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Association between fluoride exposure and cardiometabolic risk in peripubertal Mexican children

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ABSTRACT

Background: Several animal studies have suggested that fluoride exposure may increase the levels of cardiometabolic risk factors, but little is known about whether fluoride exposure is associated with such risk in humans.

Objectives: We examined the cross-sectional association between peripubertal exposure to fluoride and markers of cardiometabolic risk in 280 girls and 256 boys at age 10–18 years living in Mexico City.

Methods: We measured plasma fluoride concentration using a microdiffusion method. We collected data on anthropometry including BMI, waist circumference (WC) and trunk fat percentage. We measured serum markers of cardiometabolic risk, including fasting glucose, insulin and lipids. All the indicators of outcome were converted to age- and sex-specific z-scores. We also calculated a summary cardiometabolic risk score for each participant. Multivariable linear regression models were used to examine these associations.

Results: The geometric mean (95% confidence interval (CI)) of plasma fluoride was 0.21 μmol/L (0.20, 0.23 μmol/L) in the total sample. In girls, plasma fluoride concentrations were associated with higher z-scores for all the individual markers (except for lipids) and for the combined cardiometabolic risk score (risk score: $\beta = 1.28$, 95% CI: 0.57–2.00, p-sex interaction = 0.02)), adjusting for covariates. No associations were found in boys.

Conclusions: We found that higher peripubertal fluoride exposure at the levels observed in this study population was significantly associated with increased levels of cardiometabolic risk factors in Mexican girls but not boys. Future studies with a longitudinal design are needed to confirm our findings and further elucidate the role of fluoride in cardiometabolic risk.

1. Introduction

Metabolic syndrome is characterized by a cluster of physiologic abnormalities including abdominal obesity, dysregulated glucose homeostasis, insulin resistance, dyslipidemia, and elevated blood pressure (Kassi et al., 2011). It has been recognized that childhood and adolescence are particularly vulnerable periods to increased cardiometabolic risk and development of cardiovascular disease, type 2 diabetes and all-cause mortality later in life (DeBoer et al., 2015; Liu and Peterson, 2015; Morrison et al., 2008; O'Neill and O'Driscoll, 2015).

The proportion of metabolic syndrome is growing rapidly in children and adolescents worldwide (Al-Hamad and Raman, 2017); a systematic review of 85 studies reported the median prevalence worldwide as 3.3%, ranging from 0% to 19.2% (Friend et al., 2013). In Mexico, metabolic syndrome occurs in 2.4–45.9% of children depending on the definition (Pena-Espinoza et al., 2017), which indicates that the current diagnostic criteria are not effective in defining pediatric metabolic syndrome among Mexican children.

Fluoride has been added to drinking water and table salt in the U.S. and Mexico respectively to reduce the incidence of dental caries for decades. In 2006, after conducting a thorough review of available animal, clinical and epidemiologic data on fluoride, the National Research

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Council (NRC) called for future research to study the health risk from exposure to fluoride in order to protect vulnerable populations, especially children (National Research Council (U.S.). Committee on Fluoride in Drinking Water, 2006). A growing number of studies have linked fluoride exposure to several cardiometabolic risk factors. Specially, previous animal studies using rats have suggested that fluoride may affect glucose homeostasis and insulin resistance (Chehoud, 2008; Hu et al., 2012; Pereira et al., 2017). Several animal reports showed that fluoride exposure can disturb lipid homeostasis: fluoride may lead to increased low-density lipoprotein cholesterol (LDL-C) and triglycerides (Czerny et al., 2004; Ma et al., 2012; Sun et al., 2014), as well as decreased high-density lipoprotein cholesterol (HDL-C) (Afolabi et al., 2013). Fluoride has also been related to increased blood pressure and risk of hypertension in humans (Amini et al., 2011; Sun et al., 2013; Yousefi et al., 2018).

The majority of previous investigations that linked fluoride exposure to cardiometabolic risk used animal data, with only a few epidemiological studies focusing on hypertension in adults (Amini et al., 2011; Sun et al., 2013; Yousefi et al., 2018). However, these 3 studies lacked individual fluoride biomarkers to assess fluoride exposure. Furthermore, no existing studies have evaluated sex-related differences in these associations. To address these gaps, we examined the association between peripubertal plasma fluoride and multiple indicators of cardiometabolic risk in 280 girls and 256 boys aged 10–18 years residing in Mexico City.

2. Methods

2.1. Study population

The present analysis included participants of the Early Life Exposures in Mexico to ENvironmental Toxicants (ELEMENT) study, which consists of three sequentially-enrolled cohorts of pregnant women living in Mexico City between 1994 and 2005 (Hu et al., 2006). Briefly, cohort 1 participants did not have measured plasma samples and were excluded. Cohort 2 was an observational study; participants of cohort 2A were recruited between 1997 and 1999, and participants of cohort 2B were recruited between 1999 and 2001. Cohort 3 was a randomized clinical trial with calcium supplementations, and its participants were recruited between 2001 and 2003. Details on the ELEMENT study including recruitment, eligibility criteria and collection of maternal information have been published previously (Bashash et al., 2017). In 2015, a subset of the offspring (n = 550) were re-recruited if they were in peripubertal period (age 10–18 years). During the in-person visits, children participated in the anthropometric assessments, provided an 8-hour fasting blood sample, and completed interview-based questionnaires. Of the 550 participants, the present study included 536 children who had data on plasma fluoride and at least one measurement of cardiometabolic outcomes.

Research protocols of this study were approved by the Institutional Review Board at University of Michigan, Indiana University and the Mexico National Institute of Public Health. Maternal informed consent and child informed assent were obtained prior to enrollment.

2.2. Plasma fluoride

Fasting blood samples were stored in a 2 mL heparin tube at −80 °C, and shipped to Indiana University Oral Health Research Institute (OHRI) for fluoride analysis. Fluoride levels in plasma were measured using a fluoride ion-selective electrode (Orion No. 96-09; Fisher Scientific Co.) and a pH/ion meter (Orion Dual Star) following a modified hexamethyldisiloxane (HMDS, Sigma Chemical Co., St Louis, MO, USA) microdiffusion method as described previously (Martinez-Mier et al., 2011; Thomas et al., 2016). Fluoride concentrations were calculated by comparing the millivolt readings of plasma samples achieved from the ion-selective electrode and pH/ion meter to standard curves. A subset of plasma samples (n = 58) that had sufficient volume was measured in duplicate, of which 100% complied with the quality control criteria (i.e. relative standard deviation (RSD) < 10%). The average of two values was taken. Although the duplicate analyses were not conducted in the remaining samples, all these samples were included in the final analyses due to the fact that OHRI provides high quality control for fluoride analysis (Bashash et al., 2017; Thomas et al., 2016). All plasma samples were above limit of detection (LOD) at 0.25 nmol/L. Plasma fluoride can be considered as a biomarker that evaluates recent exposure or fluoride balance, although there is some evidence suggesting that fasting concentrations may be used as a proxy for estimating chronic exposure to fluoride (National Research Council (U.S.). Committee on Fluoride in Drinking Water, 2006).

2.3. Cardiometabolic risk factors

Weight, height and waist circumference (WC) were measured by trained research staff using standardized protocols (Lohman et al., 1988). Body mass index (BMI) z-scores were calculated from weight and height, and then converted to age- and sex-specific z-scores using the World Health Organization growth reference (de Onis et al., 2007). WC was measured using a non-stretchable measuring tape (QM2000; QuickMedical) at the level of the umbilicus, and was averaged across three repeated measurements. Tetrapolar bioelectrical impedance was measured using InBody 230 (Biospace Co, Ltd, South Korea) to estimate trunk fat percentage by trained staff. Systolic (SBP) and diastolic blood pressure (DBP) were measured in quintuplicate by the trained research staff using a digital automatic blood pressure monitor (BpTRU BPM-200, Canada); the five repeated measures were averaged (each participant had all 5 measurements). The average blood pressure (BP) was calculated using the mean value of SBP and DBP.

Serum concentrations of glucose, lipid and other hormones were only measured in a subset of children (n = 400) due to budget constraints. Fasting glucose, insulin and lipids including triglycerides, LDL-C and HDL-C were measured in serum at the Michigan Diabetes Research Center (MDRC) Chemistry Lab. Glucose was quantified using Glucose-SL assay employing an enzymatic method, and insulin concentrations were measured by an immunoturbidimetric assay (both from Sekisui Diagnostics, LLC, Lexington, MA). Triglycerides were measured using enzymatic colorimetric method with a Cobas Mira automated chemistry analyzer (Roche Diagnostics, Indianapolis, IN). HDL-C and LDL-C were measured by direct HDL-C (Roche Diagnostics, Indianapolis, IN) and direct LDL-C assays (Equal Diagnostics, Exton, PA), respectively. All the assays described above were in line with the National Cholesterol Education Program (NCEP) guidelines. All the serum markers were above LOD. In addition, we calculated the index of insulin resistance, namely the homeostatic model assessment of insulin resistance (HOMA-IR) using the following formula: HOMA-IR = insulin (μU/mL) * glucose (mg/dL)/405 (Yokoyama et al., 2004). All individual factors were then converted to standardized age- and sex-specific z-scores. Finally, a continuous cardiometabolic risk score was constructed by summing the five internally standardized z-scores including the indicator of central obesity (WC), blood pressure ([SBP + DBP]/2), glucose homeostasis (fasting glucose), insulin and lipid metabolism (triglycerides/HDL-C ratio). A higher score indicates a higher cardiometabolic risk (Ford and Li, 2008). This summary cardiometabolic risk score was constructed based on the previously published score by Viitasalo et al. (2014) who demonstrated its association with the main components of metabolic syndrome, the incident of type 2 diabetes and cardiovascular disease in children and adults. This cardiometabolic risk score was used due to the fact that there is no consensus definition for metabolic syndrome in children and adolescents.

2.4. Covariates

Child age and household socioeconomic status (SES) were collected

from questionnaires at the time of anthropometry measurements. SES in the Mexican population was classified using the index developed and standardized by the Mexican Association of Market and Public Opinion Research Agencies (AMAI) in 2011. AMAI 8x7 has identified seven socioeconomic levels ranging from A to E based on household possessions: A/B and C+ represent the upper class, while D and E indicate the lower class. Middle class was defined as having a socioeconomic level at C, C- or D+ (López-Romo, 2011). Birth weight of nude newborns was measured within 12 h of delivery using calibrated beam scales (Oken, Model TD16, Naucalpan, México). Maternal age, marital status and smoking history, breastfeeding duration, gestational age and number of siblings at birth were collected from questionnaires at pregnancy or postnatal visit. Puberty in both sexes was evaluated by a trained physician using Tanner staging of pubic hair growth following a standardized protocol (Liu et al., 2019b). Specifically, Tanner stage = 1 indicates prepuberty. Tanner stage = 2 indicates the onset of puberty demonstrating by the sparse growth of long pigmented downy hair (Marshall and Tanner, 1969, 1970). At stage 3, the pubic hair is considerably darker, coarser, and curlier with sparsely spreading over the junction of the pubes. At stage 4, the pubic hair is adult in type but the coverage is smaller than in adults. Tanner stage = 5 indicates fully matured stage.

2.5. Statistical analysis

We performed univariate and bivariate analyses. Continuous variables with skewed distributions were transformed by taking the natural logarithm including plasma fluoride, insulin, HOMA-IR and triglycerides. Descriptive data are presented as mean ± standard deviations (SD) or proportions (%). Geometric mean and 95% confidence intervals (CI) are presented for non-normally distributed variables. The Wilcoxon signed-rank and Kruskal-Wallis tests were used to examine the difference in the plasma fluoride concentrations by the level of each key variable in children. All the individual risk factors were standardized prior to the final analyses as described above. Extreme outliers of insulin and HOMA-IR of one participant and HDL-C of two participants were identified and excluded using generalized extreme Studentized deviate (ESD) method (Rosner 1983).

We evaluated the modifying effect of children's sex on the associations between peripubertal fluoride exposure and cardiometabolic risk by including interaction terms between plasma fluoride and sex for each outcome. Since we found evidence of children's sex as a modifier (P-interaction < 0.05), we examined sex-specific associations of plasma fluoride and each indicator of child cardiometabolic risk.

Multivariate linear regression was used to assess the association of log-transformed fluoride exposure with each measure of body fat (z-scores for BMI, WC and trunk fat percentage), blood pressure (z-scores for SBP and DBP), glucose homeostasis (z-scores for fasting glucose and insulin), insulin resistance (z-scores for HOMA-IR) and lipid metabolism (z-scores for triglycerides, HDL-C and LDL-C), as well as the cardiometabolic risk score. We chose covariates *a priori* if they are well-known to be associated with cardiometabolic risk or fluoride exposure or they were potential confounders. All models were adjusted for child birth weight, gestational age and number of siblings at birth, maternal age, breastfeeding duration, marital status and smoking history, household SES and cohort, and child age at visit. We defined statistical significance as $p \leq 0.05$. All statistical analyses were performed using SAS (version 9.4; SAS Institute Inc., Cary, NC, USA).

2.6. Sensitivity analyses

We performed multiple sensitivity analyses. First, we redid all the analyses by additionally adjusting for peripubertal calcium intake. For blood pressure, we further adjusted for peripubertal sodium intake (a proxy for salt intake) and BMI z-score. Secondly, we reanalyzed all the models with the additional adjustment for pubertal stage, which has been correlated with increased metabolic disorders in adulthood (Widen et al., 2012). Pubertal stages were assessed by an experienced pediatrician using Tanner stage ranging from 1 to 5 for pubic hair growth for both sexes (Liu et al., 2019b). Thirdly, given that low birthweight (LBW) and preterm birth have been linked to increased risk of cardiovascular disease (CVD) in later life (Luu et al., 2016; Newsome et al., 2003), we repeated our analyses by excluding children born preterm (gestational age < 37 weeks) or low birth weight (< 2.5 kg) (total n = 57). Fourthly, we re-ran the models including extreme outliers of insulin, HOMA-IR and HDL-C as part of the sensitivity analyses. Finally, we examined the adjusted prospective association between maternal plasma fluoride during pregnancy and each cardiometabolic outcome in children at age 10–18 years; however, only a small subset of our participants (n = 75 for girls and n = 64 for boys) had measured plasma fluoride during pregnancy (average of 3 trimesters) due to the insufficient volume of maternal samples, which can reduce the power to detect significant results.

3. Results

We included 536 children with a total of 280 girls and 256 boys in the final analyses. In the total sample, the geometric mean (95% confidence interval (CI)) of plasma fluoride was 0.21 μmol/L (0.20, 0.23 μmol/L). The mean age for children was 14.5 years (SD: 2.1) (Table 1). On average, mothers were 26.3 (SD: 5.4) years old at delivery, 29.2% were single, and 47.0% had a history of smoking. In this study sample, 12.8% were at stage 1 (prepubertal), 19.0% at stage 2 (pubertal onset), 24.0% at stage 3, 24.6% at stage 4, and 19.6% were at stage 5 (adult).

Table 2 shows that children > 16 years had higher plasma fluoride levels than younger children (geometric mean: 0.27 μmol/L versus 0.18 μmol/L, p < 0.0001); the fluoride concentrations were slightly higher in boys than girls (geometric mean: 0.23 μmol/L versus 0.20 μmol/L, p = 0.03); higher plasma fluoride was observed in those who had been breastfed ≥ 6 months (p = 0.002); the fluoride concentrations in plasma were slightly lower in children whose mothers had a smoking history (p = 0.03); children whose mothers enrolled in cohort 3 with calcium treatment during pregnancy had the lowest plasma fluoride levels (p < 0.0001). No significant differences in

Table 1
Characteristics of participants in Mexico City.

Characteristics	N	Mean (SD) or %
Child characteristics		
Age (y)	536	14.5 (2.1)
Female sex	280	52.2%
Birth weight (kg)	530	3.1 (0.5)
Gestational age (wk)	529	38.7 (1.6)
Number of siblings at birth	532	2.0 (1.0)
Maternal characteristics		
Maternal age (y)	531	26.3 (5.4)
Breastfeeding duration (mos)	532	8.1 (6.1)
Marital status		
Yes	375	70.8%
No	155	29.2%
Smoking history		
Ever	245	47.0%
Never	276	53.0%
Household SES		
Lower class	143	26.7%
Middle class	355	66.2%
Upper class	38	7.1%
Cohort		
Cohort 2A	125	23.3%
Cohort 2B	135	25.2%
Cohort 3-placebo	128	23.9%
Cohort 3-calcium	148	27.6%

SES: socioeconomic status

Table 2
Plasma fluoride concentrations (μmol/L) according to main covariates.

Covariate		N	Geometric mean (95% CI)	p
Children				
Age (y)	< 12	91	0.18 (0.16, 0.21)	< 0.0001
	12–16	257	0.18 (0.17, 0.20)	
	> 16	188	0.27 (0.25, 0.31)	
Sex	Male	256	0.23 (0.21, 0.25)	0.03
	Female	280	0.20 (0.18, 0.21)	
Birth weight (kg)	≥2.5	504	0.21 (0.20, 0.23)	0.59
	< 2.5	26	0.23 (0.17, 0.31)	
Gestational age (wk)	≥37	488	0.21 (0.20, 0.23)	0.24
	< 37	41	0.19 (0.15, 0.24)	
Number of siblings at birth	< 2	203	0.20 (0.18, 0.23)	0.73
	≥2	329	0.21 (0.20, 0.23)	
Mothers				
Maternal age (y)	≥25	311	0.21 (0.19, 0.23)	0.99
	< 25	220	0.21 (0.19, 0.23)	
Breastfeeding duration (mos)	≥6	334	0.23 (0.21, 0.25)	0.002
	< 6	198	0.18 (0.16, 0.20)	
Marital status	Married	375	0.22 (0.20, 0.24)	0.13
	Other	155	0.19 (0.17, 0.22)	
Smoking history	Ever	245	0.20 (0.18, 0.22)	0.03
	Never	276	0.22 (0.20, 0.24)	
Household SES	Lower class	143	0.21 (0.19, 0.24)	0.32
	Middle class	355	0.20 (0.19, 0.22)	
	Upper class	38	0.26 (0.18, 0.36)	
Cohort	Cohort 2A	125	0.22 (0.19, 0.26)	< 0.0001
	Cohort 2B	135	0.26 (0.23, 0.30)	
	Cohort 3-placebo	128	0.21 (0.18, 0.23)	
	Cohort 3-calcium	148	0.17 (0.15, 0.19)	

SES: socioeconomic status.

fluoride levels across categories of other covariates were detected. Mean ± SD or geometric mean (95% CI) is shown for each cardiometabolic outcome in Table 3.

We report the adjusted associations of log-transformed plasma fluoride with z-scores for individual cardiometabolic risk factors and the cardiometabolic risk score in girls (Table 4) and boys (Table 5) using separate models. In sex-stratified analyses, we observed significant associations between plasma fluoride and cardiometabolic outcomes only among girls (Table 4; Fig. 1). In girls, multivariate linear regression models showed that increasing plasma fluoride was significantly associated with a higher z-score for BMI, WC, trunk fat percentage and blood pressure (SBP and DBP). In addition, we observed a significant increase in fasting glucose and insulin z-scores in relation to higher plasma fluoride, adjusted for the same covariates (glucose: β = 0.25, 95% CI: 0.06–0.45, p = 0.01, p-sex interaction = 0.04; insulin: β = 0.29, 95% CI: 0.11–0.47, p = 0.002, p-sex interaction = 0.01). Plasma fluoride was associated with increased insulin resistance, as indicated by HOMA-IR (HOMA-IR: β = 0.32, 95% CI: 0.13–0.50, p = 0.001, p-sex interaction = 0.005). Using the continuous cardiometabolic risk score, we found a positive association between plasma fluoride and cardiometabolic risk (β = 1.28, 95% CI: 0.57–2.00, p = 0.0005, p-sex interaction = 0.02). However, plasma fluoride was not associated with lipids (triglycerides, HDL-C or LDL-C) in girls. No associations were detected in boys (Table 5).

In sensitivity analyses, including the extreme outliers (insulin and HOMA-IR of one participant and HDL-C of two participants) did not change our results (data not shown). Our results did not change meaningfully with additional adjustment for peripubertal calcium

Table 4
Adjusted associations of log-transformed plasma fluoride with cardiometabolic risk factors in girls.[a]

	N	β (95% CI)	p
BMI z-score	268	0.20 (0.00, 0.40)	0.05
Waist circumference z-score	268	0.16 (0.00, 0.33)	0.05
Trunk fat percentage z-score	265	0.19 (0.04, 0.34)	0.01
SBP z-score	268	0.28 (0.12, 0.44)	0.001
DBP z-score	268	0.23 (0.07, 0.40)	0.005
Fasting glucose z-score	193	0.25 (0.06, 0.45)	0.01
Insulin z-score	192	0.29 (0.11, 0.47)	0.002
HOMA-IR z-score[b]	192	0.32 (0.13, 0.50)	0.001
HDL-C z-score	191	−0.00 (−0.19, 0.18)	0.98
LDL-C z-score	193	−0.02 (−0.22, 0.17)	0.80
Triglycerides z-score	193	0.13 (−0.06, 0.32)	0.19
Cardiometabolic risk score[c]	190	1.28 (0.57, 2.00)	0.0005

[a] Adjusted for child birth weight, gestational age and number of siblings at birth, maternal age, breastfeeding duration, marital status and smoking history, household social economic status score and cohort (Cohort 3-Calcium, Cohort 3-placebo, Cohort 2A and 2B), and child age at visit

[b] HOMA-IR: homeostatic model assessment of insulin resistance.

[c] Calculated as the sum of five internally standardized age and sex-specific z-scores for waist circumference, the average of SBP and DBP, fasting glucose, insulin and the triglycerides /HDL-C ratio; CI, confidence interval; SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol.

Table 3
Distributions of adiposity and cardiometabolic risk factors overall and by child sex.

	Total		Girls		Boys	
	N	Mean (SD)	N	Mean (SD)	N	Mean (SD)
BMI z-score	536	0.5 (1.2)	280	0.6 (1.2)	256	0.4 (1.3)
Waist circumference (cm)	536	79.5 (11.4)	280	80.3 (11.4)	256	78.8 (11.4)
Trunk fat percentage (%)	528	26.3 (11.1)	276	31.6 (8.8)	252	20.5 (10.4)
SBP (mmHg)	536	98.6 (9.9)	280	96.8 (9.2)	256	100.5 (10.4)
DBP (mmHg)	536	63.0 (6.9)	280	62.2 (6.6)	256	63.8 (7.2)
Fasting glucose (mg/dL)	399	77.8 (7.3)	201	76.7 (7.1)	198	78.9 (7.3)
Insulin (μU/mL)*	398	16.6 (15.9, 17.4)	200	17.9 (16.7, 19.0)	198	15.5 (14.5, 16.6)
HOMA-IR[a]*	398	3.2 (3.0, 3.3)	200	3.4 (3.1, 3.6)	198	3.0 (2.8, 3.2)
HDL-C (mg/dL)	397	42.8 (8.0)	199	43.8 (8.2)	198	41.8 (7.7)
LDL-C (mg/dL)	399	92.0 (21.1)	201	95.1 (20.8)	198	88.9 (21.0)
Triglycerides (mg/dL)*	399	93.2 (89.2, 97.5)	201	99.0 (93.1, 105.3)	198	87.7 (82.3, 93.5)
Cardiometabolic risk score[b]	396	−0.03 (3.81)	198	−0.03 (3.78)	198	−0.02 (3.86)

[a] HOMA-IR: homeostatic model assessment.

[b] Calculated as the sum of five internally standardized age and sex-specific z-scores for waist circumference, the average of SBP and DBP, fasting glucose, insulin and the triglycerides /HDL-C ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol.

* Geometric mean and 95% confidence intervals (CI).

Table 5
Adjusted associations of log-transformed plasma fluoride with cardiometabolic risk factors in boys.[a]

	N	β (95% CI)	p
BMI z-score	249	0.03 (−0.17, 0.22)	0.80
Waist circumference z-score	249	−0.03 (−0.19, 0.13)	0.73
Trunk fat percentage z-score	245	−0.02 (−0.19, 0.15)	0.83
SBP z-score	249	0.09 (−0.07, 0.26)	0.24
DBP z-score	249	0.04 (−0.12, 0.20)	0.65
Fasting glucose z-score	192	−0.08 (−0.26, 0.11)	0.42
Insulin z-score	192	−0.05 (−0.23, 0.14)	0.63
HOMA-IR z-score[b]	192	−0.06 (−0.25, 0.13)	0.52
HDL-C z-score	192	0.05 (−0.12, 0.22)	0.57
LDL-C z-score	192	−0.07 (−0.26, 0.12)	0.46
Triglycerides z-score	192	−0.02 (−0.21, 0.18)	0.86
Cardiometabolic risk score[c]	192	0.08 (−0.65, 0.82)	0.83

[a] Adjusted for child birth weight, gestational age and number of siblings at birth, maternal age, breastfeeding duration, marital status and smoking history, household social economic status score and cohort (Cohort 3-Calcium, Cohort 3-placebo, Cohort 2A and 2B), and child age at visit.
[b] HOMA-IR: homeostatic model assessment of insulin resistance.
[c] Calculated as the sum of five internally standardized age and sex-specific z-scores for waist circumference, the average of SBP and DBP, fasting glucose, insulin and the triglycerides /HDL-C ratio; CI, confidence interval; SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol.

intake (Supplemental Table 1 and 2) or additional adjustment for both peripubertal sodium intake and BMI z-score for blood pressure (Supplemental Table 1 and 2). When further adjusting for pubertal stage (Supplementary Table 3) or excluding children who were born preterm or low birth weight (total n = 57) (Supplementary Table 4), the associations with BMI z-score and WC became borderline significant ($p < 0.10$); the magnitude, direction and significance of other observed associations were not altered significantly. We examined the prospective association of prenatal fluoride exposure and cardiometabolic risk factors in a much smaller subset of our participants who had available data and found no significant associations (Supplementary Table 5).

4. Discussion

Our study is the first to examine the association between fluoride exposure using a biological marker and multiple indicators of cardiometabolic risk in children. We found sex-specific associations of childhood fluoride exposure (as indicated by plasma fluoride) with increased child cardiometabolic risk. In our study of Mexican children, higher plasma fluoride was significantly associated with higher body fat, blood pressure, glucose, insulin, insulin resistance and the continuous cardiometabolic risk score in girls, but not in boys.

No previous studies have directly analyzed the association between fluoride and cardiometabolic risk score, but several animal investigations and a few epidemiological studies have shed light on the association with each individual component of metabolic syndrome. To date, two published studies have examined the association of fluoride exposure with adiposity in humans and reported no associations, which was inconsistent with our results. A study of 149 Indian children aged 6–18 years living in West Bengal showed that fluoride exposure through drinking water (mean: 2.11 mg/L) was not associated with child BMI at age 6–18 years (Das and Mondal, 2016). Another investigation reported no association of fluoride in drinking water (mean: ranged from 0.68 to 10.30 mg/L across 4 villages) with BMI or WC in 346 adults from four villages of northwestern Iran (Yousefi et al., 2018). Unlike our study that included individual biomarker of fluoride (i.e. plasma), these studies were limited by estimating fluoride exposure using drinking water concentrations at the population level. Our participants were exposed to fluoridated table salt at 200–250 mg/kg salt, while other studies were conducted in the countries where drinking water is the major source of fluoride exposure. In addition, these two investigations were not able to control for potential confounders and were limited by the relatively small sample sizes (n = 149 and 346, respectively) (Das and Mondal 2016; Yousefi et al., 2018). Furthermore, neither of these studies examined whether sex serves as a potential modifier.

We found a positive association between fluoride exposure and blood pressure, which was consistent with earlier epidemiological studies. Specifically, with the increase in the level of fluoride in ground water (mean: 0.53 mg/L), the prevalence of hypertension and SBP were increased in an Iranian population across 30 provinces (Amini et al., 2011), which was supported by another investigation conducted among Iranian adults (Yousefi et al., 2018). Similarly, a Chinese study of 487 adults aged 40 to 75 years residing in Heilongjiang Province found that higher fluoride in the drinking water (≥3.01 mg/L versus ≤ 1.20 mg/L) was associated with an increased risk of hypertension (Sun et al., 2013). A cross-sectional study of 417 children aged 5–12 years in Chihuahua, Mexico suggested that fluoride exposure may be associated with alterations in vascular biomarkers in childhood (Jimenez-Cordova et al., 2019).

We showed that plasma fluoride was associated with higher levels of glucose, insulin and insulin resistance in girls. Although no epidemiological studies have examined such associations, previous animal investigations suggested that fluoride exposure may cause a rise in the concentrations of glucose, insulin and its resistance in rats. Specifically, Hu et al. showed that high fluoride exposure increased serum insulin concentrations in 50 rats starting at two months of age that received fluoride in drinking water at 100 mg/L for a year compared with a control group (Hu et al., 2012). Their findings were confirmed by another study using two-month-old rats (n = 32) that were treated with fluoride through drinking water at 50 mg/L for 42 days (Pereira et al., 2017). The later study also provided evidence that fluoride causes a rise in insulin resistance measured by HOMA-IR (Pereira et al., 2017). One study using one dosage of fluoride (1.0 mg/kg per body weight) by gavage in 40 older rats (11 months of age) revealed that high fluoride exposure induced hyperglycemia (Chehoud, 2008).

In our cohort, fluoride exposure was not associated with serum lipid concentrations. No human studies have examined these associations, but previous investigations using animal data reported mixed findings (Afolabi et al., 2013; Czerny et al., 2004; Ma et al., 2012; Sun et al., 2014). Seventy-two rabbits who received fluoride at 50 and 100 mg/L in drinking water for five months had higher serum LDL-C, compared with those with no treatment (Sun et al., 2014). Similarly, another report found that 24 rats aged 9–10 weeks who were treated with fluoride in drinking water at the same dosage experienced increased LDL-C concentrations and decreased HDL-C in plasma (Afolabi et al., 2013). Czerny et al. confirmed these findings on LDL-C using 50 rats treated with fluoride orally at the level of 20 mg/kg per body weight for three months (Czerny et al., 2004). Increased serum LDL-C and the LDL-to-HDL ratio were also observed in 32 rabbits with fluoride at 50 mg/L in drinking water for six months, but no changes were found in the levels of triglycerides and HDL-C (Ma et al., 2012). It has been suggested that 5 mg/L fluoride in drinking water for rats may correspond to about 1 mg/L for humans (Dunipace et al., 1995).

The mechanisms by which fluoride exerts its impact on metabolic syndrome are not entirely understood: however, fluoride may induce oxidative stress (Lu et al., 2017; Oyagbemi et al., 2017) and inflammation (Afolabi et al., 2013; Ma et al., 2012), and disrupt sex hormones (Duan et al., 2016; Ortiz-Perez et al., 2003; Zhou et al., 2013), all of which have been recognized to play a major role in obesity, insulin resistance, hyperglycemia, dyslipidemia, and hypertension. Together, these cardiometabolic risk factors significantly increase the risk of cardiovascular disease (Siti et al., 2015). Given that previous analyses showed sex differences in regulating glucose, insulin sensitivity and lipid metabolism (Kim and Halter 2014; Macotela et al., 2009), it is reasonable to examine the sex-specific association of

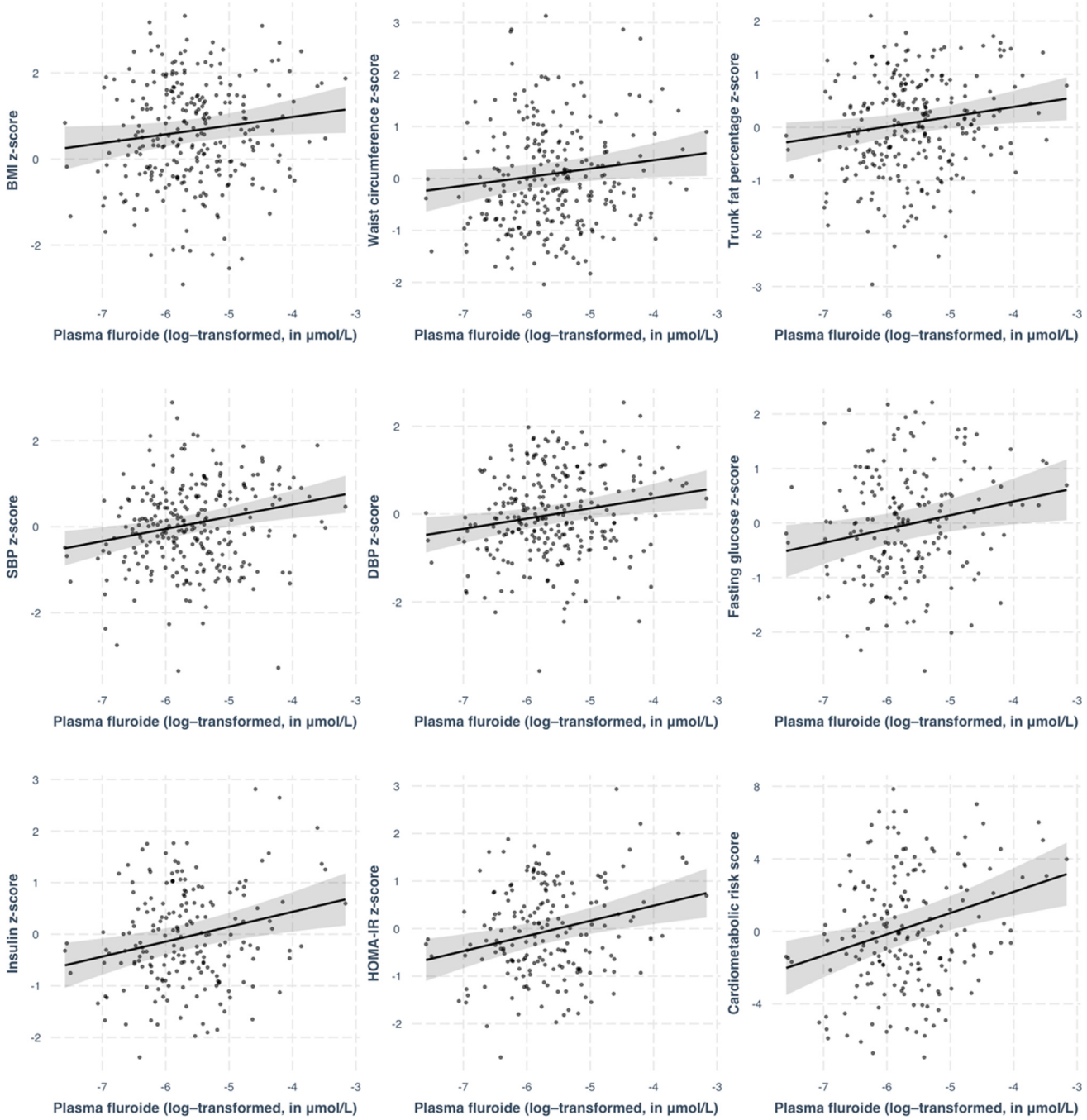

Fig. 1. Adjusted associations of log-transformed plasma fluoride with cardiometabolic risk factors in girls. SBP, systolic blood pressure; DBP, diastolic blood pressure; HOMA-IR, homeostatic model assessment of insulin resistance; Adjusted for child birth weight, gestational age and number of siblings at birth, maternal age, breastfeeding duration, marital status and smoking history, household social economic status score and cohort, and child age at visit.

fluoride with metabolic risk and its components.

In our study, the significant associations between fluoride exposure and child cardiometabolic risk were only observed in girls but not in boys. These findings are biologically plausible. It has been shown that exposure to fluoride may lead to decreased serum estrogen and progesterone levels in females (Zhou et al., 2013), which may play a protective role in the development of cardiovascular disease in women of reproductive age (Bhupathy et al., 2010; dos Santos et al., 2014). Further studies that include these sex hormones as mediators are needed.

The geometric mean of plasma fluoride in our study sample was 0.21 μmol/L (95% CI: 0.20–0.23 μmol/L). The fluoride levels in plasma in our study population are comparable to the levels reported by other populations. Here, we compared the plasma fluoride levels in our cohort with other populations; however, available studies measuring fluoride concentrations in plasma were quite limited. A U.S. study using data from the National Health and Nutrition Examination Survey (NHANES) 2013–2014 reported a geometric mean at 0.41 μmol/L (95% CI: 0.39–0.44 μmol/L) and 0.40 μmol/L (95% CI: 0.36–0.44 μmol/L) in 1075 children aged 6–11 years and 1250 adolescents aged 12–19 years, respectively (Jain, 2017). Fifteen adults aged 25–35 years from Brazil had a mean value of plasma fluoride at 0.44 μmol/L (SD: 0.10 μmol/L) or 0.55 μmol/L (SD: 0.10 μmol/L), depending on the communities of living (Cardoso et al., 2006).

According to our sensitivity analyses, pubertal stage might confound the associations of fluoride exposure with children's adiposity,

which is consistent with our previous findings suggesting fluoride may affect pubertal development (Liu et al., 2019a). It is possible that puberty might serve as a mediator of these associations; however, due to the cross-sectional design of this study, we lack the ability to demonstrate that puberty is part of the causal chain between fluoride exposure and cardiometabolic health. On the other hand, excluding children who were born preterm or low birth weight might also confound such associations. Fluoride exposure has been related to adverse birth outcomes (Zhang et al., 2019). In addition, infants who were born preterm are more sensitive to have altered renal function in later life, and the major excretion pathway of fluoride exposure is through kidney (Sanders et al., 2018). Furthermore, infants with smaller body size are exposed to higher dosage of fluoride during fetal life and subsequently accumulate fluoride in their bones (National Research Council (U.S.). Committee on Fluoride in Drinking Water, 2006). During puberty, fluorides stored in skeletons are released to children's blood stream (van Coeverden et al., 2002). Therefore, it is possible that preterm birth or low birth weight may be associated with greater fluoride levels in childhood.

Our study has some limitations. First, due to budget constraints, serum concentrations of glucose, lipid and other hormones were only measured in a subset of children (n = 400). Second, we did not collect information on salt intake so we used sodium intake as a proxy; it is reasonable because salt is the major source of sodium intake. Third, the cross-sectional design does not reflect any causal association. It is possible that children with high levels of cardiometabolic risk factors may be exposed to a high salt diet and high consumption of sugar-sweetened beverages that contain high levels of fluoride (Cantoral et al., 2019). Finally, given that the study population represents low-to-middle class among Mexican population, our findings may not be generalizable to other socioeconomic groups or populations with different ethnic composition. Despite these limitations, this study has several strengths. We were able to use multiple individual markers and a combined score of cardiometabolic risk, as well as an individual biomarker of fluoride. Several potential confounders and covariates associated with fluoride or cardiometabolic risk were considered in our study. Finally, we examined the sex-specific association between fluoride and cardiometabolic risk and found that these associations varied by child sex, which has not been previously reported.

5. Conclusions

In conclusion, our study suggested that higher fluoride exposure may increase the risk of cardiometabolic risk in children following a sex-specific pattern. Given that household SES and ethnicity are important risk factors for cardiometabolic risk, our findings may not be generalizable to other populations or socioeconomic groups. In addition, our results may not be generalizable to other populations exposed to fluoride through water supply or other vectors, since our participants received fluoride from table salt. Studies with a longitudinal design conducted in different populations and socioeconomic groups and including sex hormones are needed to confirm our findings and further elucidate the potential sex differential effect of fluoride exposure on the risk of cardiovascular disease.

Declaration of Competing Interest

The authors have no conflicts of interest to disclose.

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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.2019.105302.

References

Afolabi, O.K., Oyewo, E.B., Adekunle, A.S., Adedosu, O.T., Adedeji, A.L., 2013. Oxidative indices correlate with dyslipidemia and pro-inflammatory cytokine levels in fluoride-exposed rats. Arh. Hig. Rada. Toksikol. 64, 521–529.
Al-Hamad, D., Raman, V., 2017. Metabolic syndrome in children and adolescents. Transl. Pediatr. 6, 397–407.
Amini, H., Taghavi Shahri, S.M., Amini, M., Ramezani Mehrian, M., Mokhayeri, Y., Yunesian, M., 2011. Drinking water fluoride and blood pressure? An environmental study. Biol. Trace Elem. Res. 144, 157–163.
Bashash, M., Thomas, D., Hu, H., Martinez-Mier, E.A., Sanchez, B.N., Basu, N., 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, 097017.
Bhupathy, P., Haines, C.D., Leinwand, L.A., 2010. Influence of sex hormones and phytoestrogens on heart disease in men and women. Womens Health (Lond) 6, 77–95.
Cantoral, A., Luna-Villa, L., Mantilla-Rodriguez, A.A., Mercado, A., Lippert, F., Liu, Y., et al., 2019. Fluoride content in foods and beverages from Mexico City markets & supermarkets. Food Nutr. Bull.
Cardoso, V.E., Whitford, G.M., Buzalaf, M.A., 2006. Relationship between daily fluoride intake from diet and the use of dentifrice and human plasma fluoride concentrations. Arch. Oral Biol. 51, 552–557.
Chehoud, K.A.C.F., Sassaki, K.T., Garbin, S.A.S., Sumidaa, D.H., 2008. Effect of fluoride intake on insulin sensitivity and insulin signal transduction. Fluoride 41 270-207.
Czerny, B., Pawlik, A., Juzyszyn, Z., Mysliwiec, Z., 2004. The effect of tamoxifen and fluoride on bone mineral density, biomechanical properties and blood lipids in ovariectomized rats. Basic Clin. Pharmacol. Toxicol. 95, 162–165.
Das, K., Mondal, N.K., 2016. Dental fluorosis and urinary fluoride concentration as a reflection of fluoride exposure and its impact on IQ level and BMI of children of Laxmisagar, Simlapal Block of Bankura District, W.B., India. Environ. Monit. Assess. 188, 218.
de Onis, M., Onyango, A.W., Borghi, E., Siyam, A., Nishida, C., Siekmann, J., 2007. Development of a WHO growth reference for school-aged children and adolescents. Bull. World Health Organ. 85, 660–667.
DeBoer, M.D., Gurka, M.J., Woo, J.G., Morrison, J.A., 2015. Severity of the metabolic syndrome as a predictor of type 2 diabetes between childhood and adulthood: the Princeton Lipid Research Cohort Study. Diabetologia 58, 2745–2752.
dos Santos, R.L., da Silva, F.B., Ribeiro Jr., R.F., Stefanon, I., 2014. Sex hormones in the cardiovascular system. Horm. Mol. Biol. Clin. Investig. 18, 89–103.
Duan, L., Zhu, J., Wang, K., Zhou, G., Yang, Y., Cui, L., et al., 2016. Does fluoride affect serum testosterone and androgen binding protein with age-specificity? A population-based cross-sectional study in Chinese male farmers. Biol. Trace Elem. Res. 174, 294–299.
Dunipace, A.J., Brizendine, E.J., Zhang, W., Wilson, M.E., Miller, L.L., Katz, B.P., et al., 1995. Effect of aging on animal response to chronic fluoride exposure. J. Dent. Res. 74, 358–368.
Ford, E.S., Li, C., 2008. Defining the metabolic syndrome in children and adolescents: will the real definition please stand up? J. Pediatr. 152, 160–164.
Friend, A., Craig, L., Turner, S., 2013. The prevalence of metabolic syndrome in children: a systematic review of the literature. Metabolic Syndr. Relat. Disord. 11, 71–80.
Hu, C.Y., Ren, L.Q., Li, X.N., Wu, N., Li, G.S., Liu, Q.Y., et al., 2012. Effect of fluoride on insulin level of rats and insulin receptor expression in the MC3T3-E1 cells. Biol. Trace Elem. Res. 150, 297–305.
Hu, H., Tellez-Rojo, M.M., Bellinger, D., Smith, D., Ettinger, A.S., Lamadrid-Figueroa, H., et al., 2006. Fetal lead exposure at each stage of pregnancy as a predictor of infant mental development. Environ. Health Perspect. 114, 1730–1735.
Jain, R.B., 2017. Concentrations of fluoride in water and plasma for US children and adolescents: data from NHANES 2013–2014. Environ. Toxicol. Pharmacol. 50, 20–31.
Jimenez-Cordova, M.I., Gonzalez-Horta, C., Ayllon-Vergara, J.C., Arreola-Mendoza, L., Aguilar-Madrid, G., Villareal-Vega, E.E., et al., 2019. Evaluation of vascular and kidney injury biomarkers in Mexican children exposed to inorganic fluoride. Environ. Res. 169, 220–228.
Kassi, E., Pervanidou, P., Kaltsas, G., Chrousos, G., 2011. Metabolic syndrome: definitions and controversies. BMC Med. 9, 48.
Kim, C., Halter, J.B., 2014. Endogenous sex hormones, metabolic syndrome, and diabetes in men and women. Curr. Cardiol. Rep. 16, 467.
Liu, Y., Peterson, K.E., 2015. Maternal exposure to synthetic chemicals and obesity in the offspring: recent findings. Curr. Environ. Health Rep. 2, 339–347.
Liu, Y., Tellez-Rojo, M., Hu, H., Sanchez, B.N., Martinez-Mier, E.A., Basu, N., et al., 2019a.

Fluoride exposure and pubertal development in children living in Mexico City. Environ. Health 18, 26.
Liu, Y., Téllez-Rojo, M.M., Sánchez, B.N., Zhang, Z., Afeiche, M.C., Mercado-García, A., et al., 2019b. Early lead exposure and pubertal development in a Mexico City population. Environ. Int. 125C, 445–451.
Lohman, T.G., Roche, A.F., Martorell, R., 1988. Anthropometric standardization reference manual. Human Kinetics Books, Champaign, IL.
López-Romo, H., 2011. Actualización regla amai nse 8x7. México, DF.
Lu, Y., Luo, Q., Cui, H., Deng, H., Kuang, P., Liu, H., et al., 2017. Sodium fluoride causes oxidative stress and apoptosis in the mouse liver. Aging (Albany NY) 9, 1623–1639.
Luu, T.M., Katz, S.L., Leeson, P., Thebaud, B., Nuyt, A.M., 2016. Preterm birth: risk factor for early-onset chronic diseases. CMAJ 188, 736–746.
Ma, Y., Niu, R., Sun, Z., Wang, J., Luo, G., Zhang, J., et al., 2012. Inflammatory responses induced by fluoride and arsenic at toxic concentration in rabbit aorta. Arch. Toxicol. 86, 849–856.
Macotela, Y., Boucher, J., Tran, T.T., Kahn, C.R., 2009. Sex and depot differences in adipocyte insulin sensitivity and glucose metabolism. Diabetes 58, 803–812.
Marshall, W.A., Tanner, J.M., 1969. Variations in pattern of pubertal changes in girls. Arch. Dis. Child. 44, 291–303.
Marshall, W.A., Tanner, J.M., 1970. Variations in the pattern of pubertal changes in boys. Arch. Dis. Child. 45, 13–23.
Martinez-Mier, E.A., Cury, J.A., Heilman, J.R., Katz, B.P., Levy, S.M., Li, Y., et al., 2011. Development of gold standard ion-selective electrode-based methods for fluoride analysis. Caries Res. 45, 3–12.
Morrison, J.A., Friedman, L.A., Wang, P., Glueck, C.J., 2008. Metabolic syndrome in childhood predicts adult metabolic syndrome and type 2 diabetes mellitus 25 to 30 years later. J. Pediatr. 152, 201–206.
National Research Council (U.S.). Committee on Fluoride in Drinking Water, 2006. Fluoride in drinking water: a scientific review of EPA's standards. National Academies Press, Washington, D.C.
Newsome, C.A., Shiell, A.W., Fall, C.H., Phillips, D.I., Shier, R., Law, C.M., 2003. Is birth weight related to later glucose and insulin metabolism?– A systematic review. Diabet. Med. 20, 339–348.
O'Neill, S., O'Driscoll, L., 2015. Metabolic syndrome: a closer look at the growing epidemic and its associated pathologies. Obes. Rev. 16, 1–12.
Ortiz-Perez, D., Rodriguez-Martinez, M., Martinez, F., Borja-Aburto, V.H., Castelo, J., Grimaldo, J.I., et al., 2003. Fluoride-induced disruption of reproductive hormones in men. Environ. Res. 93, 20–30.
Oyagbemi, A.A., Omobowale, T.O., Asenuga, E.R., Adejumobi, A.O., Ajibade, T.O., Ige, T.M., et al., 2017. Sodium fluoride induces hypertension and cardiac complications through generation of reactive oxygen species and activation of nuclear factor kappa beta. Environ. Toxicol. 32, 1089–1101.
Pena-Espinoza, B.I., Granados-Silvestre, M.L.A., Sanchez-Pozos, K., Ortiz-Lopez, M.G., Menjivar, M., 2017. Metabolic syndrome in Mexican children: low effectiveness of diagnostic definitions. Endocrinol. Diabetes Nutr. 64, 369–376.
Pereira, A.G., Chiba, F.Y., de Lima Coutinho Mattera, M.S., Pereira, R.F., de Cassia Alves Nunes, R., Tsosura, T.V.S., et al., 2017. Effects of fluoride on insulin signaling and bone metabolism in ovariectomized rats. J. Trace Elem. Med Biol. 39, 140–146.
Rosner, B., 1983. Percentage points for a generalized ESD many-outlier procedure. Technometrics 25, 165–172.
Sanders, A.P., Svensson, K., Gennings, C., Burris, H.H., Oken, E., Amarasiriwardena, C., et al., 2018. Prenatal lead exposure modifies the effect of shorter gestation on increased blood pressure in children. Environ. Int. 120, 464–471.
Siti, H.N., Kamisah, Y., Kamsiah, J., 2015. The role of oxidative stress, antioxidants and vascular inflammation in cardiovascular disease (a review). Vasc. Pharmacol. 71, 40–56.
Sun, L., Gao, Y., Liu, H., Zhang, W., Ding, Y., Li, B., et al., 2013. An assessment of the relationship between excess fluoride intake from drinking water and essential hypertension in adults residing in fluoride endemic areas. Sci. Total Environ. 443, 864–869.
Sun, L., Gao, Y., Zhang, W., Liu, H., Sun, D., 2014. Effect of high fluoride and high fat on serum lipid levels and oxidative stress in rabbits. Environ. Toxicol. Pharmacol. 38, 1000–1006.
Thomas, D.B., Basu, N., Martinez-Mier, E.A., Sanchez, B.N., Zhang, Z., Liu, Y., et al., 2016. Urinary and plasma fluoride levels in pregnant women from Mexico City. Environ. Res. 150, 489–495.
van Coeverden, S.C., Netelenbos, J.C., de Ridder, C.M., Roos, J.C., Popp-Snijders, C., Delemarre-van de Waal, H.A., 2002. Bone metabolism markers and bone mass in healthy pubertal boys and girls. Clin. Endocrinol. (Oxf) 57, 107–116.
Viitasalo, A., Lakka, T.A., Laaksonen, D.E., Savonen, K., Lakka, H.M., Hassinen, M., 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, 940–949.
Widen, E., Silventoinen, K., Sovio, U., Ripatti, S., Cousminer, D.L., Hartikainen, A.L., et al., 2012. Pubertal timing and growth influences cardiometabolic risk factors in adult males and females. Diabetes Care 35, 850–856.
Yokoyama, H., Emoto, M., Fujiwara, S., Motoyama, K., Morioka, T., Komatsu, M., et al., 2004. Quantitative insulin sensitivity check index and the reciprocal index of homeostasis model assessment are useful indexes of insulin resistance in type 2 diabetic patients with wide range of fasting plasma glucose. J. Clin. Endocrinol. Metab. 89, 1481–1484.
Yousefi, M., Yaseri, M., Nabizadeh, R., Hooshmand, E., Jalilzadeh, M., Mahvi, A.H., 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 Elem. Res. 185, 282–288.
Zhang, X., Lu, E., Stone, S.L., Diop, H., 2019. Dental cleaning, community water fluoridation and preterm birth, Massachusetts: 2009–2016. Matern. Child Health J. 23, 451–458.
Zhou, Y., Zhang, H., He, J., Chen, X., Ding, Y., Wang, Y., et al., 2013. Effects of sodium fluoride on reproductive function in female rats. Food Chem. Toxicol. 56, 297–303.