the observed fluoride concentration, SG*i* is the SG of the individual urine sample, and SG*m* is the median SG for the sample.

### 2.3. Assessment of cardiometabolic outcomes

We measured cardiometabolic outcomes (lipids, adipokines, glucose, HbA1c, insulin, anthropometric measures, and blood pressure) in PROGRESS children at 4, 6, and 8 years of age following previously published methods. (Kupsco et al., 2021; Jáuregui et al., 2020).

Trained research staff collected fasting blood from children used to measure total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides in mg/dL (Roche Diagnostics, Indianapolis, IN). Low-density lipoprotein cholesterol (LDL) was calculated as the difference between total cholesterol and HDL cholesterol following the Friedewald equation (LDL cholesterol = [total cholesterol] − [HDL cholesterol] − [triglycerides/5], in mg/dL) (Friedewald et al., 1972). Adipokine hormones (in pg/mL) were measured with ultrasensitive ELISA. An enzymatically amplified "two-step" sandwich-type immunoassay was used to measure leptin (R&D Systems, Minneapolis, MN) and an ELISA method from ALPCO Diagnostics Inc. (Salem, NH) was used to measure adiponectin. Levels of HbA1c (%) were determined using the Miura 200 automated analyzer (ISE S.r.l., Rome, Italy). Fasting glucose in plasma was measured in mg/dL using an enzymatic photometric assay (Trinder's method) using a Response 910 automated analyzer (DiaSys Diagnostic Systems, Holzheim, Germany). Plasma insulin levels (uUI/mL) at year 8 were also available and were measured using a solid-phase, enzyme-labeled chemiluminiscent immunoassay (Immulite 1000 analyzer, Siemens, Germany). We calculated insulin resistance using the homeostatic model assessment (HOMA-IR) from fasting insulin and glucose at year 8, when both insulin and glucose levels were available.

Anthropometric measures (weight, height, and waist circumference [WC]), and blood pressure were measured by a trained physician and staff members at each follow-up visit. Body fat percentage was measured at 4, 6, and 8 years of age using tetrapolar bioelectrical impedance instruments InBody 370 or 230 (Biospace Co., Ltd.). At ages 4 and 6, participants had measures from both Inbody 370 and Inbody 230 instruments, so analytical adjustments were made so that all measures were consistent across instruments by using a robust linear model fit on a calibration set of 36 children with concurrent measures ($R^2 = 0.96$), as reported previously (Renzetti et al., 2017). At age 8, all body fat percentage and weight measures were consistently analyzed by the Inbody 230 instrument. Height was measured twice using a SECA mechanical wall stadiometer to the nearest 0.5 cm (Hamburg, Germany) model 206. We then used the weight and height measures to calculate sex- and age-standardized BMI z-scores (zBMI) following World Health Organization (WHO) guidelines as follows: for children under 5 years of age, overweight was defined as a weight-for-height greater than 2 standard deviations (SD) above WHO Child Growth Standards median, (WHO, 2023*a*) whereas for above 5 years of age overweight was defined as 1 SD above. (WHO, 2023*b-c*) Mean WC was calculated from the average of two measurements above the iliac crest using a SECA measuring tape. Resting systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured multiple times at each visit and averaged. At ages 4 and 6, we used both SpaceLabs Healthcare automated oscillo-metric device (Ambulatory BP 90207 monitor, Washington) with a child-sized cuff (Sanders et al., 2018), as well as a Baumanometer. Both SpaceLabs and Baumanometer measurements were collected twice each and averaged. Then, the average of the mean of the two measurements was calculated to obtain the final mean SBP and DBP at each follow-up (at 4 and 6 years). At age 8, we averaged two blood pressure measurements collected using SpaceLabs.

With the exception of BMI, for which we used WHO growth references z-scores for children (WHO, 2023*a-c*) as described above, other anthropometric biomarkers that are known to vary by age and sex specifically during childhood (WC, SBP, and DBP) (Jiang et al., 2014; Grajda et al., 2017; Muyumba et al., 2018; Fernández et al., 2004; Noubiap et al., 2022) were age and sex z-score transformed using internal cohort standardization. We also combined multiple cardiometabolic risk components to calculate two cardiometabolic risk scores (with and without insulin due to low sample size of the latter outcome), using the sum of z-score transformed WC, BP (average of SBP and DBP), glucose and triglycerides-to-HDL ratio at ages 4, 6, and 8, with and without including insulin at age 8, following Viitasalo et al. method (Viitasalo et al., 2014).

### 2.4. Additional covariates

Information on demographic factors (age of the mother and child) was self-reported at recruitment during the 2nd trimester of pregnancy, follow-up, or at childbirth (child sex). Lifestyle information was collected during pregnancy including information on smoking (passive or active smoking) and parity. Pre-pregnancy BMI was estimated in accordance to validated methods using the height at recruitment (during pregnancy) and the last menstrual period reported weight (Thomas et al., 2019). Socio-economic status (SES) was estimated using the Mexican Association of Market and Public Opinion Research Agencies (Spanish acronym AMAI) index used in Mexico (Villegas Carrasco, 2002). The AMAI index considered a total of 13 factors including the education of the head of household and number of appliances in the household, car ownership, and so forth. The index classifies families into 6 levels, which were further collapsed into 3 SES categories: lower, medium, and high. Puberty in PROGRESS children was captured by the Tanner scale for follow-up year 8. Trained study pediatricians performed Tanner staging as follows: they Tanner staged pubic hair for both sexes, genitals for boys using an orchidometer, and breast development for girls, and assigned values ranging from 1 to 5, where stage 1 indicated that an individual did not initiate development while stages above 1 indicated puberty onset or sexual maturity (Marshall and Tanner, 1970; Marshall and Tanner, 1969).

### 2.5. Statistical analyses

Descriptive analyses included children characteristics and biomarker distributions by follow-up examinations. Influential outliers (implausible values) were observed in body fat measures for two participants who were excluded from subsequent statistical analyses. An overview of the statistical methods used to estimate associations between fluoride exposure and cardiometabolic outcomes across multiple ages is shown in

Figure S2. We also conducted diagnostic tests when conducting analyses, which indicated normal distribution of errors centering at 0, as well as no visible heteroskedasticity (Figure S3-S4) nor multicollinearity among variables ($\rho < 0.44$).

We used covariate-adjusted Linear Mixed-Effects Models to assess annual changes in cardiometabolic outcomes (between continuous 4 and 8 years of age) per fluoride unit increase. First, we ln-transformed biomarkers that did not follow a normal distribution to improve normality (triglycerides, HbA1c, insulin, HOMA-IR, and adipokines leptin and adiponectin). We then re-transformed results by exponentiating the value, subtracting 1, and multiplying by 100 yielding estimates in percent changes for these outcomes. Then, we modeled fluoride exposure $\log_2$-transformed and in tertiles at ages 4 (urinary and dietary), 6 (dietary), and 8 (dietary) and evaluated changes in the following cardiometabolic risk components measured at 4, 6, and 8 years of age: total cholesterol (in mg/dL), HDL (in mg/dL), LDL (in mg/dL), and triglycerides (%), as well as glucose (mg/dL), HbA1c (%), body fat (%), adipokines leptin and adiponectin (%), and age- and sex-adjusted z-scores of body mass index (zBMI), waist circumference (zWC), and systolic and diastolic blood pressure (zSBP and zDBP). In addition, we used linear regression models to estimate cross-sectional fluoride-outcome associations: linear models examined the effect of dietary fluoride on the aforementioned cardiometabolic outcomes cross-sectionally at each visit (ages 4, 6, and 8), as well as the effect of urinary fluoride at age 4 and dietary fluoride at ages 4, 6, and 8 on insulin (%) and HOMA-IR (%) measured at age 8. Linearity of associations was evaluated using univariate Generalized Additive Models (GAM) when applicable.

We selected confounders adjusted for in the statistical models based on *a priori* knowledge and/or statistically significant associations with the exposures and outcomes of interest in our sample. Given that participants' follow-up visits were not exactly at age 4, 6, or 8, we also centered the baseline age in the study at age 4 for all analyses. Models controlled for continuous baseline-centered age, SES, and sex of child and maternal smoking status during pregnancy, continuous age at partum, parity status, and continuous pre-pregnancy BMI. We additionally controlled for children's total energy intake (kcal) as continuous in models examining dietary fluoride exposures. Children with higher levels of fast food consumption tend to accumulate higher caloric intake (Vikraman et al., 2015) but also tend to consume foods with higher fluoride content (Cantoral et al., 2019). Associations between fluoride exposures and cardiometabolic risk score (with insulin at age 8 and without insulin) were also evaluated. Covariate-adjusted Poisson Generalized Linear Mixed-Effects Models (GLMM) were used to assess fluoride effects on incidence rates of overweight/obesity status (compared to normal and underweight) between the ages of 4 and 8. Incidence rate ratios were obtained by exponentiating the Poisson regression coefficients. Covariate-adjusted Poisson GLM models were conducted to assess cross-sectional associations.

We restricted all analyses to children with available fluoride exposure at the time-point of interest and outcomes across all visits. Additional sensitivity analyses included: 1) analyses restricted to children with both complete dietary fluoride and outcome data across ages to facilitate a direct comparison of effect estimates at year 8 with similar

**Table 1**
**Children characteristics by follow-up examinations** [a]

| Variable | Follow-Up Year 4 ~Age 4 [b] | | Follow-Up Year 6 ~Age 6 [c] | Follow-Up Year 8 ~Age 8 [d] |
|---|---|---|---|---|
| | **Study Sample with Urinary Fluoride** N = 491 | **Study Sample with Dietary Fluoride** N = 500 | **Study Sample with Dietary Fluoride** N = 532 | **Study Sample with Dietary Fluoride** N = 562 |
| **Fluoride Levels (µg/mL or mcg/day)** | 0.74 (0.41) [0.00, 5.36] | 693 (315) [148, 2,770] | 745 (265) [169, 2,333] | 795 (312) [152, 2,431] |
| **Maternal Age at Partum** | 28.1 (5.6) [18.4, 44.4] | 28.1 (5.7) [18.3, 44.4] | 28.2 (5.6) [18.3, 44.4] | 28.1 (5.6) [18.3, 44.4] |
| **Age (Years)** | 4.82 (0.56) [4.01, 6.70] | 4.81 (0.55) [4.01, 6.70] | 6.74 (0.57) [5.98, 9.66] | 9.70 (0.69) [8.09, 12.08] |
| **Socioeconomic Status (AMAI Index)** | | | | |
| Low | 258 (53 %) | 267 (53 %) | 286 (54 %) | 302 (54 %) |
| Medium | 186 (38 %) | 186 (37 %) | 195 (37 %) | 205 (36 %) |
| High | 47 (9.6 %) | 47 (9.4 %) | 51 (9.6 %) | 55 (10 %) |
| **Maternal Smoking during Pregnancy** [e] | | | | |
| No Smoking (Any) | 306 (62 %) | 312 (62 %) | 333 (63 %) | 351 (62 %) |
| Smoking (Any) | 185 (38 %) | 188 (38 %) | 199 (37 %) | 211 (38 %) |
| **Maternal Pre-pregnancy BMI ($kg/m^2$)** | 26.5 (4.1) [18.4, 43.5] | 26.5 (4.1) [18.4, 43.5] | 26.5 (4.1) [18.4, 43.5] | 26.5 (4.1) [18.4, 43.5] |
| **Maternal Parity Status at Enrollment** | | | | |
| 1 pregnancy | 192 (39 %) | 195 (39 %) | 204 (38 %) | 215 (38 %) |
| 2 pregnancies | 168 (34 %) | 168 (34 %) | 185 (35 %) | 198 (35 %) |
| 3 + pregnancies | 131 (27 %) | 137 (27 %) | 143 (27 %) | 149 (27 %) |
| **Caloric Intake (kcal)** | – | 1,683 (639) [606, 4,883] | 1,902 (592) [518, 4,309] | 1,982 (744) [554, 5,309] |
| **Sex** | | | | |
| Female | 241 (49 %) | 251 (50 %) | 262 (49 %) | 274 (49 %) |
| Male | 250 (51 %) | 249 (50 %) | 270 (51 %) | 288 (51 %) |
| **Obesity or Overweight Status** [f] | 58 (12 %) | 55 (11 %) | 152 (29 %) | 260 (46 %) |
| **zBMI** [f] | 0.30 (1.11) [-3.83, 6.63] | 0.27 (1.11) [-3.83, 6.63] | 0.45 (1.33) [-4.33, 7.13] | 0.88 (1.30) [-3.01, 4.50] |

[a] Mean (SD) [Range]; n (%). Restricted in participants with available data on fluoride exposure (urinary in µg/mL and dietary in mcg/day) and BMI.
[b] Restricted to participants with fluoride exposure at follow-up year 4 and BMI at years 4, 6, and 8.
[c] Restricted to participants with fluoride exposure at year 6 and BMI at years 6 and 8.
[d] Restricted to participants with fluoride exposure and BMI at year 8.
[e] Any maternal smoking (active or passive) during pregnancy.
[f] Continuous 2007 WHO BMI z-scores: underweight (<-2SD), normal (between ≥ -2 SD and ≤ +1SD), overweight (> +1 SD and ≤ +2 SD), and obese (> +2SD).

**Table 2**
**Children anthropometric and cardiometabolic biomarker distributions by follow-up examinations** [a]

| Variable | Biomarker Level at Follow-Up Year 4 ~Age 4 [b] | | Biomarker Level at Follow-Up Year 6 ~ Age 6 [c] | Biomarker Level at Follow-Up Year 8 ~ Age 8 [d] |
|---|---|---|---|---|
| | Study Sample with Urinary Fluoride | Study Sample with Dietary Fluoride | Study Sample with Dietary Fluoride | Study Sample with Dietary Fluoride |
| ***Time-varying Outcomes*** | | | | |
| **zBMI** [e] | 0.30 (1.11) | 0.27 (1.11) | 0.45 (1.33) | 0.88 (1.30) |
| | [-3.83, 6.63] | [-3.83, 6.63] | [-4.33, 7.13] | [-3.01, 4.50] |
| | n = 491 | n = 500 | n = 532 | n = 562 |
| **zWC** | 0.05 (1.01) | 0.03 (1.00) | 0.02 (1.01) | 0.00 (0.98) |
| | [-1.94, 5.41] | [-1.94, 5.41] | [-1.81, 4.16] | [-1.89, 3.39] |
| | n = 496 | n = 505 | n = 532 | n = 563 |
| **Body Fat (%)** | 24.2 (6.28) | 24.1 (6.17) | 25.0 (7.95) | 30.6 (8.82) |
| | [3.00, 47.8] | [3.00, 47.78] | [6.80, 50.4] | [3.00, 52.4] |
| | n = 478 | n = 483 | n = 516 | n = 560 |
| **zDBP** [f] | −0.02 (0.97) | −0.02 (0.96) | −0.05 (0.98) | 0.00 (0.98) |
| | [-2.67, 3.15] | [-2.67, 3.15] | [-2.54, 3.03] | [-2.60, 3.57] |
| | n = 473 | n = 483 | n = 511 | n = 538 |
| **zSBP** [f] | −0.03 (0.96) | −0.03 (0.97) | −0.03 (0.99) | 0.00 (0.98) |
| | [-2.38, 2.51] | [-2.38, 3.17] | [-2.49, 2.65] | [-2.61, 3.33] |
| | n = 473 | n = 483 | n = 511 | n = 538 |
| **Leptin (ng/mL)** [g] | 3.13 (3.16) | 3.11 (3.15) | 5.49 (6.85) | 10.8 (10.9) |
| | [0.11, 27.2] | [0.11, 27.2] | [0.35, 60.5] | [0.72, 68.6] |
| | n = 320 | n = 326 | n = 428 | n = 514 |
| **Adiponectin (ng/mL)** [g] | 15.5(7.16) | 15.4 (7.20) | 12.5 (6.47) | 13.8 (16.0) |
| | [0.72, 45.1] | [0.72, 45.1] | [0.98, 45.8] | [0.03, 107.8] |
| | n = 317 | n = 323 | n = 429 | n = 513 |
| **Cholesterol (mg/dL)** | 162.2 (27.1) | 162.5 (27.4) | 163.3 (20.5) | 158.3 (30.6) |
| | [101.0, 254.7] | [101.0, 254.7] | [87.0, 267.0] | [74.0, 323.0] |
| | n = 331 | n = 338 | n = 441 | n = 524 |
| **HDL (mg/dL)** | 49.7 (9.26) | 49.7 (9.29) | 51.5 (12.0) | 49.4 (11.5) |
| | [26.5, 79.0] | [26.5, 79.0] | [5.53, 127.6] | [22.1, 92.0] |
| | n = 330 | n = 337 | n = 441 | n = 524 |
| **LDL (mg/dL)** | 96.1 (24.5) | 96.5 (24.7) | 95.8 (18.5) | 90.4 (26.3) |
| | [16.6, 186.2] | [16.6, 186.2] | [23.8, 226.7] | [5.44, 245.0] |
| | n = 330 | n = 337 | n = 440 | n = 523 |
| **Triglycerides (mg/dL)** | 81.8 (46.0) | 81.8 (45.7) | 80.1 (35.8) | 92.5 (48.3) |
| | [34.0, 458.0] | [34.0, 458.0] | [27.0, 313.0] | [17.0, 420.0] |
| | n = 331 | n = 338 | n = 440 | n = 523 |
| **HbA1c (%)** | 5.23 (0.39) | 5.23 (0.39) | 5.39 (0.33) | 4.91 (0.61) |
| | [3.30, 6.60] | [3.30, 6.60] | [3.16, 6.72] | [1.09, 6.51] |
| | n = 329 | n = 335 | n = 440 | n = 524 |
| **Glucose (mg/dL)** | 86.4 (11.9) | 86.3 (11.6) | 86.8 (9.37) | 86.3 (9.84) |
| | [43.7, 136.0] | [43.7, 136.0] | [53.9, 114.4] | [58.4, 131.4] |
| | n = 330 | n = 337 | n = 440 | n = 523 |
| **Cardiometabolic Risk Score 1** [h] | −0.07 (2.28) | −0.06 (2.28) | −0.02 (2.37) | 0.01 (2.29) |
| | [-5.54, 9.07] | [-5.54, 9.07] | [-6.30, 11.2] | [-5.35, 8.46] |
| | n = 317 | n = 324 | n = 421 | n = 499 |
| ***Outcomes at Year 8 Only*** | | | | |
| **Plasma insulin at year 8 (uUI/mL)** [i] | 2.64 (3.95) | 2.65 (3.94) | 2.63 (3.93) | 2.66 (3.93) |
| | [0.38, 43.5] | [0.38, 43.5] | [0.38, 43.5] | [0.38, 43.5] |
| | n = 238 | n = 240 | n = 259 | n = 261 |
| **HOMA-IR at year 8** [i] | 0.58 (0.91) | 0.58 (0.91) | 0.58 (0.91) | 0.58 (0.91) |
| | [0.06, 10.5] | [0.06, 10.5] | [0.06, 10.5] | [0.06, 10.5] |
| | n = 238 | n = 240 | n = 259 | n = 261 |
| **Cardiometabolic Risk Score 2 at year 8** [i,j] | −0.12 (2.76) | −0.14 (2.77) | −0.13 (2.78) | −0.13 (2.78) |
| | [-5.72, 9.01] | [-5.72, 9.01] | [-5.72, 9.01] | [-5.72, 9.01] |
| | n = 228 | n = 230 | n = 250 | n = 251 |

[a] Mean (SD) [Range]; n (%). Restricted to participants with available data on fluoride exposure (urinary and dietary) and each biomarker. Biomarkers with skewed distributions (leptin, adiponectin, triglycerides, HbA1c, insulin, and HOMA-IR) are not ln-transformed in the descriptive table but are ln-transformed for analyses.

[b] Restricted to participants with fluoride exposure at follow-up year 4 and BMI at years 4, 6, and 8. Plasma insulin, HOMA-IR, and cardiometabolic risk score accounting for insulin were only available at follow-up year 8 and sample size shown restricts to participants with fluoride exposure at year 4 and biomarker data at follow-up year 8.

[c] Restricted to participants with fluoride exposure at follow-up year 6 and BMI at years 6–8. Plasma insulin, HOMA-IR, and cardiometabolic risk score accounting for insulin were only available at year 8 and sample size shown restricts to participants with fluoride exposure at year 6 and biomarker data at year 8.

[d] Restricted to participants with fluoride exposure and BMI at year 8.

[e] Continuous 2007 WHO BMI z-scores.

[f] Average of SpaceLab and Baumanometer measurements.

[g] pg/mL units divided by 1000.

[h] Score composed of waist circumference, glucose, blood pressure (average of DBP and SBP), triglyceride/HDL ratio. Age- and sex-stratified z-scores were calculated for every item.

[i] Outcome only available at follow-up year 8.

[j] Score composed of waist circumference, glucose, blood pressure (average of DBP and SBP), triglyceride/HDL ratio, and insulin. Age- and sex-stratified z-scores were calculated for every item.

sample size than at previous ages, 2) analyses using covariate-adjusted Linear Mixed-Effects Models with missing information in the outcome to facilitate comparisons of effect estimates across ages and improve efficiency in models, assuming a missing at random pattern of incomplete data, 3) stratified analyses and evaluation of potential effect modification by sex across all follow-up visits, 4) analyses additionally adjusting for puberty status at age 8 (in sex-stratified analyses), and 5) a descriptive data comparison between the subset samples with the lowest and highest sample size that were divergent in statistical significance across ages to evaluate potential selection bias from lost-to-follow-up and/or missing data.

## 3. Results

The majority of PROGRESS mothers whose children were included in analyses had low socio-economic status (53–54 %), did not smoke actively or passively during pregnancy (62–63 %), and were parous prior to enrollment (61–62 %) (Table 1). The mean (SD) age of children at follow-up year 4 (the baseline age in this study) was 4.81 (0.55) years. The prevalence of overweight or obesity in children progressively increased from 11 to 12 % at the baseline age of 4 years to 29 % at age 6 and 46 % by age 8. Similar to the increased prevalence for overweight or obesity observed by age, average distributions for the following cardiometabolic outcomes examined increased remarkably from follow-up year 4 to year 8 (Table 2): zBMI, body fat percentage, leptin, triglycerides, and overall cardiometabolic risk score. Dietary fluoride intake in children presented low ($\rho = 0.12$, $p = 0.006$, between follow-up year 4 and 8) to moderate correlations ($\rho = 0.36$, $p < 0.001$, between follow-up year 4 and 6) across visits (data not shown), and increased on average —mean (SD)— over time, from 693 (315) mg/day to 745 (265) mg/day, and 795 (312) mg/day at follow-up years 4, 6, and 8, respectively (Table 1). Higher exposure levels to fluoride were observed in boys compared to girls across all ages (**Table S1**). For instance, urinary fluoride levels in girls were on average —mean (SD)— 0.72 (0.30) μg/mL whereas in boys were 0.75 (0.50) μg/mL.

A doubling of dietary fluoride intake at age 4 was associated with an annual increase in triglycerides [β = 2.02 % (95 % CI: 0.37, 3.69)] (**Fig. 1**, **Table S2**), total cholesterol [β = 1.46 mg/dL (95 % CI: 0.52, 2.39)], HDL [β = 0.39 mg/dL (95 % CI: 0.02, 0.76)], LDL [β = 0.87 mg/dL (95 % CI: 0.02, 1.71)], and HbA1c [β = 0.76 % (95 % CI: 0.28, 1.24)], and an annual decrease in leptin [β = -3.58 % (95 % CI: −6.34, −0.75)] between the ages of 4 and 8 years. Using the dietary fluoride variable at age 4 in tertiles, we observed that associations were consistent in directionality and yielded linear trends (**Table S2**; $p$-tertile trend < 0.05) for most biomarkers: children in the top tertile intake of dietary fluoride had an annual 2.30 mg/dL (95 % CI: 0.81, 3.79) increase in levels of total cholesterol ($p$-tertile trend = 0.003), an annual 1.77 mg/dL (95 % CI: 0.43, 3.11) increase in levels of LDL ($p$-tertile trend = 0.012), and an annual 6.21 ng/mL (95 % CI: 1.76, 10.5) decrease in levels of leptin ($p$-tertile trend = 0.008), compared to children in the bottom tertile for fluoride intake. At age 6, dietary fluoride intake was associated with higher HDL levels only [β per-fluoride-doubling = 4.40 mg/dL (95 % CI: 1.70, 7.11); $p$-tertile trend = 0.035]. No associations were observed between dietary fluoride exposure at ages 4 or 6 in relation to anthropometric changes in zBMI, zWC, body fat, zSBP, and zDBP over time (**Fig. 2**).

Cross-sectional analyses examining cardiometabolic associations with dietary fluoride exposures at age 8 (**Table S2**; **Figs. 3-4**) indicated that higher tertiles of fluoride exposure were associated with higher levels of BMI z-scores ($p$-tertile trend = 0.042), triglycerides ($p$-tertile trend = 0.045), glucose ($p$-tertile trend = 0.019), leptin ($p$-tertile trend = 0.012), higher odds of overweight status ($p$-tertile trend = 0.033), and had a marginal association with cardiometabolic risk score ($p$-tertile trend = 0.074). Analyses using $\log_2$-transformed fluoride exposure at year 8 were consistent in directionality but only analyses with glucose remained statistically significant [β per-fluoride-doubling = 3.47 mg/dL (95 % CI: 1.21, 5.73)]. GAM univariate models indicated no potential deviations from linearity in any of the exposure-outcome associations examined with $p$-values ranging from $p = 0.11$ (for dietary fluoride with zBMI and glucose) to $p = 0.90$ (for dietary fluoride with HbA1c). Dietary fluoride at age 4 was also positively related to insulin at age 8 [β per-fluoride-doubling = 24.6 % (95 % CI: 1.02, 53.7)], and dietary fluoride at age 6 was also positively related to insulin resistance at age 8 (β per-fluoride-doubling = 34.6 % (95 % CI: 1.83, 77.8)] in cross-sectional analyses.

In sensitivity cross-sectional analyses at year 8 with lower sample size (for comparability with previous years), we found associations of similar direction and magnitude between dietary fluoride and cardiometabolic outcomes when we restricted analyses to children with complete data across ages to facilitate comparability of effect estimates across examination periods (**Table S2**). In sensitivity longitudinal analyses with missing information in the outcome (ages 4–8) with larger sample size (for comparability with later ages), we observed for the most part overall consistent statistically significant annual changes (**Table S3**) than in main analyses (**Table S2**; **Fig. 1**), with only fluoride effects on LDL being the most attenuated in statistical significance.

Urinary fluoride exposure measured at age 4 yielded a lower number of associations: higher levels of urinary fluoride were associated only with annual increases in HbA1c levels [β per-fluoride-doubling = 0.37 % (95 % CI: 0.08, 0.65); $p$-tertile trend = 0.035] and HDL levels [β per-fluoride-doubling = 0.23 mg/dL (95 % CI: 0.01, 0.45)], or had a negative association at baseline with triglycerides at age 4 [β per-fluoride-doubling = -5.76 % (95 % CI: −8.94, −2.47)] (**Figure S5**). Furthermore, null associations were observed between urinary fluoride exposure at age 4 and anthropometric outcomes in children between the ages of 4 and 8 (**Figure S6**).

Analyses testing for interaction effects between dietary fluoride and sex indicated stronger associations in girls compared to boys between the ages of 6 and 8, while stronger associations were found in boys compared to girls at age 8 (**Table S4**). In longitudinal models, we observed that there were consistent greater annual increases in triglyceride levels ($p$-interaction for fluoride tertile trend and sex = 0.009; $p$-interaction for $\log_2$-fluoride and sex = 0.006) and greater annual decreases in adiponectin levels ($p$-interaction for fluoride tertile trend and sex = 0.030; $p$-interaction for $\log_2$-fluoride and sex = 0.010) in girls compared to boys between the ages of 6 and 8 in relation to dietary fluoride exposures at age 6. Cross-sectional exploratory analyses at age 8 additionally adjusting for puberty onset indicated potential interaction effects by male sex, where boys had higher dietary fluoride-induced levels of total cholesterol ($p$-interaction for fluoride tertile trend and sex = 0.046), and LDL ($p$-interaction for fluoride tertile trend and sex = 0.047) compared to girls. Lastly, no consistent effect modification by sex was observed between urinary fluoride and cardiometabolic outcomes.

## 4. Discussion

Findings from this study suggest a potential role of fluoride exposure at several childhood periods on adverse cardiometabolic changes, including alteration of lipids, adipokines, glucose, glycated hemoglobin, and obesity in school-aged children approximately 4–8 years of age. These associations were observed primarily with respect to dietary fluoride rather than fluoride measured in urine, the latter being only a proxy of short-term fluoride exposure through multiple sources (other sources beyond diet). Stronger effects of dietary fluoride were particularly observed in boys compared to girls during 8 years of age, and in girls compared to boys prior to age 8.

To our knowledge, this is the first longitudinal study in young children examining both urinary and dietary fluoride exposures with multiple cardiometabolic endpoints. Most published studies evaluating the effects of fluoride exposure on cardiometabolic health were conducted in adolescents (Liu et al., 2020; Malin et al., 2019) or adults (Itai et al., 2021; Trivedi et al., 1993; Yousefi et al., 2018). For instance, Liu et al.

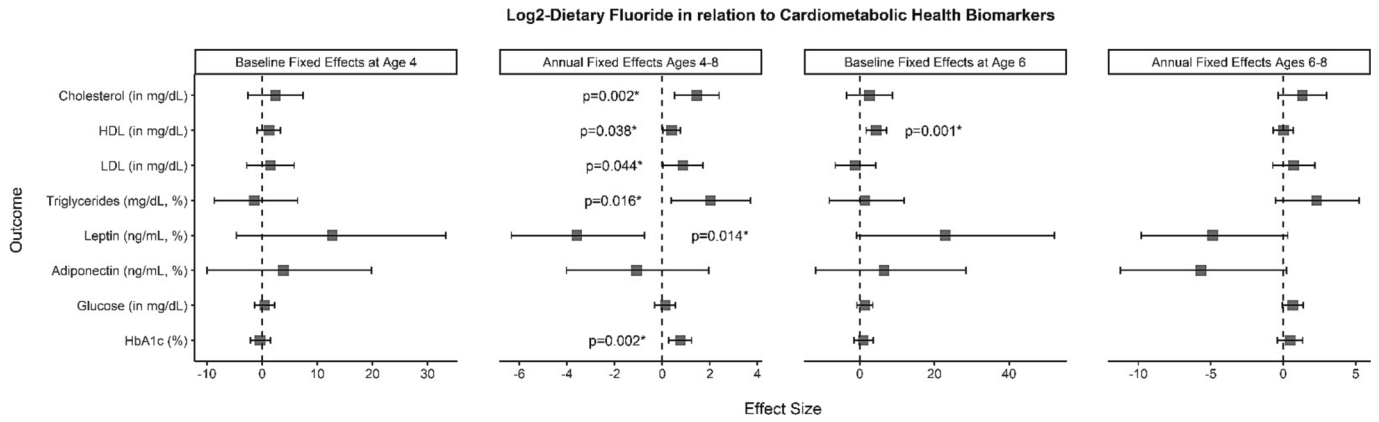


**Fig. 1. Longitudinal Associations between Dietary Fluoride Exposures at Ages 4 and 6 and Changes in Cardiometabolic Biomarkers in PROGRESS Children through Age 8. Squares represent effect estimates and whiskers represent 95% confidence intervals. * P<0.05 denotes statistical significance..**

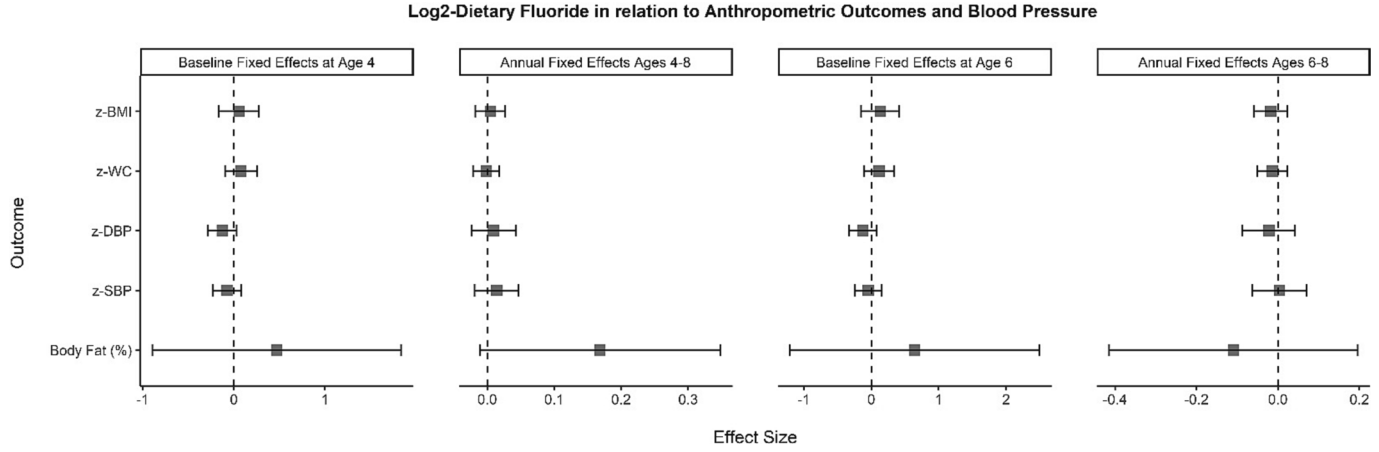


**Fig. 2. Longitudinal Associations between Dietary Fluoride Exposures at Ages 4 and 6 and Changes in Anthropometric Measures and Blood Pressure in PROGRESS Children through Age 8. Squares represent effect estimates and whiskers represent 95% confidence intervals. * P<0.05 denotes statistical significance.**

(Liu et al., 2020) observed in Mexican adolescents a cross-sectional association of plasma fluoride and higher levels for several cardiometabolic outcomes (BMI, WC, trunk fat percentage, blood pressure, glucose and insulin), but these associations were observed only in girls and no associations were observed in relation to lipids, unlike in our study in children. Only a few studies examined fluoride exposures and cardiometabolic outcomes in children or infants but these were cross-sectional: (Thippeswamy et al., 2021; Liu et al., 2019; Wang et al., 2020; Bai et al., 2020) Ballantyne et al. (Ballantyne et al., 2022) reported either null associations between plasma fluoride and blood pressure, anthropometry, lipids, glucose metabolism and inflammation markers, or an inverse association with HbA1c levels. Liu et al. (Liu et al., 2019) indicated that urinary fluoride concentrations were associated with higher weight, BMI, and higher odds of overweight and obesity, similar to our findings with dietary fluoride but not with urinary fluoride. Other cross-sectional studies on cardiometabolic outcomes in children linked fluoride (in water, plasma, or urine) to liver and kidney function alterations (Xiong et al., 2007) and hormones, such as sex steroid hormones (Bai et al., 2020) and thyroid hormones: Thippeswamy et al. (Thippeswamy et al., 2021) observed an alteration of parathyroid hormones in cord blood of newborns with high fluoride exposure from drinking water and Wang et al. (Wang et al., 2020) also observed low-to-moderate fluoride exposures in drinking water and in urine with dysregulations in thyroid hormone function.

In our study, we observed changes in lipids, diabetes biomarkers (glucose and glycated hemoglobin), and hormone leptin that were primarily affected by dietary fluoride exposures. Mechanisms that may increase the risk of fluoride-induced adverse cardiometabolic outcomes include inflammation, oxidative stress, and hormone disruption mechanisms. Preliminary experimental studies in animals suggest that chronic fluoride exposure can alter membrane lipids of rat liver (Wang et al., 2000) and (Wang et al., 2000; Oncü et al., 2007) can increase inflammatory cytokine response in rabbit aorta (Ma et al., 2012). Toxicological studies also suggest that fluoride exposure increases cardiotoxicity (Miltonprabu and Thangapandiyan, 2015) and insulin resistance (Lima Leite et al., 2014), and can alter circulating thyroid hormone levels (Basha et al., 2011; Shoback and McGhee, 1988; Chen et al., 1988; Puranik et al., 2015) and lipid peroxidation (Oncü et al., 2007). Furthermore, fluoride can increase serum triglycerides and LDL cholesterol levels, while decreasing HDL, individually (Hassan and Yousef, 2009; Miltonprabu and Thangapandiyan, 2015). Levels of HDL, also known as “good” cholesterol, were also altered but were positively related to fluoride exposure in our study, though this association disappeared by follow-up year 8. Endocrine disrupting chemicals can alter levels of lipids during early life and may not manifest deleterious effects until later in life (Heindel et al., 2017; Sarr et al., 2012; Padmanabhan et al., 2016; Barouki et al., 2012). This premise should be followed up by evaluating early life fluoride exposures in relation cardiometabolic effects at later years in adolescence and in adulthood. Our study is also the first study that links fluoride with changes in levels of adipokines across

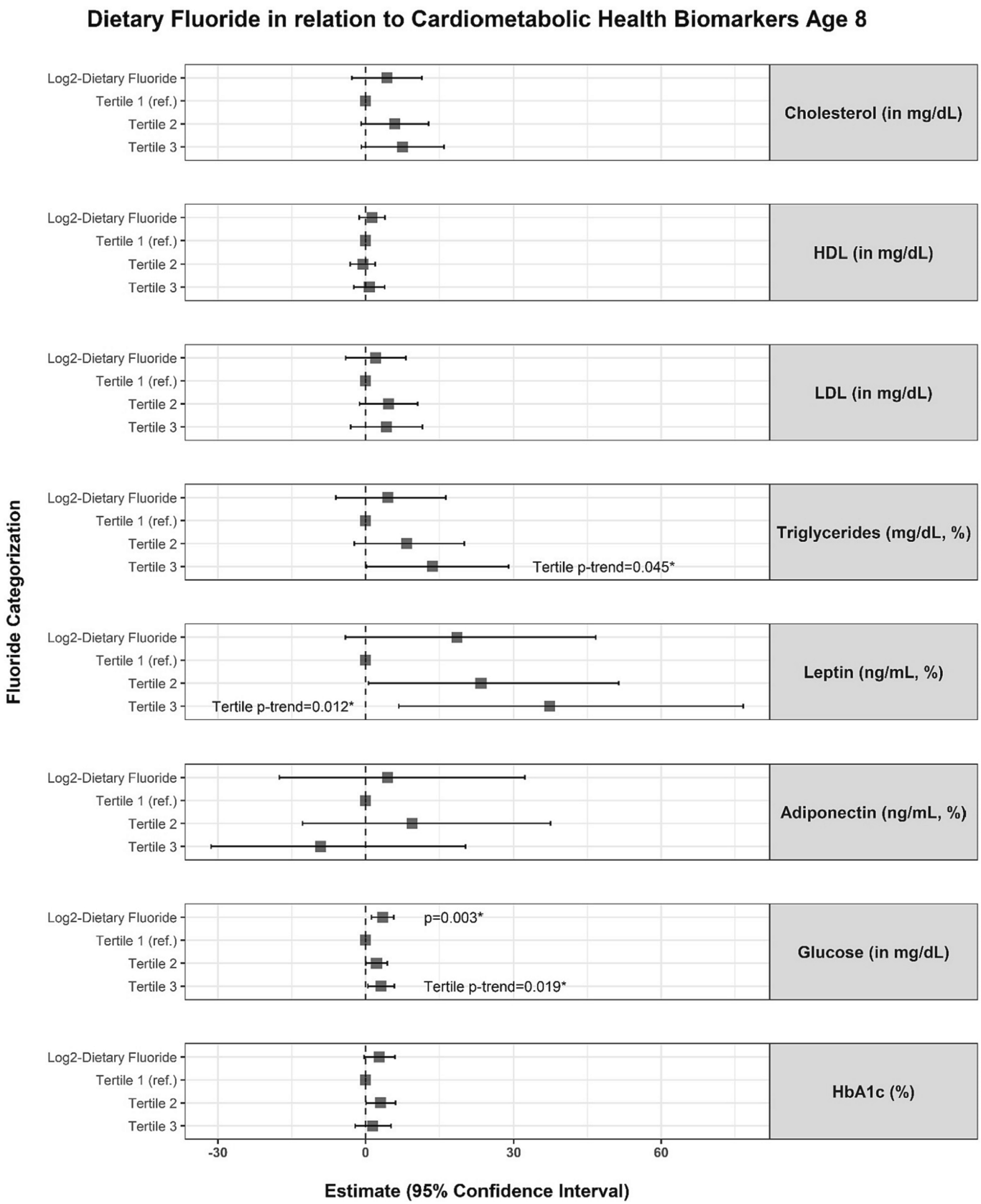


**Fig. 3. Cross-sectional Associations between Dietary Fluoride Exposures at Age 8 and Cardiometabolic Biomarkers in PROGRESS Children at Age 8. Squares represent effect estimates and whiskers represent 95% confidence intervals. * P<0.05 denotes statistical significance.**

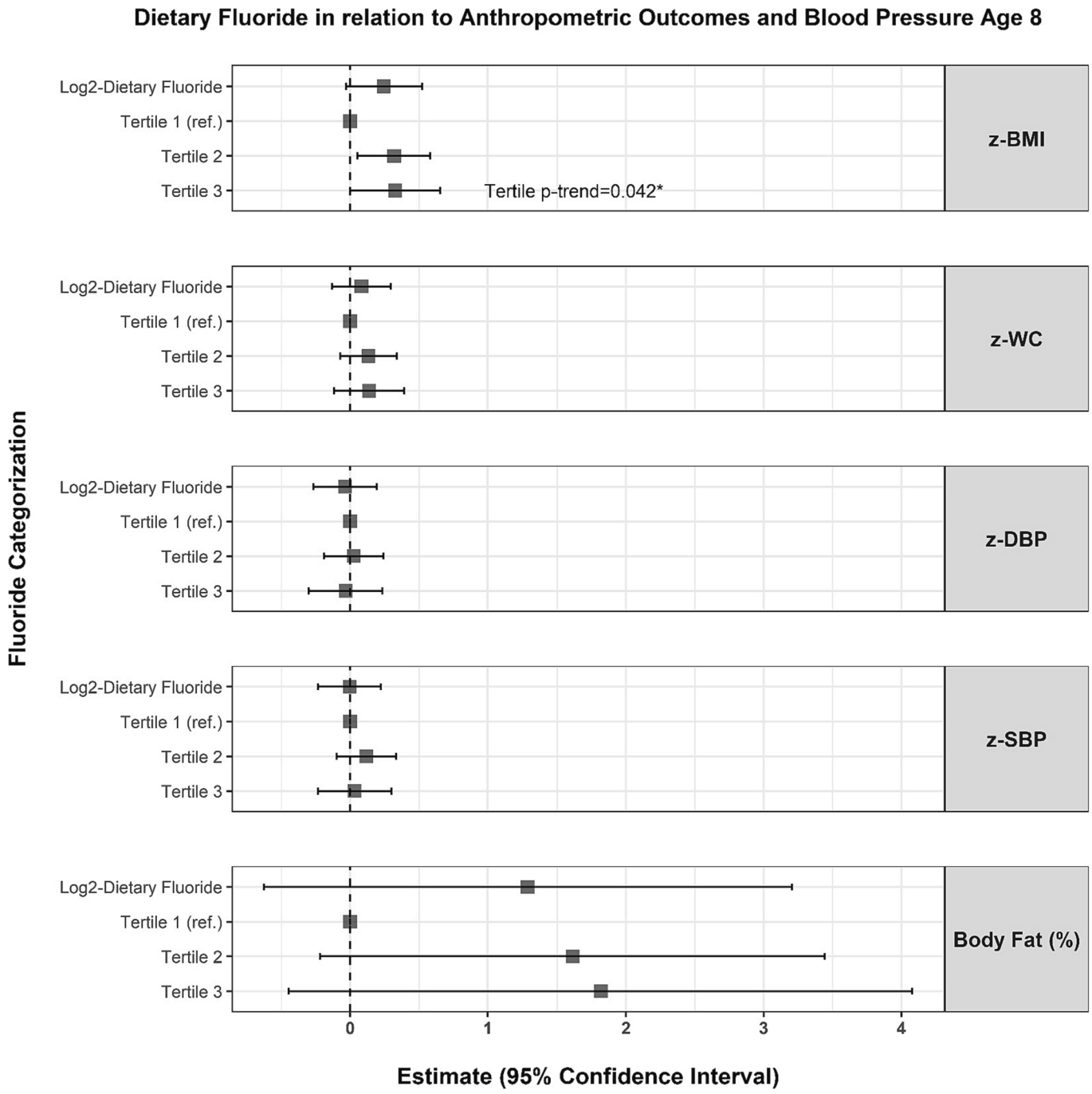


**Fig. 4. Cross-sectional Associations between Dietary Fluoride Exposures at Age 8 and Anthropometric Measures and Blood Pressure in PROGRESS Children at Age 8. Squares represent effect estimates and whiskers represent 95% confidence intervals. * P<0.05 denotes statistical significance.**

childhood. Adipokines have been implicated in the development of metabolic syndrome (MetS): higher leptin levels can promote insulin resistance (Campos et al., 2018). Several studies conducted in youth, including pre-pubertal children, linked elevated levels of leptin with higher levels of MetS-related markers and obesity (Madeira et al., 2017; Yoshinaga et al., 2008; Mi et al., 2010; Valle-Martos et al., 2021), consistent with our findings in Mexican children at year 8.

We also found evidence that dietary fluoride during mid- to late-childhood (~around 8 years of age) was related to higher BMI levels. Previous findings in the Mexican PROGRESS cohort have further linked fluoride with worsened kidney function (Saylor et al., 2022) and IQ (Cantoral et al., 2021) in children. A previous marginally significant association was reported in PROGRESS between fluoride exposure and lower glomerular filtration rate in a subset of children with obesity (Saylor et al., 2022). This may suggest that fluoride's potential metabolism-disrupting effects in children could be exacerbated by BMI. Furthermore, experimental research indicates a potential role of the gut microbiome in mediating fluoride-induced effects on obesity (Chen et al., 2022). On the other hand, our study did not observe any associations with other anthropometric factors such as body fat, blood pressure, and waist circumference in pre-pubertal children. This is consistent with a few of the abovementioned cross-sectional studies in children (Ballantyne et al., 2022) but inconsistent with the evidence in adolescents (Liu et al., 2020). Yet, it is noteworthy that both anthropometric markers and biomarker data at year 8, particularly body fat, WC, and cholesterol, shared a positive directionality with dietary fluoride,

consistent with our hypothesis that fluoride may induce overall deleterious cardiometabolic effects.

Dietary fluoride exposure did not correlate with urinary fluoride at age 4 in our study and clear association patterns with cardiometabolic outcomes were not as evident with respect to urinary fluoride. This could likely be explained by fluoride's short half-life in the body of only a few hours (Carlson et al., 1960; Ekstrand and Ehrnebo, 1983) and associated measurement error stemming from a single spot urine sample collection (Idowu et al., 2019; Kumah et al., 2022). FFQ data represent an estimation of dietary-based exposure over a longer period (one week), and thus, it could be more representative of long-term fluoride exposures. The lack of correlation between urinary fluoride concentrations and estimated dietary fluoride could also suggest potential differences in adsorbed fluoride retained in body tissues, organs, and bones and levels of excreted fluoride in urine. Only a fraction of fluoride exposure is excreted in urine while the rest is partially retained in the skeletal system (Villa et al., 2010). Urinary fluoride concentrations represent recent absorption and releases from long-term osseous accumulation due to continuous bone tissue remodeling (Grandjean, 2019; Fawell et al., 2006). Urinary fluoride concentration is representative of recent exposure, fluoride intake from multiple sources other than diet, such as dental hygiene products, but also representative of potentially non-adsorbed exposure (Zohouri et al., 2006; Gillespie et al., 2022; Liu et al., 2011), which could explain the non-significant positive correlation between urinary and dietary fluoride at age 4 observed in our study (Spearman $\rho = 0.062$, p-value $= 0.137$). A previous study indicated a lack of correlation between fluoride intake and urinary fluoride excretion in children 6–7 years of age (Zohoori et al., 2013), similar to our cohort. While our primary findings were observed primarily with dietary fluoride exposures and less so with urinary fluoride, associations for lipids (HDL and triglycerides) tended to be in the same direction as those observed for dietary fluoride exposures.

We found evidence to suggest a potential dysregulated metabolism induced by fluoride that differed by sex in pre-pubertal children. In Mexico, fluoride exposure primarily originates from fluoridation in salt, (Cantoral et al., 2019) followed by naturally occurring drinking-water fluoride exposure (Armienta and Segovia, 2008; Farías et al., 2021). Greater consumption of fluoride-based diets or salty foods in boys at later ages could explain higher fluoride levels in this population and subsequent altered metabolism as evidenced by a higher level of fluoride estimated in boys than girls across all ages and media. Most importantly, we observed that fluoride associations in boys were stronger than in girls during the last follow-up visit where fluoride levels were the highest across time points. However, differences in exposure levels may not fully explain sex interactions, and divergent effects could also be attributed to sex-differences in fluoride absorption, metabolism and/or secretion. During the start of puberty, a wide array of metabolic changes can occur and create susceptibility to endocrine-disrupting chemicals, which can result in delayed or advanced puberty onset (Chen et al., 2011; Grandjean et al., 2012; Harley et al., 2017). In our study, we only found consistent effect modification effects by male sex in cross-sectional analyses around 8 years of age in the last follow-up, though we observed for some biomarkers (triglycerides and adiponectin) interactions by female sex prior to age 8. Previous cross-sectional studies conducted in a Mexican population of adolescents ages 10–18 (Liu et al., 2020) and in a Chinese cohort of children ages 7–13 (Liu et al., 2019) indicated fluoride-induced cardiometabolic effects in girls but not in boys. Even though these studies did not measure dietary fluoride, the latter study in Chinese children measuring urinary fluoride indicated substantially lower average exposure concentrations of about half (0.4 mg/L) the levels in our cohort (0.7 μg/mL). Estimated urinary fluoride levels in our cohort are consistent with previously reported levels in other populations living in areas with water fluoride levels below the recommended safety standard limits (Green et al., 2020; Zohoori et al., 2013; Czarnowski et al., 1996) established in drinking water by the WHO (1.5 mg/L) and the CDC (2.0 mg/L) in the general population (Boehmer et al., 2023; WHO, 2017). The inconsistent results found in Mexican boys around age 8 compared to the results from the aforementioned cross-sectional studies indicating stronger association at later ages in girls may suggest possible susceptible windows for fluoride exposure at different concentrations that could affect cardiometabolic health outcomes distinctly across the life course. This warrants further investigation in other longitudinal cohorts with longer follow-up through adolescence and with variability in fluoride exposures.

Despite these novel findings in a longitudinal cohort of children, we acknowledge several limitations in our study. The FFQ-derived estimates for fluoride intake may be prone to measurement error as well as the availability of only one spot-urine sample at age 4. In the fluoride intake assessment, we used one average value from drinking water because of a lack of more detailed information on drinking water sources in our cohort, which may not fully capture individual variability of fluoride intake in drinking water. However, the cohort comes from the same geographical municipal region (Mexico City) as the drinking samples from wells and municipal water obtained, and therefore, our estimates based on the quantity of drinking water measured in the FFQ can serve as a proxy of individual drinking water fluoride level. Results for our FFQ-derived fluoride estimates in association with cardiometabolic outcomes have been partly replicated previously in another Mexican population which provides additional confidence in our findings (Liu et al., 2020). Another study limitation is the lack of data pertaining to fluoride intake earlier in infancy. We also did not directly measure the frequency of use of fluoridated toothpaste or dental hygiene products in PROGRESS, which could be an important source of fluoride exposure in children and were only indirectly captured in the fluoride levels measured in urine in our study. Similarly, we did not have available information about the brand of salt consumed which is a possible limitation of our study. Furthermore, sample size restrictions with complete-case analyses (for exposure and outcome) between ages 4 and 8 may have impeded us from detecting associations. Our sensitivity analyses with missing values in the outcome (**Table S3**) as well as descriptive data comparing analytical subset samples (**Tables S5-S7**) showed that all lifestyle factors and non-time-varying covariates were equally distributed across samples, indicating that missing values were missing at random and complete-case analyses were not subject to selection bias. Moreover, our cardiometabolic risk score in the larger sample size did not account for insulin, and the results between the two cardiometabolic risk scores (with and without accounting for insulin) differed, yet sample size was substantially lower when accounting for insulin and likely prone to lower statistical power. Last, our sample representing children from Mexico City is relatively homogenous and findings may not be generalizable to other ages or different races and ethnicities. On the other hand, important strengths of this study include the longitudinal, prospective design, the evaluation of repeated dietary-based fluoride measurements, and the assessment of a comprehensive panel of cardiometabolic risk factors.

Fluoridation of drinking water has been implemented in multiple countries to promote dental health. These findings can help inform whether fluoridation practices are an optimal cost-effective measure to improve health. Even though fluoride can be excreted from the body within hours, a potential long-lasting endocrine-disrupting role of the chemical should be considered in future studies. In this study, we identified an environmental and modifiable potential risk factor for cardiometabolic disease in the sensitive childhood period, which is of public health relevance due to the rising cardiometabolic disease epidemic worldwide and the particular high prevalence (>38 %) and rising trend of overweight and obesity in Mexican children ages 5–11 (INSP, 2020; Shamah-Levy et al., 2018). Furthermore, since 1993, Mexico has had a national program of iodized and fluoridated salt, which mandates that for every kg of salt there be approximately 200 mg to 250 mg of fluoride mixture (Secretaría de Salud, 1994; Gobierno de México, 2023). These results could be of high relevance in a country like Mexico where in the general population the intake of salt, the primary

source of fluoride other than dental products, largely exceeds the WHO recommendation (Vargas-Meza et al., 2016), and can be informative for public health interventions.

## 5. Conclusion

Fluoride exposure from diet during early- and mid-childhood was primarily associated with potentially adverse cardiometabolic outcomes in school-aged children between 4 and 8 years of age. We particularly observed dietary fluoride-associated increases in lipids, diabetes-related biomarkers, and BMI, and found evidence of potential sex-specific associations at different periods during childhood. More longitudinal studies are needed to corroborate these findings and associations at later ages.

## CRediT authorship contribution statement

**Sandra India Aldana:** Conceptualization, Data curation, Formal analysis, Software, Visualization, Methodology, Writing – original draft. **Elena Colicino:** Methodology, Writing – review & editing. **Alejandra Cantoral Preciado:** Conceptualization, Writing – review & editing. **Maricruz Tolentino:** Writing – review & editing. **Andrea A. Baccarelli:** Writing – review & editing. **Robert O. Wright:** Funding acquisition, Writing – review & editing. **Martha María Téllez Rojo:** Conceptualization, Funding acquisition, Writing – review & editing. **Damaskini Valvi:** Conceptualization, Funding acquisition, Methodology, 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

Data will be made available on request.

## Acknowledgments

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## Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2023.108375.

## References

Armienta, M.A., Segovia, N., 2008. Arsenic and fluoride in the groundwater of Mexico. Environ Geochem Health. 30 (4), 345–353. https://doi.org/10.1007/s10653-008-9167-8.

Bai, R., Huang, Y., Wang, F., Guo, J., 2020. Associations of fluoride exposure with sex steroid hormones among U.S. children and adolescents, NHANES 2013-2016. Environ Pollut. May 2020;260:114003. doi:10.1016/j.envpol.2020.114003.

Ballantyne, J.A., Coyle, G., Sarwar, S., Kühn, T., 2022. Fluoride status and cardiometabolic health: findings from a representative survey among children and adolescents. Nutrients 14(7) (2022). doi: 10.3390/nu14071459.

Barouki, R., Gluckman, P.D., Grandjean, P., Hanson, M., Heindel, J.J., 2012. Developmental origins of non-communicable disease: Implications for research and public health. Environ. Health. 2012/06/20 2012;11(1):42. doi:10.1186/1476-069X-11-42.

Basha, P.M., Rai, P., Begum, S., Dec 2011. Fluoride toxicity and status of serum thyroid hormones, brain histopathology, and learning memory in rats: a multigenerational assessment. Biol. Trace Elem. Res. 144 (1–3), 1083–1094. https://doi.org/10.1007/s12011-011-9137-3.

Bashash, M., Thomas, D., Hu, H., et al., 2017. Prenatal fluoride exposure and cognitive outcomes in children at 4 and 6–12 years of age in Mexico. Environ. Health Perspect. 125 (9) https://doi.org/10.1289/EHP655.

Boehmer, T.J., LS, Espinoza, L., Ladva, C.N., 2023. Community Water Fluoridation Levels To Promote Effectiveness and Safety in Oral Health — United States, 2016–2021. Vol. 72:593–596. 2023. MMWR Morb Mortal Wkly Rep.

Bucher, J.R., Hejtmancik, M.R., Toft 2nd, J.D., Persing, R.L., Eustis, S.L., Haseman, J.K., 1991. Results and conclusions of the national toxicology program's rodent carcinogenicity studies with sodium fluoride. Int. J. Can. 48 (5), 733–737. https://doi.org/10.1002/ijc.2910480517.

Campos, R., Masquio, D.C.L., Corgosinho, F.C., et al., Jun 2018. Relationship between adiponectin and leptin on osteocalcin in obese adolescents during weight loss therapy. Arch. Endocrinol. Metab. 62 (3), 275–284. https://doi.org/10.20945/2359-3997000000039.

Cantoral, A., Luna-Villa, L.C., Mantilla-Rodriguez, A.A., et al., Dec 2019. Fluoride content in foods and beverages from Mexico city markets and supermarkets. Food Nutr Bull. 40 (4), 514–531. https://doi.org/10.1177/0379572119858486.

Cantoral, A., Luna-Villa, L.C., Mantilla-Rodriguez, A.A., et al., 2019. Fluoride content in foods and beverages from Mexico City markets and supermarkets. Food Nutrit. Bull. 40 (4), 514–531. https://doi.org/10.1177/0379572119858486.

Cantoral, A., Téllez-Rojo, M.M., Malin, A.J., et al., Dec 2021. Dietary fluoride intake during pregnancy and neurodevelopment in toddlers: a prospective study in the progress cohort. Neurotoxicology. 87, 86–93. https://doi.org/10.1016/j.neuro.2021.08.015.

Carlson, C.H., Armstrong, W.D., Singer, L., 1960. Distribution and excretion of radiofluoride in the human. Proceedings of the Society for Experimental Biology and Medicine. 1960/06/01 1960;104(2):235-239. doi:10.3181/00379727-104-25791.

Chattopadhyay, A., Podder, S., Agarwal, S., Bhattacharya, S., 2011. Fluoride-induced histopathology and synthesis of stress protein in liver and kidney of mice. Archives of Toxicology Archiv für Toxikologie. Apr 2011 2014-08-02 2011;85(4):327-35. doi: 10.1007/s00204-010-0588-7.

Chen, C.J., Anast, C.S., Brown, E.M., Jun 1988. Effects of fluoride on parathyroid hormone secretion and intracellular second messengers in bovine parathyroid cells. J. Bone Miner. Res. 3 (3), 279–288. https://doi.org/10.1002/jbmr.5650030306.

Chen, A., Chung, E., DeFranco, E.A., Pinney, S.M., Dietrich, K.N., 2011. Serum PBDEs and age at menarche in adolescent girls: Analysis of the National Health and Nutrition Examination Survey 2003–2004. Environmental Research. 2011/08/01/ 2011;111(6):831-837. Doi: 10.1016/j.envres.2011.05.016.

Chen, J., Cao, J., Wang, J., et al., 2013. Effects of fluoride on growth, body composition, and serum biochemical profile in a freshwater teleost, Cyprinus carpio. Environ. Toxicol. Chem. 2013;32(10):2315-21. doi:10.1002/etc.2305.

Chen, G., Peng, Y., Huang, Y., et al., 2022. Fluoride induced leaky gut and bloom of Erysipelatoclostridium ramosum mediate the exacerbation of obesity in high-fat-diet fed mice. J. Adv. Res. https://doi.org/10.1016/j.jare.2022.10.010.

Choi, A.L., Sun, G., Zhang, Y., Grandjean, P., 2012. Developmental fluoride neurotoxicity: a systematic review and meta-analysis. Environ. Health Perspect. 120 (10), 1362–1368.

Czarnowski, W., Wrześniowska, K., Krechniak, J., 1996. Fluoride in drinking water and human urine in northern and central Poland. Sci Total Environ. 191 (1–2), 177–184. https://doi.org/10.1016/0048-9697(96)05259-x.

de Camargo, A.M., Merzel, J., 1980. Histological and histochemical appearance of livers and kidneys of rats after long-term treatment with different concentrations of sodium fluoride in drinking water. Acta Anat (basel). 108 (3), 288–294. https://doi.org/10.1159/000145310.

Denova-Gutiérrez, E., Ramírez-Silva, I., Rodríguez-Ramírez, S., Jiménez-Aguilar, A., Shamah-Levy, T., Rivera-Dommarco, J.A., 2016. Validity of a food frequency questionnaire to assess food intake in Mexican adolescent and adult population. Salud Pública de México. 12/15 2016;58(6):617-628. doi:10.21149/spm.v58i6.7862.

Ding, Y., YanhuiGao, S.H., et al., 2011. The relationships between low levels of urine fluoride on children's intelligence, dental fluorosis in endemic fluorosis areas in Hulunbuir, Inner Mongolia, China. J. Hazard. Mater. 186 (2–3), 1942–1946. https://doi.org/10.1016/j.jhazmat.2010.12.097.

Ekstrand, J., Ehrnebo, M., Oct 1983. The relationship between plasma fluoride, urinary excretion rate and urine fluoride concentration in man. J. Occup. Med. 25 (10), 745–748. https://doi.org/10.1097/00043764-198310000-00014.

Everett, E.T., 2011. Fluoride's effects on the formation of teeth and bones, and the influence of genetics. J. Dent. Res. 90 (5), 552–560. https://doi.org/10.1177/0022034510384626.

Farías, P., Estevez-García, J.A., Onofre-Pardo, E.N., et al., 2021. Fluoride exposure through different drinking water sources in a contaminated basin in Guanajuato, Mexico: a deterministic human health risk assessment. Int. J. Environ. Res. Public Health. Oct 31 2021;18(21)doi:10.3390/ijerph182111490.

Fawell, J., Bailey, K., Chilton, J., Dahi, E., Magara, Y., 2006. Fluoride in drinking-water. IWA Publishing.

Fernández, J.R., Redden, D.T., Pietrobelli, A., Allison, D.B., Oct 2004. Waist circumference percentiles in nationally representative samples of African-American, European-American, and Mexican-American children and adolescents. J Pediatr. 145 (4), 439–444. https://doi.org/10.1016/j.jpeds.2004.06.044.

Friedewald, W.T., Levy, R.I., Fredrickson, D.S., Jun 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 18 (6), 499–502.
Gillespie, G., Jackson Rudd, D., Zhang, S., et al., Feb 2022. A phase 1 trial to evaluate the relationship between fluoride intake and urinary fluoride excretion in healthy participants. J Clin Pharmacol. 62 (2), 190–198. https://doi.org/10.1002/jcph.1956.
Gobierno de México. Sal yodada fluorurada. 2023. https://www.gob.mx/cofepris/acciones-y-programas/sal-yodada-fluorurada#:~:text=Coadyuvar%20a%20la%20prevenci%C3%B3n%20de%20los%20casos%20de,consumo%20de%20sal%20yodada%20o%20sal%20yodada%20fluorurada.
Grajda, A., Kułaga, Z., Gurzkowska, B., Wojtyło, M., Góźdź, M., Litwin, M., Jun 2017. Preschool children blood pressure percentiles by age and height. J Hum Hypertens. 31 (6), 400–408. https://doi.org/10.1038/jhh.2016.90.
Grandjean, P., Grønlund, C., Kjær, I.M., et al., 2012. Reproductive hormone profile and pubertal development in 14-year-old boys prenatally exposed to polychlorinated biphenyls. Reproduct. Toxicol. 34 (4), 498–503. https://doi.org/10.1016/j.reprotox.2012.07.005.
Grandjean, P., 2019. Developmental fluoride neurotoxicity: an updated review. Environ. Health. 2019;18(1):110. doi:10.1186/s12940-019-0551-x.
Green, R., Till, C., Cantoral, A., et al., 2020. Associations between urinary, dietary, and water fluoride concentrations among children in Mexico and Canada. Toxics 2020;8(4). doi:10.3390/toxics8040110.
Guo, X.-y., Sun, G.-f., Sun, Y.-c., 2003. Oxidative stress from fluoride-induced hepatotoxicity in rats. Fluoride 2003;2011-12-13 2003;36(1):25-29.
Harley, K.G., Rauch, S.A., Chevrier, J., et al., 2017. Association of prenatal and childhood PBDE exposure with timing of puberty in boys and girls. Environ. Int. 100, 132–138. https://doi.org/10.1016/j.envint.2017.01.003.
Hassan, H.A., Yousef, M.I., 2009. Mitigating effects of antioxidant properties of black berry juice on sodium fluoride induced hepatotoxicity and oxidative stress in rats. Food Chem. Toxicol.. 47 (9), 2332–2337. https://doi.org/10.1016/j.fct.2009.06.023.
Hauser, R., Meeker, J.D., Park, S., Silva, M.J., Calafat, A.M., 2004. Temporal variability of urinary phthalate metabolite levels in men of reproductive age. Environ. Health Perspect. 112 (17), 1734–1740. https://doi.org/10.1289/ehp.7212.
Heindel, J.J., Blumberg, B., Cave, M., et al., 2017. Metabolism disrupting chemicals and metabolic disorders. Reproduct. Toxicol. 68, 3–33. https://doi.org/10.1016/j.reprotox.2016.10.001.
Holt, R.D., Morris, C.E., Winter, G.B., Downer, M.C., Aug 1994. Enamel opacities and dental caries in children who used a low fluoride toothpaste between 2 and 5 years of age. Int Dent J. 44 (4), 331–341.
Idowu, O.S., Azevedo, L.B., Valentine, R.A., et al., 2019. The use of urinary fluoride excretion to facilitate monitoring fluoride intake: a systematic scoping review. PLOS ONE. 14 (9), e0222260.
INSP, 2020 Instituto Nacional de Salud Pública (INSP). Encuesta Nacional de Salud y Nutrición 2020 sobre Covid-19. Resultados nacionales. 2020. https://ensanut.insp.mx/encuestas/ensanutcontinua2020/doctos/informes/ensanutCovid19ResultadosNacionales.pdf.
Itai, K., Onoda, T., Nohara, M., et al., Aug 2021. Slightly elevated serum ionic fluoride levels inhibit insulin secretion and increase glucose levels in a general Japanese population: a cross-sectional study. Biol. Trace. Elem Res. 199 (8), 2819–2825. https://doi.org/10.1007/s12011-020-02415-1.
Jáuregui, A., Salvo, D., García-Olvera, A., et al., 2020. Physical activity, sedentary time and cardiometabolic health indicators among Mexican children. Clin. Obes. 10 (1), e12346.
Jiang, X., Cao, Z., Shen, L., et al., 2014. Blood pressure tables for Chinese adolescents: justification for incorporation of important influencing factors of height, age and sex in the tables. BMC Pediatr. 2014;14:10. doi:10.1186/1471-2431-14-10.
Kheradpisheh, Z., Mirzaei, M., Mahvi, A.H., et al., 2018. Impact of drinking water fluoride on human thyroid hormones: a case- control study. Sci. Rep. 8 (1), 2674. https://doi.org/10.1038/s41598-018-20696-4.
Kumah, E.A., Eskandari, F., Azevedo, L.B., John, S., Zohoori, F.V., 2022. Mapping the evidence for monitoring fluoride exposure in community prevention programmes for oral health using nail clippings and spot urine samples: a scoping review. BMC Oral Health. https://doi.org/10.1186/s12903-022-02615-2.
Kumar, V., Chahar, P., Kajjari, S., Rahman, F., Bansal, D.K., Kapadia, J.M., 2018. Fluoride, thyroid hormone derangements and its correlation with tooth eruption pattern among the pediatric population from endemic and non-endemic fluorosis areas. J. Contemp. Dent. Pract. 19 (12), 1512–1516.
Kupsco, A., Wu, H., Calafat, A.M., et al., Jan 2021. Prenatal maternal phthalate exposures and child lipid and adipokine levels at age six: a study from the PROGRESS cohort of Mexico City. Environ Res. 192, 110341 https://doi.org/10.1016/j.envres.2020.110341.
Li, Y., Liang, C., Slemenda, C.W., et al., May 2001. Effect of long-term exposure to fluoride in drinking water on risks of bone fractures. J. Bone Miner. Res. 16 (5), 932–939. https://doi.org/10.1359/jbmr.2001.16.5.932.
Lima Leite, A., Gualiume Vaz Madureira Lobo, J., 2014.Barbosa da Silva Pereira HA, et al. Proteomic analysis of gastrocnemius muscle in rats with streptozotocin-induced diabetes and chronically exposed to fluoride. PLoS One. 2014;9(9):e106646. doi:10.1371/journal.pone.0106646.
Liu, H.-Y., Chen, J.-R., Hung, H.-C., Hsiao, S.-Y., Huang, S.-T., Chen, H.-S., 2011. Urinary fluoride concentration in children with disabilities following long-term fluoride tablet ingestion. Res. Dev. Disabilities 32 (6), 2441–2448. https://doi.org/10.1016/j.ridd.2011.07.016.
Liu, L., Wang, M., Li, Y., et al., 2019. Low-to-moderate fluoride exposure in relation to overweight and obesity among school-age children in China. Ecotoxicology and Environmental Safety. 2019/11/15/ 2019;183:109558. Doi: 10.1016/j.ecoenv.2019.109558.
Liu, Y., Téllez-Rojo, M., Sánchez, B.N., et al., 2020. Association between fluoride exposure and cardiometabolic risk in peripubertal Mexican children. Environ Int. 134, 105302. https://doi.org/10.1016/j.envint.2019.105302.
Lu, Y., Luo, Q., Cui, H., et al., 2017. Sodium fluoride causes oxidative stress and apoptosis in the mouse liver. Aging (albany NY). 9 (6), 1623–1639. https://doi.org/10.18632/aging.101257.
Ma, Q., Huang, H., Sun, L., et al., Dec 2017. Gene-environment interaction: Does fluoride influence the reproductive hormones in male farmers modified by ERα gene polymorphisms? Chemosphere. 188, 525–531. https://doi.org/10.1016/j.chemosphere.2017.08.166.
Ma, Y., Niu, R., Sun, Z., et al., Jun 2012. Inflammatory responses induced by fluoride and arsenic at toxic concentration in rabbit aorta. Arch Toxicol. 86 (6), 849–856. https://doi.org/10.1007/s00204-012-0803-9.
Madeira, I., Bordallo, M.A., Rodrigues, N.C., et al., 2017. Leptin as a predictor of metabolic syndrome in prepubertal children. Arch. Endocrinol. Metabol. 61.
Malin, A.J., Till, C., 2015. Exposure to fluoridated water and attention deficit hyperactivity disorder prevalence among children and adolescents in the United States: an ecological association. Environ. Health. 2015;14:17. doi:10.1186/s12940-015-0003-1.
Malin, A.J., Lesseur, C., Busgang, S.A., Curtin, P., Wright, R.O., Sanders, A.P., Nov 2019. Fluoride exposure and kidney and liver function among adolescents in the United States: NHANES, 2013–2016. Environ Int. 132, 105012 https://doi.org/10.1016/j.envint.2019.105012.
Marshall, W.A., Tanner, J.M., Jun 1969. Variations in pattern of pubertal changes in girls. Arch Dis Child. 44 (235), 291–303. https://doi.org/10.1136/adc.44.235.291.
Marshall, W.A., Tanner, J.M., 1970. Variations in the pattern of pubertal changes in boys. Arch. Dis. Child. 45 (239), 13–23. https://doi.org/10.1136/adc.45.239.13.
Martínez-Mier, E.A., Soto-Rojas, A.E., Buckley, C.M., Margineda, J., Zero, D.T., Dec 2009. Evaluation of the direct and diffusion methods for the determination of fluoride content in table salt. Commun. Dent Health. 26 (4), 204–210.
Martínez-Mier, E.A., Cury, J.A., Heilman, J.R., et al., 2011. Development of gold standard ion-selective electrode-based methods for fluoride analysis. Caries Res. 45 (1), 3–12. https://doi.org/10.1159/000321657.
Mi, J., Munkonda, M.N., Li, M., et al., 2010. Adiponectin and Leptin Metabolic Biomarkers in Chinese Children and Adolescents. J. Obesity. 2010/10/31 2010;2010:892081. doi:10.1155/2010/892081.
Miltonprabu, S., Thangapandiyan, S., Jan 2015. Epigallocatechin gallate potentially attenuates Fluoride induced oxidative stress mediated cardiotoxicity and dyslipidemia in rats. J Trace Elem Med Biol. 29, 321–335. https://doi.org/10.1016/j.jtemb.2014.08.015.
Muyumba, E.K., Nkulu, D.N., Mukeng, C.K., et al., 2018. Oscillometric blood pressure by age and height for non overweight children and adolescents in Lubumbashi, Democratic Republic of Congo. BMC Cardiovasc. Disord. 2018;18(1):9. doi:10.1186/s12872-018-0741-4.
National Institutes of Health (NIH). Fluoride. https://ods.od.nih.gov/factsheets/Fluoride-HealthProfessional/#en1.
Noubiap, J.J., Nansseu, J.R., Lontchi-Yimagou, E., et al., 2022. Global, regional, and country estimates of metabolic syndrome burden in children and adolescents in 2020: a systematic review and modelling analysis. Lancet Child Adolesc Health. 6(3), 158–170. https://doi.org/10.1016/s2352-4642(21)00374-6.
Oncü, M., Kocak, A., Karaoz, E., Darici, H., Savik, E., Gultekin, F., Sep 2007. Effect of long-term fluoride exposure on lipid peroxidation and histology of testes in first- and second-generation rats. Biol. Trace Elem Res. 118 (3), 260–268. https://doi.org/10.1007/s12011-007-0036-6.
Ortiz-Pérez, D., Rodríguez-Martínez, M., Martínez, F., et al., Sep 2003. Fluoride-induced disruption of reproductive hormones in men. Environ. Res. 93 (1), 20–30. https://doi.org/10.1016/s0013-9351(03)00059-8.
Padmanabhan, V., Cardoso, R.C., Puttabyatappa, M., 2016. Developmental programming, a pathway to disease. Endocrinology. 157 (4), 1328–1340. https://doi.org/10.1210/en.2016-1003.
Puranik, C.P., Ryan, K.A., Yin, Z., Martinez-Mier, E.A., Preisser, J.S., Everett, E.T., 2015. Fluoride modulates parathyroid hormone secretion in vivo and in vitro. Cells Tissues Organs. 200 (6), 413–423. https://doi.org/10.1159/000438699.
Renzetti, S., Just, A.C., Burris, H.H., et al., Jan 2017. The association of lead exposure during pregnancy and childhood anthropometry in the Mexican PROGRESS cohort. Environ Res. 152, 226–232. https://doi.org/10.1016/j.envres.2016.10.014.
Rugg-Gunn, A.J., Villa, A.E., Buzalaf, M.R.A., 2011. Contemporary biological markers of exposure to fluoride. Monogr Oral Sci. 22, 37–51. https://doi.org/10.1159/000325137.
Sanders, A.P., Svensson, K., Gennings, C., et al., Nov 2018. Prenatal lead exposure modifies the effect of shorter gestation on increased blood pressure in children. Environ Int. 120, 464–471. https://doi.org/10.1016/j.envint.2018.08.038.
Sarr, O., Yang, K., Regnault, T.R.H. *In Utero* Programming of later adiposity: the role of fetal growth restriction. J. Preg.. 2012/11/28 2012;2012:134758. doi:10.1155/2012/134758.
Saylor, C., Malin, A.J., Tamayo-Ortiz, M., et al., Mar 2022. Early childhood fluoride exposure and preadolescent kidney function. Environ Res. 204 (Pt A), 112014 https://doi.org/10.1016/j.envres.2021.112014.
Secretaría de Salud., 1994. Norma Oficial Mexica NOM-040-SSA1-1993, Bienes y Services. Sal Yodada Y Sal Yodada Fluorurada. Especificaciones Sanitarias.
Shamah-Levy, T., Cuevas-Nasu, L., Gaona-Pineda, E.B., et al., 2018. Overweight and obesity in children and adolescents, 2016 halfway national health and nutrition survey update. Salud Publica Mex. 60 (3), 244–253.

Shanthakumari, D., Srinivasalu, S., Subramanian, S., 2004. Effect of fluoride intoxication on lipidperoxidation and antioxidant status in experimental rats. Toxicology 2004; 2022-01-26 2004;204(2-3):219-228.

Shoback, D.M., McGhee, J.M., Jun 1988. Fluoride stimulates the accumulation of inositol phosphates, increases intracellular free calcium, and inhibits parathyroid hormone release in dispersed bovine parathyroid cells. Endocrinology 122 (6), 2833–2839. https://doi.org/10.1210/endo-122-6-2833.

Skórka-Majewicz, M., Goschorska, M., Żwierełło, W., et al., Dec 2020. Effect of fluoride on endocrine tissues and their secretory functions – review. Chemosphere. 260, 127565 https://doi.org/10.1016/j.chemosphere.2020.127565.

Tavener, J.A., Davies, G.M., Davies, R.M., Ellwood, R.P., 2006. The prevalence and severity of fluorosis in children who received toothpaste containing either 440 or 1,450 ppm F from the age of 12 months in deprived and less deprived communities. Caries Res. 40 (1), 66–72. https://doi.org/10.1159/000088909.

Thippeswamy, H.M., Devananda, D., Nanditha Kumar, M., Wormald, M.M., Prashanth, S. N., Jan 2021. The association of fluoride in drinking water with serum calcium, vitamin D and parathyroid hormone in pregnant women and newborn infants. Eur J Clin Nutr. 75 (1), 151–159. https://doi.org/10.1038/s41430-020-00707-2.

Thomas, D.M., Oken, E., Rifas-Shiman, S.L., et al., 2019. Do women know their prepregnancy weight? Obesity. 27 (7), 1161–1167. https://doi.org/10.1002/oby.22502.

Trivedi, N., Mithal, A., Gupta, S.K., Godbole, M.M., 1993. The Fluoride Collaborative Study G. Reversible impairment of glucose tolerance in patients with endemic fluorosis. Diabetologia. 36 (9), 826–828. https://doi.org/10.1007/BF00400357.

[91] US Department of Health and Human Services (USDHHS). U.S. Public Health Service Recommendation for Fluoride Concentration in Drinking Water for the Prevention of Dental Caries. Public Health Reports. 2015;130(4):318-331. doi: 10.1177/003335491513000408.

Valle-Martos, R., Valle, M., Martos, R., Cañete, R., Jiménez-Reina, L., Cañete, M.D., 2021. Liver enzymes correlate with metabolic syndrome, inflammation, and endothelial dysfunction in prepubertal children with obesity. Original research. Front. Pediatr. 2021. https://doi.org/10.3389/fped.2021.629346.

Vargas-Meza, J., Cervantes-Armenta, M.A., Campos-Nonato, I., et al., 2016. Dietary sodium and potassium intake: data from the Mexican National health and nutrition survey 2016. Nutrients. Jan 11 2022;14(2)doi:10.3390/nu14020281.

Viitasalo, A., Lakka, T.A., Laaksonen, D.E., et al., 2014. Validation of metabolic syndrome score by confirmatory factor analysis in children and adults and prediction of cardiometabolic outcomes in adults. Diabetologia. 57 (5), 940–949. https://doi.org/10.1007/s00125-014-3172-5.

Vikraman, S., Fryar, C.D., Ogden, C.L., Sep 2015. Caloric Intake from fast food among children and adolescents in the United States, 2011–2012. NCHS Data Brief. 213, 1–8.

Villa, A., Anabalon, M., Zohouri, V., Maguire, A., Franco, A.M., Rugg-Gunn, A., 2010. Relationships between Fluoride Intake, Urinary Fluoride Excretion and Fluoride Retention in Children and Adults: An Analysis of Available Data. Caries Research. 44 (1), 60–68. https://doi.org/10.1159/000279325.

Wang, S.X., Wang, Z.H., Cheng, X.T., et al., 2007. Arsenic and fluoride exposure in drinking water: children's IQ and growth in Shanyin county, Shanxi province, China. Environ Health Perspect. Apr 2007;115(4):643-7. doi:10.1289/ehp.9270.

Wang, A., Ma, Q., Gong, B., et al., 2021. DNA methylation and fluoride exposure in school-age children: Epigenome-wide screening and population-based validation. Ecotoxicol. Environ. Saf. Oct 15 2021;223:112612. doi:10.1016/j.ecoenv.2021.112612.

Villegas Carrasco, A., 2002. The AMAI system of classifying households by socio-economic level. ESOMAR.

Wang, M., Liu, L., Li, H., et al., Jan 2020. Thyroid function, intelligence, and low-moderate fluoride exposure among Chinese school-age children. Environ Int. 134, 105229 https://doi.org/10.1016/j.envint.2019.105229.

Wang, Y.N., Xiao, K.Q., Liu, J.L., Dallner, G., Guan, Z.Z., 2000. Effect of long term fluoride exposure on lipid composition in rat liver. Toxicology. 146 (2–3), 161–169. https://doi.org/10.1016/s0300-483x(00)00167-0.

Wang, X., Zheng, R., Yao, Q., Liang, Z., Wu, M., Wang, H., Nov 2019. Effects of fluoride on the histology, lipid metabolism, and bile acid secretion in liver of Bufo gargarizans larvae. Environ Pollut. 254 (Pt B), 113052 https://doi.org/10.1016/j.envpol.2019.113052.

Warren, J.J., Levy, S.M., Broffitt, B., Cavanaugh, J.E., Kanellis, M.J., Weber-Gasparoni, K., 2009. Considerations on optimal fluoride intake using dental fluorosis and dental caries outcomes–a longitudinal study. J. Publ. Health Dent. Spring 69 (2), 111–115. https://doi.org/10.1111/j.1752-7325.2008.00108.x.

Wong, M.C., Glenny, A.M., Tsang, B.W., Lo, E.C., Worthington, H.V., Marinho, V.C., 2010. Topical fluoride as a cause of dental fluorosis in children. Cochrane Database Syst. Rev. 20 2010;2010(1):Cd007693. doi:10.1002/14651858.CD007693.pub2.

World Health Organization (WHO). Basic methods for assessing renal fluoride excretion in community prevention programmes for oral health. 2014. https://www.who.int/publications/i/item/9789241548700.

World Health Organization (WHO). *Guidelines for drinking-water quality, 4th edition, incorporating the 1st addendum*. 2017. https://www.who.int/publications/i/item/9789241549950.

World Health Organization (WHO). Child growth standards: WHO Anthro Survey Analyser and other tools. https://www.who.int/tools/child-growth-standards/software.

World Health Organization (WHO). Obesity and overweight. https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight.

World Health Organization (WHO). Growth reference data for 5-19 years. https://www.who.int/tools/growth-reference-data-for-5to19-years/application-tools.

Xiong, X., Liu, J., He, W., et al., Jan 2007. Dose-effect relationship between drinking water fluoride levels and damage to liver and kidney functions in children. Environ. Res. 103 (1), 112–116. https://doi.org/10.1016/j.envres.2006.05.008.

Xu, K., An, N., Huang, H., et al., 2020. Fluoride exposure and intelligence in school-age children: evidence from different windows of exposure susceptibility. BMC Publ. Health. 2020;20(1):1657. doi: 10.1186/s12889-020-09765-4.

Yoshinaga, M., Sameshima, K., Tanaka, Y., et al., 2008. Adipokines and the prediction of the accumulation of cardiovascular risk factors or the presence of metabolic syndrome in elementary school children. Circulat. J. 72 (11), 1874–1878. https://doi.org/10.1253/circj.CJ-08-0180.

Yousefi, M., Yaseri, M., Nabizadeh, R., et al., 2018. Association of hypertension, body mass index, and waist circumference with fluoride intake; water drinking in residents of fluoride endemic areas, Iran. Biol. Trace Element Res. Oct 2018 2021-10-06 2018;185(2):282-288. doi: 10.1007/s12011-018-1269-2.

Yousefi, M., Yaseri, M., Nabizadeh, R., et al., 2018. Association of Hypertension, body mass index, and waist circumference with fluoride intake; water drinking in residents of fluoride endemic areas, Iran. Biol. Trace Element Res. 185 (2), 282–288. https://doi.org/10.1007/s12011-018-1269-2.

Yu, X., Chen, J., Li, Y., et al., Sep 2018. Threshold effects of moderately excessive fluoride exposure on children's health: A potential association between dental fluorosis and loss of excellent intelligence. Environ Int. 118, 116–124. https://doi.org/10.1016/j.envint.2018.05.042.

Zhou, T., Yang, R., Li, S., et al., Mar 2013. Influence of water fluoride exposure on sex hormone binding globulin and testosterone in adult male. Wei Sheng Yan Jiu. 42 (2), 241–244.

Zohoori, F.V., Walls, R., Teasdale, L., et al., 2013. Fractional urinary fluoride excretion of 6–7-year-old children attending schools in low-fluoride and naturally fluoridated areas in the UK. Br J Nutr. 109 (10), 1903–1909. https://doi.org/10.1017/s0007114512003583.

Zohouri, F.V., Swinbank, C.M., Maguire, A., Moynihan, P.J., 2006. Is the fluoride/creatinine ratio of a spot urine sample indicative of 24-h urinary fluoride? Community Dent Oral Epidemiol. 34 (2), 130–138. https://doi.org/10.1111/j.1600-0528.2006.00269.x.