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Prenatal and postnatal manganese teeth levels and neurodevelopment at 7, 9, and 10.5 years in the CHAMACOS cohort

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ABSTRACT

Background: Numerous cross-sectional studies of school-age children have observed that exposure to manganese (Mn) adversely affects neurodevelopment. However, few prospective studies have looked at the effects of both prenatal and postnatal Mn exposure on child neurodevelopment.
Methods: We measured Mn levels in prenatal and early postnatal dentine of shed teeth and examined their association with behavior, cognition, memory, and motor functioning in 248 children aged 7, 9, and/or 10.5 years living near agricultural fields treated with Mn-containing fungicides in California. We used generalized linear models and generalized additive models to test for linear and nonlinear associations, and generalized estimating equation models to assess longitudinal effects.
Results: We observed that higher prenatal and early postnatal Mn levels in dentine of deciduous teeth were adversely associated with behavioral outcomes, namely internalizing, externalizing, and hyperactivity problems, in boys and girls at 7 and 10.5 years. In contrast, higher Mn levels in prenatal and postnatal dentine were associated with better memory abilities at ages 9 and 10.5, and better cognitive and motor outcomes at ages 7 and 10.5 years, among boys only. Higher prenatal dentine Mn levels were also associated with poorer visuospatial memory outcomes at 9 years and worse cognitive scores at 7 and 10.5 years in children with higher prenatal lead levels (≥0.8 μg/dL). All these associations were linear and were consistent with findings from longitudinal analyses.
Conclusions: We observed that higher prenatal and early postnatal Mn levels measured in dentine of deciduous teeth, a novel biomarker that provides reliable information on the developmental timing of exposures to Mn, were associated with poorer behavioral outcomes in school-age boys and girls and better motor function, memory, and/or cognitive abilities in school-age boys. Additional research is needed to understand the inconsistencies in the neurodevelopmental findings across studies and the degree to which differences may be associated with different Mn exposure pathways and biomarkers.

Abbreviations: ADHD, Attention Deficit Hyperactivity Disorder; AUC, Area Under the Curve; BASC-2, Behavior Assessment Scale for Children, 2nd edition; CADS, Conners' ADHD/DSM-IV Scales, Parent and Teacher versions; CAVLT-2, Children's Auditory Verbal Learning Test, 2nd edition; CES-D, Center for Epidemiologic Studies Depression Scale; CHAM1, Initial CHAMACOS cohort (recruited 1999–2000 during pregnancy); CHAM2, Second CHAMACOS cohort (recruited 2009–2011 at child age 9); CHAMACOS, Center for the Health Assessment of Mothers and Children of Salinas; CI, Confidence Interval; CPT-II, Conners' Continuous Performance Test II, Version 5; DAP, Dialkyl Phosphate; DSM-IV, Diagnostic and Statistical Manual of Mental Disorders, 4th edition; GEE, Generalized Estimating Equations; GM, Geometric Mean; HOME, Home Observation for Measurement of the Environment; IQ, Intelligence Quotient; LOD, Limit of Detection; Mn, Manganese; NEPSY-II, A Developmental Neuropsychological Assessment, 2nd edition; OP, Organophosphate; PBDE, Polybrominated Diphenyl Ether; PPVT, Peabody Picture Vocabulary Test; TVIP, Test de Vocabulario en Imágenes Peabody; SD, Standard Deviation; SE, Standard Error; WISC-IV, Wechsler Intelligence Scale for Children, 4th edition; WRAVMA, Wide Range Assessment of Visual Motor Ability.

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1. Introduction

Manganese (Mn) is an essential element involved in important enzymatic reactions (Aschner, 2000; Gwiazda et al., 2002), but in excess, it is a potent neurotoxicant (Menezes-Filho et al., 2009a; Mergler and Baldwin, 1997; Roels et al., 2012). Food is the main source of Mn for the general population (ATDSR, 2012), but environmental exposure to Mn can occur from water naturally high in Mn or contaminated by industrial waste (Bouchard et al., 2007; Bouchard et al., 2011b; He et al., 1994; Kondakis et al., 1989), combustion of anti-knock additives in gasoline (Zayed et al., 1999), Mn mining operations (Riojas-Rodriguez et al., 2010), ferromanganese production facilities (Haynes et al., 2010; Menezes-Filho et al., 2009b), and spraying of Mn-containing fungicides (Gunier et al., 2013; Mora et al., 2014). Absorption and distribution of ingested Mn are closely regulated through homeostatic mechanisms (Papavasiliou et al., 1966; Roth, 2006). However, inhaled Mn can directly enter the systemic circulation through the lungs (Vitarella et al., 2000) and access the brain directly through the olfactory bulb (Dorman et al., 2002; Elder et al., 2006; Leavens et al., 2007), bypassing biliary excretion mechanisms.

Children and infants may be particularly susceptible to the neurotoxic effects of Mn exposure as their Mn homeostatic mechanisms are poorly developed (Aschner, 2000; Ljung and Vahter, 2007; Yoon et al., 2009) and Mn can enter their developing brains by crossing the blood–brain barrier (Aschner, 2000; Aschner and Dorman, 2006). Multiple studies have reported associations between exposure to Mn and neurodevelopmental problems in children. Higher in utero Mn levels measured in blood and teeth have been associated with attention problems (Ericson et al., 2007; Takser et al., 2003), behavioral disinhibition (Ericson et al., 2007), impaired non-verbal memory (Takser et al., 2003), and poor cognitive and language development (Lin et al., 2013) in toddlers and preschoolers, and with externalizing behavior and attention problems (Ericson et al., 2007) in school-aged children. Postnatal Mn exposure has been associated with poor language development in toddler boys (Rink et al., 2014), and behavioral problems in school-aged boys and girls (Ericson et al., 2007). Studies of school-aged children and adolescents (6–14 year olds) have linked elevated Mn levels in drinking water, blood, and hair samples with oppositional behavior and hyperactivity (Bouchard et al., 2007), impaired cognitive abilities (Bouchard et al., 2011b; Kim et al., 2009; Menezes-Filho et al., 2011; Riojas-Rodriguez et al., 2010; Wasserman et al., 2006), and poor memory (He et al., 1994; Torres-Agustin et al., 2013), motor coordination (He et al., 1994; Hernandez-Bonilla et al., 2011; Lucchini et al., 2012), and visuoperceptive speed (He et al., 1994; Zhang et al., 1995). To date, only one epidemiologic study has assessed exposure to Mn both prenatally and postnatally (Ericson et al., 2007).

Blood Mn has typically been used as a biomarker of exposure to Mn in occupational and population-based studies of adults and children (Mergler et al., 1999; Takser et al., 2003), while studies in environmentally-exposed children have also measured Mn levels in hair (Bouchard et al., 2007; Bouchard et al., 2011b; Eastman et al., 2013; Menezes-Filho et al., 2011; Riojas-Rodriguez et al., 2010; Wright et al., 2006), in the exposure medium (e.g., water) (Bouchard et al., 2011b; Khan et al., 2012; Wasserman et al., 2006), or in teeth (Arora et al., 2012; Ericson et al., 2007). Studies on Mn toxicokinetics suggest that blood may best reflect recent exposures (i.e., days), while teeth may integrate longer-term exposures (e.g., months or longer) (Arora et al., 2011; Arora et al., 2012; Ericson et al., 2007; Smith et al., 2007). Deciduous teeth incorporate Mn in an incremental pattern and dentine, unlike enamel, can provide reliable information on the developmental timing of exposures to Mn that occur between the second trimester of pregnancy (starting at 13–16 weeks gestation, when incisors begin forming) and 10–11 months after birth (when molars stop developing) (Arora et al., 2012).

In this study, we measured prenatal and early postnatal dentine Mn levels in children's deciduous teeth, and examined the association of Mn levels with behavior, cognition, memory, and motor development in 7-, 9-, and 10.5-year-old children living in an agricultural community in California where large amounts of Mn-containing fungicides are applied.

2. Methods

2.1. Study population

The Center for the Health Assessment of Mother and Children of Salinas (CHAMACOS) is a birth cohort study examining the health effects of pesticide and other environmental exposures in Mexican-American children living in the Salinas Valley, California. Common crops in this agricultural region include lettuce, strawberries, grapes, and broccoli. About 110,000 kg of Mn-containing fungicides, mancozeb and maneb (20% Mn by weight) (FAO, 1980), were used in Monterey County in 2012 (CDPR, 2014), but almost 160,000 kg were applied in 1999–2000, when study participants were pregnant (CDPR, 2001).

Detailed methods for the CHAMACOS study have been described elsewhere (Eskenazi et al., 2004; Eskenazi et al., 2006). Briefly, eligible pregnant women (≥18 years old, <20 weeks of gestation, Spanish- or English-speaking, qualified for low-income health insurance, and planning to deliver at the county hospital) were recruited in community clinics between September 1999 and December 2000. Six hundred and one pregnant women were enrolled and 526 of them delivered live-born singletons (referred to henceforth as the CHAM1 cohort).

A second cohort of 300 9 year-olds (referred to henceforth as the CHAM2 cohort) was recruited between September 2009 and August 2011. CHAM2 children were born between February 2000 and August 2002 to approximately match the birth dates of CHAM1 children. Children were eligible to participate if their mother, when pregnant, was ≥18 years old, Spanish- or English-speaking, qualified for low-income health insurance, and received prenatal care at any low-income provider in the Salinas Valley.

Because CHAM2 enrollment began at age 9, only CHAM1 children completed the neurobehavioral test battery at age 7 ($n = 339$). CHAM1 and CHAM2 children completed identical neurobehavioral assessments at ages 9 ($n = 634$) and 10.5 ($n = 615$). Standardized assessments were conducted by bilingual psychometricians who were trained and supervised by a pediatric neuropsychologist. Subtests were administered in the dominant language of the child, which was determined through administration of the Oral Vocabulary subtest of the Woodcock–Johnson/Woodcock–Muñoz Tests of Cognitive Ability in both English and Spanish (Woodcock and Johnson, 1990).

Teeth were collected for 282 CHAM1 and 173 CHAM2 children, but due to financial and logistical constraints, only teeth for 227 CHAM1 children and 70 CHAM2 children were analyzed. For this study, we excluded 39 children who provided a shed molar instead of an incisor, four children with a medical condition that would affect the neurobehavioral assessment (i.e., one with autism, and three with history of seizures), three children who were twins, and three children missing all neurobehavioral assessments. Children included in these analyses ($n = 248$) did not differ significantly from the full sample of CHAM1 ($n = 335$) and CHAM2 ($n = 309$) children on most attributes, including maternal marital status, poverty category at age 9, and child's birth weight. However, children included in these analyses had older mothers (mean age = 26.8 vs. 25.6 years, $p < 0.01$) with poorer cognitive abilities [mean maternal Peabody Picture Vocabulary Test (PPVT) score = 88.9 vs. 93.2 points, $p < 0.01$] than the full sample of CHAM1 and CHAM2 children.

All study activities were approved by the University of California at Berkeley Committee for the Protection of Human Subjects, and written informed consent was obtained from all mothers. Child verbal assent was obtained at 7, 9, and 10.5 years of age.

2.2. Maternal interviews and assessments

CHAM1 mothers were interviewed twice during pregnancy (median, 13 and 26 weeks gestation), shortly after delivery, and when children were 6 months, and 1, 2, 3.5, 5, 7, 9, and 10.5 years old. CHAM2 mothers were interviewed when their children were 9 and 10.5 years old. Interviews were conducted in English or Spanish by trained bilingual interviewers. CHAM1 mothers were administered the Revised PPVT or Test de Vocabulario en Imágenes Peabody (TVIP) of Verbal Intelligence (Dunn and Dunn, 1981) at the 6-month and 9-year visits; CHAM2 mothers completed the PPVT/TVIP at the 9-year visit only. CHAM1 mothers also completed the Center for Epidemiologic Studies Depression Scale (CES-D) (Radloff, 1977) at the 7- and 9-year visits, and the Middle Childhood and Early Adolescence Home Observation for Measurement of the Environment (HOME) inventory short form (Caldwell and Bradley, 1984) at the 7-, 9-, and 10.5-year visits, while CHAM2 mothers completed these scales only at the 9- and 10.5-year visits. Additional information, such as birth weight and gestational duration, was abstracted from prenatal and delivery medical records for both CHAM1 and CHAM2 participants. Data on maternal hemoglobin during pregnancy (median, 25 weeks gestation) were abstracted for CHAM1 children only.

2.3. Behavior

Mothers and teachers of CHAM1 children were administered either the English or Spanish version of the Parent and Teacher Rating Scales of the Behavior Assessment System for Children, 2nd edition (BASC-2) (Reynolds and Kamphaus, 2004) and the Conners' Attention Deficit Hyperactivity Disorder (ADHD)/Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV) Scales (CADS) (Conners, 2001) when children were 7 years old. CHAM1 and CHAM2 mothers were administered the CADS at the 9-year visit and the BASC-2 at the 10.5-year visit. Scores for four CADS subscales (Conners ADHD index, and DSM-IV-based Inattentive, Hyperactive/Impulsive, and Total ADHD), two BASC-2 subscales (Hyperactivity and Attention Problems), and two BASC-2 composite scales (Internalizing and Externalizing problems) were calculated and standardized to a nonclinical population (age-standardized *T*-scores, mean ± SD = 50 ± 10), with higher values indicating more frequent behavioral problems.

At 9 years of age, CHAM1 and CHAM2 children completed the Conners' Continuous Performance Test II, Version 5 (CPT-II) (Conners, 2002), a computerized test that assesses accuracy and impulse control. Scores for errors of commission (false positive) and errors of omission (false negative) were analyzed as continuous, sex- and age-standardized *T*-scores (mean ± SD = 50 ± 10). A continuous ADHD Confidence Index score, indicating the probability of children being correctly classified as having clinical ADHD, was also examined.

At the 10.5-year visit, CHAM1 and CHAM2 children were administered the BASC-2 Self-Report of Personality, Child Version (Reynolds and Kamphaus, 2004). Scores for two subscales (Hyperactivity and Attention problems) were compared to national norms to generate age-standardized *T*-scores (mean ± SD = 50 ± 10), with higher scores indicating more frequent behavioral problems.

2.4. Cognition

CHAM1 children were administered either the English or Spanish version of the Wechsler Intelligence Scale for Children, 4th edition (WISC-IV) (Wechsler, 2003) at the 7-year study visit. At 10.5 years of age CHAM1 and CHAM2 children were also administered the WISC-IV. Scores for four domains were calculated at both time points: Verbal Comprehension, Perceptual Reasoning, Working Memory, and Processing Speed. A Full-Scale intelligence quotient (IQ) was also calculated. WISC-IV scores were standardized against U.S. population-based norms for English- and Spanish-speaking children and analyzed as continuous variables.

2.5. Visuospatial and verbal memory

At the 9-year visit, CHAM1 and CHAM2 children completed a test of visuospatial memory, the NEPSY-II Memory for Designs (Korkman et al., 2007). We calculated continuous scaled scores (mean ± SD = 10 ± 3) for immediate and delayed memory using normative values for the corresponding chronological age.

At age 10.5 years, children's verbal learning and memory abilities were assessed using either the English or Spanish version of the Children's Auditory Verbal Learning Test, 2nd edition (CAVLT-2) (Talley, 1997; Torres-Agustin et al., 2013). We analyzed four subscales as continuous standardized scores (mean ± SD = 100 ± 15): Learning curve (learning progression), Immediate recall (susceptibility of new information to be disrupted), Delayed recall (long-term memory and retrieval ability), Immediate memory span (short-term memory), and Level of learning (long-term memory coding abilities) (Torres-Agustin et al., 2013).

2.6. Motor functioning

CHAM1 children were administered finger-tapping (Reitan Neuropsychology Laboratory, Tucson, AZ) and pegboard (Wide Range Assessment of Visual Motor Ability, WRAVMA) (Adams and Sheslow, 1995) tests at age 7 to assess fine motor dexterity. Finger-tap scores were standardized within our study population (*z*-scores, mean ± SD = 0 ± 1), but pegboard scores were age-standardized to a mean of 100 (SD = 15).

At ages 9 and 10.5 years, CHAM1 and CHAM2 children were administered parts of the Luria Nebraska Motor Battery (Golden et al., 1980). We selected for analysis seven subtests that have shown sensitivity to Mn exposure (Lucchini et al., 2012): dominant hand clench, non-dominant hand clench, alternative hand clench, finger-thumb touching with dominant hand, finger-thumb touching with non-dominant hand, alternative hand tapping twice with dominant hand and once with non-dominant hand, and alternative hand tapping twice with non-dominant hand and once with dominant hand. The sum of the scores of the five subtests administered by Lucchini et al. (2012) and the sum of all seven subtests were standardized within our study population (*z*-scores, mean ± SD = 0 ± 1).

2.7. Tooth Mn measurements

Tooth collection started at the 7-year visit for CHAM1 children and at the 9-year visit for the CHAM2 children. Participants were asked to mail or bring to the study visits the child's shed teeth. Detailed methods for measuring Mn in teeth dentine and its validation as a biomarker of prenatal and early postnatal Mn exposure have been described elsewhere (Arora et al., 2011; Arora et al., 2012). Briefly, incisors were sectioned in a vertical plane, cleaned in an ultrasonic bath of Milli-Q water, and dried in an oven at 60 °C for 24 h. Then the neonatal line, a histological feature used to demarcate prenatally and postnatally formed regions of enamel and dentine (Sabel et al., 2008), was identified using light microscopy. Mn levels and spatial distribution in prenatal and postnatal mantle dentine were determined with laser ablation–inductively coupled plasma-mass spectrometry using the neonatal line as a reference. Because multiple measurements were taken in prenatal and postnatal dentine, we calculated the area under the curve (AUC) to estimate cumulative Mn exposure in prenatal (from 13 to 16 weeks of gestation to birth) and postnatal (from birth to approximately 2.5 months of age) periods. Mn levels were normalized to ^{43}Ca to adjust for variations in mineralization. Coefficients of variation for five teeth measured on three different days ranged from 4.5% to 9.5% indicating good reproducibility of ^{55}Mn:^{43}Ca dentine measurements. Mn levels

below the limit of detection (LOD = 0.001 ^{55}Mn:^{43}Ca AUC x 10^4) were set at LOD/$\sqrt{2}$ ($n = 4$ postnatal dentine samples). In addition, four children had Mn measurements in prenatal dentine but no measurements in postnatal dentine due to tooth wear.

2.8. Other environmental toxicants

We examined the potential confounding or effect modification of known or suspected neurotoxicants, including organophosphorous (OP) pesticides, lead, and polybrominated diphenyl ether flame retardants (PBDEs), in CHAM1 children. Prenatal exposure to OP pesticides, indicated by urinary dialkyl phosphate (DAP) metabolite levels, was measured in maternal urine samples collected at approximately 13 and 26 weeks of gestation using an isotope dilution gas chromatography–tandem mass spectrometry method (Bradman et al., 2005). Blood lead levels were quantified in cord blood, maternal samples collected at about 26 weeks of gestation, or maternal samples collected at delivery ($n = 59$, 53, and 53, respectively) using graphite furnace atomic absorption spectrometry. Lead was also quantified in blood samples collected from children at 12 ($n = 161$) and 24 months ($n = 176$). PBDEs were measured in maternal blood samples at approximately 26 weeks of gestation using high-resolution gas chromatography/high-resolution mass spectrometry with isotope dilution quantification (Sjodin et al., 2004). PBDE levels were expressed on a serum lipid basis. Total lipids were quantified by measuring triglycerides and total cholesterol in serum (Phillips et al., 1989).

2.9. Data analysis

Prenatal and postnatal dentine Mn levels were transformed to the $\log_2$ scale to normalize the residuals and reduce the influence of outliers. We examined the association between teeth Mn levels and neurodevelopment using multivariable linear regression models. We also examined potential non-linear associations using generalized additive models with a three-degrees-of-freedom cubic spline function. If a potentially nonlinear association between dentine Mn levels and any of the neurodevelopmental outcomes was identified ($p_{GAM} < 0.05$), we created indicator variables for both tertiles and quintiles of Mn levels and included them in the adjusted regression models (we modeled tertiles separately from quintiles). We used generalized estimating equation (GEE) models to examine relationships of prenatal and postnatal dentine Mn levels with outcomes that were examined in two of the three neurobehavioral assessments.

We built separate models for behavioral, cognitive, memory, and motor outcomes, and used the same covariates in the model for all outcomes within a category. Main covariates of interest were selected using directed acyclic graphs and based on statistical considerations if covariates were associated with the exposure and any of the outcomes in the bivariate analyses ($p < 0.20$). We retained the following variables as covariates for all analyses (modeled as shown in Table 1, unless defined below): maternal education, intelligence (PPVT score, continuous), years in the US (continuous), depression at time of assessment (dichotomous: <16 vs. ≥16 points in CES-D); child's sex and age at neurobehavioral assessment or at maternal interview (continuous); child language of the assessment or maternal language at interview (dichotomous); psychometrician (one, two, or three categories); HOME z-score at time of assessment (continuous); household income at time of assessment, and number of children in the home at time of assessment (continuous). Missing values (<10%) for covariates were imputed using data from the nearest available visit when available or by randomly selecting a value from the dataset.

We conducted several sensitivity analyses to assess the robustness of our results. First, we reran models after excluding outliers defined by studentized residuals (residuals divided by the model standard error) greater than three standard units. Second, we reran the analyses excluding the CHAM2 children to assess whether differences between CHAM1 and CHAM2 influenced the associations of prenatal and postnatal dentine Mn with neurodevelopmental outcomes. Third, we fitted the adjusted regression models excluding preterm ($n = 22$) and other low birth weight ($n = 2$) children given that these variables may mediate the associations between Mn exposure and neurodevelopmental outcomes. Fourth, we fitted the adjusted regression models for postnatal dentine Mn including prenatal dentine Mn as a confounder for participants with both measurements. Fifth, in the subset of CHAM1 children for whom we had measured levels during pregnancy, we examined the confounding effect of potential neurotoxicants measured during pregnancy (i.e., DAPs and PBDEs) (Bouchard et al., 2011a; Eskenazi et al., 2013; Marks et al., 2010) by adding them individually to the prenatal dentine Mn final models. We also assessed the confounding effect of lead exposure during childhood by adding child blood lead levels measured at 12 and 24 months to the postnatal dentine Mn models.

We evaluated effect modification of the associations of prenatal and postnatal dentine Mn with neurodevelopmental outcomes by child sex. Because there is evidence of synergism between lead and Mn, we also examined effect modification of the associations between prenatal dentine Mn and neurodevelopmental outcomes by prenatal lead exposure (blood lead levels above or below the median < 0.8 vs. ≥0.8 μg/dL) in the subset of CHAM1 children for whom these measurements were available. Interactions were assessed using cross-product terms and were considered statistically significant if $p < 0.10$.

3. Results

Most women in the present study were young (mean age = 26.8 ± 5.1 years at delivery of their CHAMACOS child), born in Mexico (88%), multiparous (67%), did not complete high school (76%), did not work in agriculture during pregnancy (63%), and had a family income below the U.S. poverty threshold (60%, Table 1). Many women reported sufficient symptoms at the 7- and 9-year follow-up visits to qualify as "at-risk" of depression on the CES-D scale (21% and 27%, respectively; data not shown). Geometric means (geometric standard deviation, GSD) of prenatal and postnatal dentine Mn levels were 0.46 (1.48) and 0.14 (2.47) ^{55}Mn:^{43}Ca AUC × 10^4, respectively (Table A.1). Prenatal and postnatal dentine Mn levels were moderately correlated ($r_s = 0.49$, $p < 0.001$, $n = 244$). Maternal intelligence, parity, gestational anemia, child sex, and family income were not associated with the child's prenatal or postnatal dentine Mn levels (Table 1). However, higher prenatal and postnatal dentine Mn levels were observed in children of mothers aged 25–34 years, born in Mexico, poorly educated, and who had lived for ≤10 years in the U.S. Higher prenatal dentine Mn levels were also found in children of mothers who worked in agriculture during pregnancy. Conversely, prenatal dentine Mn levels were lower among children whose mothers reported smoking during pregnancy, had higher blood lead levels during pregnancy (≥0.8 μg/dL), and low birth weight or preterm children. CHAM1 and CHAM2 families were similar demographically (comparisons not shown), but CHAM1 children showed higher prenatal and early postnatal Mn levels in dentine compared to CHAM2 children (Table 1). Summary statistics for the children's performance on the various neurobehavioral tests are presented in Table A.2.

In our cubic spline analysis, we found evidence of a small number of nonlinear associations of dentine Mn levels with neurodevelopmental outcomes (marked with $p_{GAM} < 0.05$), but when we categorized Mn levels in either tertiles or quintiles we did not observe clear dose–response relationships (data not shown). We therefore report results from multivariate linear regression and GEE models with prenatal and postnatal dentine Mn levels parameterized as continuous variables.

Table 1
Study population characteristics and children's prenatal and postnatal dentine Mn levels (^{55}Mn:^{43}Ca AUC × 10^4).

Characteristic	Prenatal Mn		Postnatal Mn	
	n (%)[a]	GM (95% CI)	*n* (%)[b]	GM (95% CI)
All participants	248 (100.0)	0.46 (0.44, 0.49)	244 (100.0)	0.14 (0.13, 0.16)
Maternal characteristics				
Age (years)				
18–24	91 (36.7)	0.43 (0.40, 0.47)*	90 (36.9)	0.13 (0.10, 0.16)**
25–29	91 (36.7)	0.48 (0.44, 0.53)	88 (36.1)	0.16 (0.14, 0.18)
30–34	44 (17.7)	0.50 (0.44, 0.56)	44 (18.0)	0.16 (0.12, 0.22)
35–45	22 (8.9)	0.44 (0.38, 0.50)	22 (9.0)	0.10 (0.06, 0.18)
Education				
≤6th grade	113 (45.6)	0.51 (0.47, 0.54)**	110 (45.1)	0.16 (0.13, 0.19)*
7th–12th grade	75 (30.2)	0.46 (0.43, 0.50)	75 (30.7)	0.13 (0.11, 0.15)
Completed high school	60 (24.2)	0.39 (0.35, 0.43)	59 (24.2)	0.14 (0.11, 0.17)
Intelligence (PPVT score)[c]				
≤74	47 (19.0)	0.44 (0.39, 0.49)	47 (19.2)	0.16 (0.14, 0.18)
75–99	83 (33.4)	0.47 (0.43, 0.52)	80 (32.8)	0.15 (0.12, 0.18)
≥100	118 (47.6)	0.47 (0.44, 0.50)	117 (48.0)	0.13 (0.11, 0.16)
Country of birth				
Mexico	219 (88.3)	0.48 (0.45, 0.50)	215 (88.1)	0.15 (0.13, 0.17)**
Other	29 (11.7)	0.36 (0.31, 0.42)	29 (11.9)	0.12 (0.10, 0.14)
Years in US				
≤5	121 (48.8)	0.47 (0.44, 0.51)*	119 (48.8)	0.15 (0.13, 0.18)
6–10	67 (27.0)	0.48 (0.44, 0.51)	65 (26.6)	0.14 (0.11, 0.17)
≥11	60 (24.2)	0.42 (0.38, 0.47)	60 (24.6)	0.13 (0.11, 0.17)
Parity				
0	82 (33.1)	0.47 (0.43, 0.51)	81 (33.2)	0.14 (0.12, 0.17)
≥1	166 (66.9)	0.46 (0.43, 0.49)	163 (66.8)	0.14 (0.12, 0.16)
Smoking during pregnancy				
No	236 (95.2)	0.47 (0.45, 0.49)**	232 (95.1)	0.14 (0.13, 0.16)
Yes	12 (4.8)	0.33 (0.23, 0.46)	12 (4.9)	0.14 (0.10, 0.20)
Gestational anemia (hemoglobin < 11.6 g/dL)[d]				
No	82 (53.2)	0.49 (0.45, 0.53)	80 (53.3)	0.14 (0.11, 0.18)
Yes	72 (46.8)	0.49 (0.44, 0.54)	70 (46.7)	0.17 (0.15, 0.21)
Higher lead exposure during pregnancy (blood lead ≥ 0.8 μg/dL)[d]				
No	86 (50.9)	0.51 (0.47, 0.55)**	85 (51.2)	0.16 (0.13, 0.19)
Yes	83 (49.1)	0.44 (0.40, 0.49)	81 (48.8)	0.14 (0.12, 0.17)
Agricultural work during pregnancy[d]				
No	124 (62.9)	0.45 (0.42, 0.49)**	123 (63.7)	0.13 (0.11, 0.16)
Yes	73 (37.1)	0.52 (0.48, 0.57)	70 (36.3)	0.17 (0.13, 0.21)
Household income[d]				
At or below poverty level	118 (59.9)	0.50 (0.47, 0.53)	115 (59.6)	0.14 (0.11, 0.18)
Above poverty level	79 (40.1)	0.45 (0.41, 0.49)	78 (40.4)	0.15 (0.13, 0.16)
Child characteristics				
Child's sex				
Boy	108 (43.5)	0.46 (0.43, 0.49)	105 (43.0)	0.13 (0.11, 0.16)
Girl	140 (56.5)	0.46 (0.43, 0.50)	139 (57.0)	0.15 (0.13, 0.17)
Low birth weight (<2500 g)				
No	235 (94.8)	0.47 (0.44, 0.49)*	231 (94.7)	0.14 (0.13, 0.16)
Yes	13 (5.2)	0.39 (0.30, 0.51)	13 (5.3)	0.13 (0.09, 0.18)
Preterm birth (<37 weeks)				
No	226 (91.1)	0.47 (0.45, 0.49)*	222 (91.0)	0.14 (0.13, 0.16)
Yes	22 (8.9)	0.39 (0.30, 0.49)	22 (9.0)	0.15 (0.11, 0.21)
Cohort				
CHAM1	197 (79.4)	0.48 (0.45, 0.50)**	193 (79.1)	0.14 (0.12, 0.17)*
CHAM2	51 (20.6)	0.41 (0.37, 0.45)	51 (20.9)	0.13 (0.12, 0.15)

Abbreviations: AUC, area under the curve; GM, geometric mean; CI, confidence interval; PPVT, Peabody Picture Vocabulary Test; CHAM1, initial CHAMACOS cohort (recruited 1999–2000 during pregnancy); and CHAM2, second CHAMACOS cohort (recruited 2009–2011 at child age 9).
p-values are for Mann–Whitney or Kruskal–Wallis tests across the different categories of each characteristic.

[a] Children who completed the 7-, 9-, or 10.5-year neurobehavioral assessment and had prenatal dentine Mn levels measured in shed incisors.

[b] Children who completed the 7-, 9-, or 10.5-year neurobehavioral assessment and had postnatal dentine Mn levels measured in shed incisors.

[c] Analyzed as continuous variable in multivariable models.

[d] Information was missing for several mother–child pairs with prenatal dentine ($n = 51$ for family income at enrollment, $n = 51$ for maternal agricultural work during pregnancy, $n = 94$ for gestational anemia, $n = 79$ for blood lead levels during pregnancy) and postnatal dentine Mn measurements ($n = 51$ for family income at enrollment, $n = 51$ for maternal agricultural work during pregnancy, $n = 94$ for gestational anemia, and $n = 78$ for blood lead levels during pregnancy).

* $p < 0.10$.

** $p < 0.05$.

3.1. Behavior

3.1.1. Prenatal Mn

No associations were observed between prenatal dentine Mn levels and behavioral outcomes at ages 7, 9, or 10.5 years in cross-sectional (Table 2) or longitudinal (Table A.3) analyses of boys and girls combined. However, when we stratified by child sex, we found that higher prenatal dentine Mn levels were associated with more frequent maternal reports of internalizing, externalizing, and hyperactivity problems on BASC-2 at age 10.5 years among boys [β for a two-fold increase in Mn levels = 4.0, 95% confidence interval (CI): 0.6, 7.4; β = 2.7, 95% CI: −0.2, 5.6; and β = 3.7, 95% CI: 0.2, 7.2; respectively] but not

Table 2
Adjusted linear models for behavioral outcomes in children at 7, 9, and 10.5 years, per two-fold increase in prenatal dentine Mn ($^{55}Mn:^{43}Ca$ AUC × 10^4) in all children and stratified by sex.

Outcomes[a]	All children		Boys		Girls		p_{INT}
	n	β (95% CI)	*n*	β (95% CI)	*n*	β (95% CI)	
7-year assessment							
CADS — maternal report (T-scores)[b]							
ADHD index	198	−0.7 (−2.4, 1.1)	83	0.9 (−1.9, 3.8)	115	−1.3 (−3.9, 1.3)	0.12
DSM-IV total scale	198	−1.3 (−2.9, 0.4)	83	−0.5 (−3.4, 2.4)	115	−1.5 (−4.0, 0.9)	0.40
Inattentive subscale	198	−1.4 (−3.0, 0.1)*	83	−0.3 (−3.2, 2.5)	115	−2.2 (−4.4, 0.1)*	0.18
Hyperactive/impulsive subscale	198	−1.1 (−2.9, 0.7)	83	−0.5 (−3.5, 2.6)	115	−1.1 (−3.5, 1.4)	0.60
BASC-2 — maternal report (T-scores)[b]							
Internalizing problems	193	0.4 (−1.7, 2.5)	83	1.5 (−1.6, 4.6)	110	−0.5 (−3.8, 2.7)	0.19
Externalizing problems	193	−0.3 (−2.2, 1.6)	83	0.9 (−2.4, 4.1)	110	−0.7 (−3.3, 1.9)	0.43
Attention problems	193	0.4 (−2.3, 3.0)	83	0.6 (−2.3, 3.6)	110	0.7 (−1.8, 3.3)	0.91
Hyperactivity	193	0.4 (−1.4, 2.3)	83	1.5 (−2.6, 5.7)	110	−0.3 (−3.8, 3.3)	0.35
CADS — teacher report (T-scores)[c]							
ADHD index	170	−1.0 (−3.5, 1.5)	70	0.6 (−3.2, 4.4)	100	−1.9 (−5.7, 2.0)	0.29
DSM-IV total scale	170	−0.1 (−2.3, 2.1)	70	0.8 (−2.4, 4.1)	100	−0.6 (−4.1, 2.8)	0.50
Inattentive subscale	173	0.8 (−1.1, 2.6)	71	1.3 (−2.2, 4.8)	102	0.7 (−1.6, 3.0)	0.71
Hyperactive/impulsive subscale	173	−1.2 (−3.6, 1.1)	71	0.1 (−3.5, 3.6)	102	−2.0 (−5.5, 1.6)	0.45
BASC-2 — teacher report (T-scores)[c]							
Internalizing problems	173	−2.8 (−6.2, 0.5)*	71	−2.1 (−5.6, 1.4)	102	−3.8 (−9.0, 1.5)	0.55
Externalizing problems	173	−0.5 (−2.6, 1.6)	71	0.1 (−4.2, 4.4)	102	−1.1 (−3.8, 1.5)	0.88
Attention problems	173	−0.7 (−2.3, 0.9)	71	0.1 (−4.3, 4.5)	102	−2.0 (−4.6, 0.6)	0.66
Hyperactivity	173	−1.0 (−3.2, 1.1)	71	−0.6 (−3.2, 2.0)	102	−0.5 (−2.7, 1.6)	0.74
9-year assessment							
CADS — maternal report (T-scores)[b]							
ADHD index	243	0.1 (−1.8, 2.1)	107	2.0 (−0.9, 4.9)	136	−0.5 (−3.5, 2.6)	0.17
DSM-IV total scale	242	0.8 (−1.5, 3.0)	107	2.8 (−0.5, 6.0)*	136	0.0 (−3.4, 3.5)	0.29
Inattentive subscale	242	0.3 (−1.7, 2.2)	107	1.6 (−1.1, 4.3)	136	−0.2 (−3.4, 2.9)	0.30
Hyperactive/impulsive subscale	242	1.1 (−1.5, 3.7)	107	3.7 (−0.4, 7.7)*	136	0.2 (−3.7, 4.2)	0.35
CPT-II (T-scores)[d]							
Errors of omission	238	−2.0 (−7.0, 3.0)	104	−5.1 (−11.6, 1.5)	134	−0.4 (−7.4, 6.6)	0.22
Errors of commission	238	−2.0 (−4.2, 0.2)*	104	−2.4 (−6.2, 1.4)	134	−2.2 (−5.2, 0.8)	0.49
ADHD confidence index	238	−3.0 (−8.7, 2.7)	104	−10.0 (−17.2, −2.7)**	134	3.2 (−5.0, 11.3)	0.01
10.5-year assessment							
BASC-2 — maternal report (T-scores)[b]							
Internalizing problems	232	1.0 (−1.0, 2.9)	99	4.0 (0.6, 7.4)**	133	0.3 (−2.3, 2.8)	0.12
Externalizing problems	227	0.6 (−1.0, 2.2)	96	2.7 (−0.2, 5.6)*	131	0.0 (−2.0, 2.0)	0.10
Attention problems	232	0.1 (−2.2, 2.4)	99	1.0 (−1.8, 3.9)	133	0.2 (−1.7, 2.2)	0.79
Hyperactivity	232	0.1 (−1.4, 1.7)	99	3.7 (0.2, 7.2)**	133	−1.7 (−4.8, 1.3)	0.02
BASC-2 — self-report (T-scores)[d]							
Attention problems	225	−0.5 (−2.9, 1.8)	98	−0.6 (−5.2, 4.0)	130	−0.7 (−3.8, 2.3)	0.87
Hyperactivity	228	−0.9 (−3.6, 1.8)	97	1.5 (−3.2, 6.1)	128	−1.2 (−3.9, 1.5)	0.22

Abbreviations: AUC, area under the curve; CADS, Conners' ADHD/DSM-IV Scales; ADHD, Attention Deficit/Hyperactivity Disorder; BASC-2, Behavior Assessment System for Children 2nd edition; and CPT-II, Continuous Performance Test 2nd edition.

[a] Higher scores indicate poorer performance or more symptomatic behavior.

[b] Adjusted for maternal education, intelligence (PPVT score), years in the US, and depression at time of assessment; child's age at maternal interview; language of maternal interview; HOME z-score, household income, and number of children in the home at time of assessment.

[c] Adjusted for maternal education, intelligence (PPVT score), years in the US, and depression at time of assessment; child's age at interview; HOME z-score, household income, and number of children in the home at time of assessment.

[d] Adjusted for maternal education, intelligence (PPVT score), years in the US, and depression at time of assessment; child's age at assessment, and language of assessment; HOME z-score, household income, number of children in the home, and psychometrician at time of assessment (9-year and 10.5-year assessments).

* *p*Linear < 0.10.

** *p*Linear < 0.05.

among girls (Table 2). We also observed that higher prenatal dentine Mn levels were associated with more frequent maternal reports of internalizing problems on BASC-2 at ages 7 (β = 3.9, 95% CI: −0.5, 8.4) and 10.5 years (β = 5.1, 95% CI: 1.9, 8.3), but less frequent teacher reports of inattention on CADS at age 7 years (β = −2.4, 95% CI: −7.0, 2.1), among children with lower prenatal lead levels (<0.8 μg/dL; Table A.4).

3.1.2. Postnatal Mn

Higher postnatal dentine Mn levels were associated with more frequent maternal reports of behavioral problems at 7 years in cross-sectional analyses of boys and girls combined (Table 3). Effect sizes were small, but stronger for some BASC-2 outcomes, specifically internalizing problems (β = 0.8, 95% CI: 0.0, 1.6), externalizing problems (β = 0.6, 95% CI: 0.0, 1.2), and hyperactivity (β = 0.8, 95% CI: 0.1, 1.4). Similarly, adjusted GEE analyses of repeated behavior measures at ages 7 and 10.5 years showed that higher postnatal dentine Mn levels were related to slightly worse internalizing (β = 0.6, 95% CI: 0.0, 1.2) and externalizing problems (β = 0.4, 95% CI: −0.1, 0.8) BASC-2 scores (Table A.3). We did not observe consistent sex differences in the associations between postnatal dentine Mn levels and behavioral outcomes (Table 2).

3.2. Cognition

3.2.1. Prenatal Mn

No consistent and statistically significant associations between prenatal dentine Mn levels and cognitive outcomes were observed in cross-sectional (Table 4) or longitudinal (Table A.3) analyses of boys and girls combined. However, when we stratified by child sex, we observed that higher prenatal dentine Mn levels were associated with better cognitive outcomes at 7 and 10.5 years among boys than among

Table 3
Adjusted linear models for behavioral outcomes in children at 7, 9, and 10.5 years, per two-fold increase in postnatal dentine Mn (^{55}Mn:^{43}Ca AUC × 10^4) in all children and stratified by sex.

Outcomes[a]	All children		Boys		Girls		p_{INT}
	n	β (95% CI)	*n*	β (95% CI)	*n*	β (95% CI)	
7-year assessment							
CADS — maternal report (T-scores)[b]							
ADHD index	194	0.4 (−0.3, 1.1)	80	0.7 (−0.2, 1.6)	114	0.3 (−0.9, 1.6)	0.87
DSM-IV total scale	194	0.5 (−0.1, 1.1)	80	0.5 (−0.4, 1.5)	114	0.6 (−0.3, 1.5)	0.80
Inattentive subscale	194	0.2 (−0.4, 0.8)	80	0.6 (−0.2, 1.5)	114	0.1 (−0.9, 1.0)	0.62
Hyperactive/impulsive subscale	194	0.7 (0.1, 1.3)**	80	0.5 (−0.5, 1.5)	114	0.8 (0.0, 1.6)**	0.60
BASC-2 — maternal report (T-scores)[b]							
Internalizing problems	189	0.8 (0.0, 1.6)**	80	1.6 (0.8, 2.4)**	109	0.4 (−0.7, 1.4)	0.13
Externalizing problems	189	0.6 (0.0, 1.2)**	80	0.9 (−0.1, 1.9)*	109	0.5 (−0.3, 1.3)	0.40
Attention problems	189	−0.1 (−1.2, 1.0)	80	0.6 (−0.4, 1.7)	109	1.0 (0.1, 1.8)**	0.67
Hyperactivity	189	0.8 (0.1, 1.4)**	80	−0.4 (−1.7, 0.8)	109	0.8 (−0.7, 2.3)	0.16
CADS — teacher report (T-scores)[c]							
ADHD index	166	0.0 (−1.5, 1.6)	67	0.0 (−2.3, 2.2)	99	0.0 (−2.1, 2.1)	0.89
DSM-IV total scale	166	0.1 (−1.2, 1.4)	67	0.1 (−1.8, 1.9)	99	0.0 (−1.8, 1.9)	0.81
Inattentive subscale	169	0.4 (−0.7, 1.5)	68	0.3 (−1.5, 2.0)	101	0.4 (−0.9, 1.8)	0.96
Hyperactive/impulsive subscale	169	−0.1 (−1.4, 1.2)	68	−0.1 (−2.2, 2.0)	101	−0.5 (−2.4, 1.4)	0.53
BASC-2 — teacher report (T-scores)[c]							
Internalizing problems	169	−0.3 (−1.7, 1.1)	68	0.3 (−0.9, 1.5)	101	−0.9 (−4.2, 2.3)	0.43
Externalizing problems	169	0.0 (−1.3, 1.2)	68	−0.2 (−2.6, 2.1)	101	−0.1 (−1.4, 1.2)	0.95
Attention problems	169	0.0 (−1.1, 1.1)	68	−0.3 (−3.0, 2.5)	101	−0.5 (−2.2, 1.2)	0.74
Hyperactivity	169	−0.2 (−1.7, 1.2)	68	−0.3 (−1.8, 1.1)	101	0.3 (−0.9, 1.6)	0.56
9-year assessment							
CADS — maternal report (T-scores)[b]							
ADHD index	239	0.1 (−0.6, 0.8)	104	0.3 (−0.7, 1.3)	135	0.1 (−0.9, 1.1)	0.90
DSM-IV total scale	238	0.3 (−0.4, 1.0)	104	0.0 (−1.0, 1.1)	134	0.6 (−0.4, 1.6)	0.36
Inattentive subscale	238	0.2 (−0.5, 0.8)	104	−0.1 (−1.1, 0.9)	134	0.5 (−0.4, 1.5)	0.29
Hyperactive/impulsive subscale	238	0.3 (−0.5, 1.1)	104	0.1 (−1.1, 1.4)	134	0.5 (−0.6, 1.7)	0.53
CPT-II (T-scores)[d]							
Errors of omission	234	0.2 (−0.9, 1.3)	101	−0.7 (−2.4, 0.9)	133	0.5 (−1.0, 1.9)	0.27
Errors of commission	234	−0.1 (−0.8, 0.5)	101	−0.5 (−1.4, 0.4)	133	0.3 (−0.5, 1.1)	0.54
ADHD confidence index	234	0.5 (−1.1, 2.0)	101	−1.2 (−3.2, 0.8)	133	1.3 (−0.8, 3.4)	0.07
10.5-year assessment							
BASC-2 — maternal report (T-scores)[b]							
Internalizing problems	228	0.4 (−0.3, 1.2)	96	0.6 (−0.8, 2.0)	132	0.3 (−0.5, 1.1)	0.76
Externalizing problems	224	0.3 (−0.3, 0.8)	93	0.6 (−0.5, 1.7)	131	0.3 (−0.3, 0.8)	0.90
Attention problems	228	0.3 (−0.5, 1.0)	96	−0.2 (−1.4, 0.9)	132	0.1 (−0.4, 0.7)	0.51
Hyperactivity	228	−0.1 (−0.6, 0.5)	96	0.4 (−1.0, 1.7)	132	0.1 (−1.0, 1.2)	0.56
BASC-2 — self-report (T-scores)[d]							
Attention problems	221	−0.2 (−0.9, 0.4)	95	0.1 (−1.2, 1.4)	129	−0.1 (−1.1, 0.9)	0.89
Hyperactivity	224	−0.1 (−0.7, 0.6)	94	0.2 (−1.0, 1.4)	127	−0.2 (−1.0, 0.7)	0.86

Abbreviations: AUC, area under the curve; CADS, Conners' ADHD/DSM-IV Scales; ADHD, Attention Deficit/Hyperactivity Disorder; BASC-2, Behavior Assessment System for Children 2nd edition; and CPT-II, Continuous Performance Test 2nd edition.

[a] Higher scores indicate poorer performance or more symptomatic behavior.

[b] Adjusted for maternal education, intelligence (PPVT score), years in the US, and depression at time of assessment; child's age at maternal interview; language of maternal interview; HOME z-score, household income, and number of children in the home at time of assessment.

[c] Adjusted for maternal education, intelligence (PPVT score), years in the US, and depression at time of assessment; child's age at interview; HOME z-score, household income, and number of children in the home at time of assessment.

[d] Adjusted for maternal education, intelligence (PPVT score), years in the US, and depression at time of assessment; child's age at assessment, and language of assessment; HOME z-score, household income, number of children in the home, and psychometrician at time of assessment (9-year and 10.5-year assessments).

* $p_{Linear} < 0.10$.

** $p_{Linear} < 0.05$.

girls, although these sex differences were not statistically significant (Table 4). In contrast, we found consistent associations of higher prenatal Mn levels with worse cognitive outcomes at ages 7 and 10.5 years among children with higher prenatal lead blood levels (≥0.8 μg/dL; Fig. 1 and Table A.5). For example, higher prenatal dentine Mn levels were associated with 3.5-point (95% CI: −7.2, 0.2) and 3.7-point (95% CI: −7.7, 0.2) decreases in Full Scale IQ scores at 7 and 10.5 years, respectively, but only among children with higher prenatal lead exposures.

3.2.2. Postnatal Mn

We did not find statistically significant associations between postnatal dentine Mn levels and cognitive outcomes in the analyses of boys and girls combined, but we did observe significant interactions between postnatal Mn levels and child sex for most cognitive outcomes (Table 5). More specifically, we found a positive linear relationship of postnatal dentine Mn levels with Full Scale, Verbal Comprehension, and Perceptual Reasoning IQ scores at 7 and 10.5 years, and Working Memory IQ scores at 7 years, in boys but not girls. For instance, a two-fold increase in postnatal Mn levels was associated with 1.9-point (95% CI: 0.6, 3.1) and 2.0-point (95% CI: 0.7, 3.3) increases in Full Scale IQ scores at 7 and 10.5 years, respectively, in boys but not girls.

Adjusted GEE analyses of repeated outcome measures in boys and girls combined did not show associations between postnatal dentine Mn levels and cognitive outcomes (Table A.3). However, when we stratified GEE analyses by child sex, we observed that higher postnatal Mn levels were associated with better Full Scale (β = 1.7, 95% CI: 0.3, 3.1), Verbal Comprehension (β = 1.4, 95% CI: 0.6, 2.1), Perceptual Reasoning (β = 2.8, 95% CI: 0.6, 5.1), and Working Memory IQ scores (β = 1.2, 95% CI: 0.1, 2.2) in boys but not girls (β = −0.1, 95% CI: −1.3, 1.2, p_{INT} =

Table 4
Adjusted linear models for cognitive, memory, and motor outcomes in children at 7, 9, and 10.5 years, per two-fold increase in prenatal dentine Mn (^{55}Mn:^{43}Ca AUC × 10^4) in all children and stratified by sex.

Outcomes	All children		Boys		Girls		p_{INT}
	n	β (95% CI)	*n*	β (95% CI)	*n*	β (95% CI)	
Cognition							
7-year assessment							
WISC-IV Full-Scale IQ (scaled scores)	175	0.8 (−2.4, 3.9)	76	3.2 (−1.3, 7.8)	99	−0.6 (−5.2, 4.0)	0.22
Verbal comprehension IQ	193	0.9 (−2.0, 3.8)	83	1.2 (−2.8, 5.1)	110	0.6 (−3.4, 4.7)	0.84
Perceptual reasoning IQ	193	2.2 (−2.7, 7.0)[#]	83	4.7 (−4.2, 13.7)	110	0.7 (−5.5, 6.9)	0.42
Working memory IQ	176	0.7 (−2.4, 3.8)	76	2.7 (−2.7, 8.0)	100	−0.2 (−4.2, 3.7)	0.40
Processing speed IQ	176	1.6 (−1.6, 4.7)	76	4.2 (−1.0, 9.3)	100	−0.3 (−4.1, 3.5)	0.12
10.5-year assessment							
WISC-IV Full-Scale IQ (scaled scores)	231	1.2 (−1.2, 3.6)	98	1.1 (−3.0, 5.1)	133	0.3 (−3.1, 3.6)	0.42
Verbal comprehension IQ	233	−0.4 (−2.5, 1.8)	100	−2.0 (−5.6, 1.7)	133	−0.7 (−3.5, 2.1)	0.94
Perceptual reasoning IQ	233	3.0 (−0.8, 6.7)	100	3.8 (−3.6, 11.1)	133	1.7 (−3.1, 6.4)	0.32
Working memory IQ	233	1.4 (−0.9, 3.6)	100	1.6 (−2.4, 5.6)	133	0.4 (−2.7, 3.4)	0.48
Processing speed IQ	233	0.0 (−2.7, 2.7)	100	1.0 (−3.5, 5.5)	133	−0.7 (−4.5, 3.1)	0.51
Memory							
9-year assessment							
NEPSY-II Memory for Designs (scaled scores)							
Immediate total	184	0.8 (−0.2, 1.8)	78	1.9 (0.8, 3.0)[**]	106	0.3 (−1.2, 1.9)	0.31
Delayed total	185	1.0 (0.0, 1.9)[**]	78	2.2 (0.7, 3.7)[**]	107	0.3 (−1.1, 1.6)	0.17
10.5-year assessment							
CAVLT-2 (standardized scores)							
Immediate recall	233	2.1 (−2.4, 6.6)[#]	100	4.3 (−0.9, 9.4)	133	−0.3 (−7.2, 6.6)	0.25
Delayed recall	233	3.8 (−0.4, 8.0)[*]	100	6.2 (0.7, 11.8)[**]	133	1.4 (−4.8, 7.6)	0.18
Immediate memory span	233	−0.9 (−4.3, 2.5)	100	−4.4 (−9.9, 1.1)	133	0.8 (−3.7, 5.3)	0.18
Level of learning	233	3.8 (0.0, 7.6)[**]	100	4.7 (−1.1, 10.6)	133	2.6 (−2.2, 7.4)	0.61
Motor function							
7-year assessment							
WRAVMA Pegboard (scaled scores)							
Dominant hand	193	1.9 (−2.5, 6.3)	83	2.1 (−5.1, 9.3)	110	2.5 (−3.3, 8.3)	0.48
Non-dominant hand	193	2.0 (−2.5, 6.5)	83	2.6 (−4.3, 9.6)	110	2.1 (−4.1, 8.3)	0.66
Finger Tap (z-scores)							
Dominant hand	193	0.2 (0.0, 0.4)[*]	83	0.5 (0.1, 0.8)[**]	110	0.1 (−0.2, 0.4)	0.04
Non-dominant hand	193	0.1 (−0.2, 0.3)	83	0.3 (0.0, 0.7)[*]	110	−0.1 (−0.4, 0.2)	0.01
9-year assessment							
Luria-Nebraska Motor Scale (z-scores)							
All items	227	0.1 (−0.1, 0.3)	100	0.3 (−0.1, 0.6)	127	0.0 (−0.3, 0.3)	0.02
5-item sum	227	0.1 (−0.1, 0.3)	100	0.3 (0.0, 0.6)[*]	127	0.0 (−0.3, 0.2)	0.01
10.5-year assessment							
Luria-Nebraska Motor Scale (z-scores)							
All items	233	0.1 (0.0, 0.3)	100	0.3 (0.1, 0.5)[**]	133	0.0 (−0.3, 0.2)	0.01
5-item sum	233	0.2 (0.0, 0.4)[*]	100	0.5 (0.2, 0.8)[**]	133	0.0 (−0.4, 0.3)	0.02

Abbreviations: CI, confidence interval; WISC-IV, Wechsler Intelligence Scale for Children 4th edition; IQ, intellectual quotient; CAVLT-2, Children's Auditory Verbal Learning Test 2nd edition; and WRAVMA, Wide Range Assessment of Visual Motor Ability.
Models adjusted for maternal education, intelligence (PPVT score), years in the US, and depression at time of assessment; child's sex, age at assessment, and language of assessment; HOME z-score, household income, number of children in the home, and psychometrician at time of assessment (9-year and 10.5-year assessments).
[*] $p_{Linear} < 0.10$.
[**] $p_{Linear} < 0.05$.
[#] $p_{GAM} < 0.05$.

0.05; β = 0.2, 95% CI: −0.9, 1.2, $p_{INT} = 0.10$; β = −0.7, 95% CI: −2.1, 0.7, $p_{INT} = 0.01$; β = 0.1, 95% CI: −1.3, 1.4, $p_{INT} = 0.32$; respectively, data not shown).

3.3. Memory

3.3.1. Prenatal Mn

Higher prenatal dentine Mn levels were linearly associated with better memory outcomes at ages 9 and 10.5 years in the analyses of boys and girls combined (Table 4). A two-fold increase in prenatal dentine Mn levels was associated with a 1.0-point increase in NEPSY-II Memory for Designs Delayed total score (95% CI: 0.0, 1.9) at 9 years, and 3.8-point increases in CAVLT-2 Delayed recall (95% CI: −0.4, 8.0) and Level of learning scores (95% CI: 0.0, 7.6) at 10.5 years. In sex-stratified analyses, we observed that higher prenatal dentine Mn levels were associated with better NEPSY-II Memory for Designs Immediate (β = 1.9, 95% CI: 0.8, 3.0) and Delayed total scores (β = 2.2, 95% CI: 0.7, 3.7) at 9 years and improved CAVLT-2 Delayed recall scores (β = 6.2, 95% CI: 0.7, 11.8) at 10.5 years in boys but not in girls (Table 4), but these sex differences were not statistically significant. Conversely, we also found that higher prenatal dentine Mn levels were associated with poorer NEPSY-II Memory for Designs Delayed total scores (β = −1.2, 95% CI: −2.4, 0.0) at 9 years in children with higher prenatal lead levels, but not in those with lower lead levels (Fig. 1 and Table A.5).

3.3.2. Postnatal Mn

Postnatal dentine Mn levels were not linearly associated with any of the memory outcomes in combined analyses across child sex (Table 5). However, sex-stratified analyses revealed that higher postnatal dentine Mn levels were significantly associated with better memory scores at ages 9 and 10.5 years in boys, but not girls (Table 5). A two-fold increase in postnatal Mn levels was associated with a 0.5-point increase in NEPSY-II Memory for Designs Immediate total score (95% CI: 0.1, 1.0) at 9 years, and 2.9-point increase in CAVLT-2 Immediate recall score (95% CI: 0.7, 5.2), 3.0-point increase in CAVLT-2 Delayed recall score (95% CI: 0.3, 5.6), and a 2.6-point increase in CAVLT-2 Level of learning score (95% CI: 0.5, 4.8) at 10.5 years in boys.

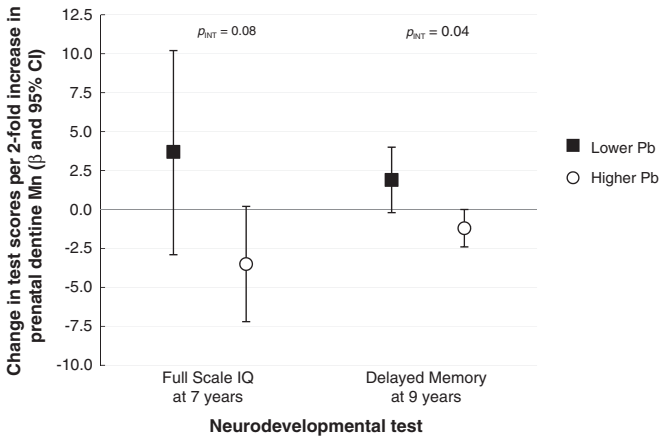

Fig. 1. Adjusted linear models for WISC-IV Full Scale IQ at 7 years and NEPSY-II Memory for Designs Delayed Memory at 9 years per two-fold increase in prenatal dentine Mn (^{55}Mn:^{43}Ca AUC × 10^4) stratified by prenatal lead exposure (maternal blood Pb levels during pregnancy <0.8 vs. ≥0.8 μg/dL). Models adjusted for maternal education, intelligence (PPVT score), years in the US, and depression at time of assessment; child's sex, age at assessment, and language of assessment; HOME z-score, household income, number of children in the home, and psychometrician at time of assessment (9-year and 10.5-year assessments).

3.4. Motor function

3.4.1. Prenatal Mn

No consistent and statistically significant associations between prenatal dentine Mn levels and motor outcomes were observed in the cross-sectional (Table 4) or longitudinal analyses (Table A.3) that combined boys and girls. However, when we stratified by child sex, we found that higher prenatal dentine Mn levels were associated with better z-scores on the Finger tapping test for both dominant (β = 0.5, 95% CI: 0.1, 0.8) and non-dominant hands (β = 0.3, 95% CI: 0.0, 0.7) at age 7, all-item (β = 0.3, 95% CI: −0.1, 0.6) and five-item sum (β = 0.3, 95% CI: 0.0, 0.6) of the Luria-Nebraska Motor Scale at age 9, and all-item (β = 0.3, 95% CI: 0.1, 0.5) and five-item sum (β = 0.5, 95% CI: 0.2, 0.8) of the Luria-Nebraska Scale at age 10.5 in boys compared to girls (Table 4). When we stratified GEE analyses by child sex, we observed that higher prenatal Mn levels were associated with better z-scores on the all-item (β = 0.3, 95% CI: 0.1, 0.5) and five-item sum (β = 0.4, 95% CI: 0.2, 0.6) of the Luria-Nebraska Motor Scale in boys but not girls (β = 0.0, 95% CI: −0.2, 0.2, p_{INT} < 0.01; β = 0.0, 95% CI: −0.3, 0.3, p_{INT} < 0.01; respectively, data not shown).

3.4.2. Postnatal Mn

We did not find statistically significant associations between postnatal dentine Mn levels and motor outcomes in cross-sectional (Table 5) or longitudinal analyses (Table A.3) that combined boys and girls, but we observed several significant associations in the sex-stratified analyses (Table 5). Higher postnatal Mn levels were associated with better scores on the WRAVMA pegboard (non-dominant hand: β = 1.4, 95% CI: −0.4, 3.2) and Finger tapping test (dominant hand: β = 0.2, 95% CI: 0.1, 0.3) at 7 years, and Luria-Nebraska Motor Scale (all-item sum: β = 0.1, 95% CI: 0.0, 0.1; five-item sum: β = 0.1, 95% CI: −0.1, 0.2) at 10.5 years in boys than in girls (Table 5).

3.5. Sensitivity analyses

In general, the point estimates did not change appreciably after removal of the outliers from the final multivariable models. Restricting the analyses to CHAM1 children yielded results similar to those obtained for the entire group. Including prenatal dentine Mn levels in the adjusted models for postnatal Mn levels and excluding preterm and other low birth weight children from the analyses only marginally altered the results (change in estimates <10%). Similarly, including prenatal DAPs and child blood lead levels measured at 12 and 24 months in the adjusted models did not change the point estimates observed in the main analyses (data not shown). However, when we included prenatal PBDE levels in the models, we observed that some associations that were not previously statistically significant became significant: higher prenatal dentine Mn levels were associated with less frequent teacher reports of hyperactivity problems (β for a two-fold increase in Mn levels = −1.9, 95% CI: −4.1, 0.3) on BASC-2, lower teacher ADHD Index (β = −2.4, 95% CI: −5.1, 0.2) and Hyperactive/impulsive scores (β = −2.6, 95% CI: −5.1, −0.1) on CADS at age 7, and lower errors of omission (β = −5.7, 95% CI: −12.5, 1.2) and ADHD Confidence index scores (β = −7.7, 95% CI: −14.7, −0.8) on CPT-II at age 9 (data not shown). Notably, the associations that we observed between prenatal and postnatal Mn levels and maternal reports of behavioral problems at ages 7 and 10.5 years remained unchanged when we included prenatal PBDE levels in the models.

4. Discussion

We found that prenatal and early postnatal Mn levels in dentine of deciduous teeth were adversely associated with behavioral outcomes, namely maternal-reported internalizing, externalizing, and hyperactivity problems, in school-age boys and girls. In contrast, we observed that prenatal and postnatal Mn dentine levels were favorably associated with several measures of cognition, visuospatial and verbal memory, and motor function in boys. We also found that higher prenatal Mn levels were associated with poorer visuospatial memory and cognition in children exposed to higher prenatal lead levels. Our results appeared to be independent of the associations of prenatal OP pesticide and PBDE exposure with child neurobehavioral development that have been previously reported in the CHAMACOS cohort (Bouchard et al., 2011a; Eskenazi et al., 2013; Marks et al., 2010).

To our knowledge, this is the largest and most comprehensive study to date on the potential neurodevelopmental effects of prenatal and early postnatal Mn status in school-age children. Few studies have prospectively examined the associations between in utero and/or postnatal exposure to Mn and neurobehavioral outcomes (Chung et al., 2015; Ericson et al., 2007; Lin et al., 2013; Takser et al., 2003) and there are some consistencies between their findings and ours. A small study of 27 U.S. children observed that higher prenatal Mn levels in enamel of deciduous teeth were associated with poorer performance in behavioral disinhibition tests at ages 3 and 4.5 years (including increased errors of commission on a continuous performance test), and more adverse maternal and teacher reports of internalizing and externalizing problems at ages 6–7

Table 5
Adjusted linear models for cognitive, memory, and motor outcomes in children at 7, 9, and 10.5 years, per two-fold increase in postnatal dentine Mn ($^{55}Mn:^{43}Ca$ AUC × 10^4) in all children and stratified by sex.

Outcomes	All children		Boys		Girls		p_{INT}
	n	β (95% CI)	*n*	β (95% CI)	*n*	β (95% CI)	
Cognition							
7-year assessment							
WISC-IV Full-Scale IQ (scaled scores)	171	0.5 (−0.7, 1.6)	73	1.9 (0.6, 3.1)**	98	−0.3 (−2.1, 1.6)	0.12
Verbal comprehension IQ	189	0.6 (−0.3, 1.6)	80	1.6 (0.1, 3.1)**	109	0.3 (−1.3, 1.9)	0.50
Perceptual reasoning IQ	189	0.7 (−0.8, 2.2)	80	3.4 (1.6, 5.2)**	109	−1.1 (−3.1, 0.9)	<0.01
Working memory IQ	172	0.4 (−1.0, 1.8)	73	1.5 (0.5, 2.6)**	99	−0.4 (−2.5, 1.7)	0.16
Processing speed IQ	172	0.5 (−0.9, 1.8)	73	−0.3 (−2.6, 2.0)	99	1.3 (−0.3, 2.8)	0.11
10.5-year assessment							
WISC-IV Full-Scale IQ (scaled scores)	227	0.7 (−0.3, 1.8)	95	2.0 (0.7, 3.3)**	132	−0.2 (−1.5, 1.2)	0.01
Verbal comprehension IQ	229	0.6 (−0.6, 1.7)	97	1.5 (0.2, 2.9)**	132	−0.4 (−1.7, 0.8)	<0.01
Perceptual reasoning IQ	229	1.2 (−0.3, 2.6)	97	3.3 (1.3, 5.4)**	132	−0.2 (−2.0, 1.5)	0.01
Working memory IQ	229	0.7 (−0.2, 1.7)	97	0.9 (−0.5, 2.3)	132	0.7 (−0.8, 2.1)	0.78
Processing speed IQ	229	−0.5 (−1.5, 0.5)	97	0.2 (−1.5, 1.8)	132	−0.6 (−2.1, 0.9)	0.72
Memory							
9-year assessment							
NEPSY-II Memory for Designs (scaled scores)							
Immediate total	180	0.1 (−0.3, 0.5)#	75	0.5 (0.1, 1.0)**	105	−0.3 (−0.8, 0.3)	0.02
Delayed total	181	0.0 (−0.5, 0.5)#	75	0.4 (−0.4, 1.2)	106	−0.3 (−0.9, 0.4)	0.25
10.5-year assessment							
CAVLT-2 (standardized scores)							
Immediate recall	229	0.9 (−1.2, 3.1)	97	2.9 (0.7, 5.2)**	132	−0.7 (−4.1, 2.7)	0.08
Delayed recall	229	1.3 (−1.4, 3.9)	97	3.0 (0.3, 5.6)**	132	−0.1 (−4.7, 4.6)	0.24
Immediate memory span	229	0.8 (−1.0, 2.6)	97	1.8 (−0.7, 4.2)	132	−0.2 (−2.8, 2.4)	0.28
Level of learning	229	1.1 (−0.8, 3.0)	97	2.6 (0.5, 4.8)**	132	−0.4 (−2.8, 2.1)	0.08
Motor function							
7-year assessment							
WRAVMA Pegboard (scaled scores)							
Dominant hand	189	−0.4 (−2.0, 1.2)	80	0.0 (−1.9, 1.8)	109	−1.3 (−4.1, 1.6)	0.44
Non-dominant hand	189	0.1 (−1.8, 2.1)	80	1.4 (−0.4, 3.2)	109	−1.8 (−4.2, 0.6)	0.04
Finger tap (z-scores)							
Dominant hand	189	0.1 (0.0, 0.1)	80	0.2 (0.1, 0.3)**	109	0.0 (−0.2, 0.1)	0.03
Non-dominant hand	189	0.0 (−0.1, 0.1)	80	0.1 (0.0, 0.2)	109	0.0 (−0.2, 0.1)	0.19
9-year assessment							
Luria-Nebraska Motor Scale (z-scores)							
All items	223	−0.1 (−0.1, 0.0)	97	0.0 (−0.1, 0.1)	126	−0.1 (−0.2, 0.1)	0.36
5-item sum	223	−0.1 (−0.1, 0.0)	97	0.0 (−0.1, 0.1)	126	−0.1 (−0.2, 0.1)	0.28
10.5-year assessment							
Luria-Nebraska Motor Scale (z-scores)							
All items	229	0.0 (−0.1, 0.1)	97	0.1 (0.0, 0.1)	132	−0.1 (−0.1, 0.0)*	<0.01
5-item sum	229	0.0 (−0.1, 0.0)	97	0.1 (−0.1, 0.2)	132	−0.1 (−0.2, 0.0)*	0.09

Abbreviations: CI, confidence interval; WISC-IV, Wechsler Intelligence Scale for Children 4th edition; IQ, intellectual quotient; CAVLT-2, Children's Auditory Verbal Learning Test 2nd edition; and WRAVMA, Wide Range Assessment of Visual Motor Ability.
Models adjusted for maternal education, intelligence (PPVT score), years in the US, and depression at time of assessment; child's sex, age at assessment, and language of assessment; HOME z-score, household income, number of children in the home, and psychometrician at time of assessment (9-year and 10.5-year assessments).
* $p_{Linear} < 0.10$.
** $p_{Linear} < 0.05$.
\# $p_{GAM} < 0.05$.

(1st grade) and 8–9 years (3rd grade) (Ericson et al., 2007). This study also found a positive association between early postnatal enamel Mn levels and teacher-reported scores of externalizing problems at ages 6–7 and 8–9 years. Unlike numerous cross-sectional studies of school-age children that reported negative associations between Mn levels and cognition (Bouchard et al., 2011b; He et al., 1994; Hernandez-Bonilla et al., 2011; Kim et al., 2009; Lucchini et al., 2012; Menezes-Filho et al., 2011; Riojas-Rodriguez et al., 2010; Torres-Agustin et al., 2013; Wasserman et al., 2006), a cohort study of 247 French children did not find significant adverse associations of maternal and child Mn levels at delivery with cognitive abilities assessed at ages 3 and 6 years (Takser et al., 2003), while we observed beneficial associations among boys.

Although our study shows some consistencies with previous prospective studies, there are also some inconsistencies. For example, a study of 232 Korean mother–child pairs observed inverted U-shaped relationships of maternal blood Mn levels measured at delivery with mental and psychomotor development at 6 months (Chung et al., 2015), but we did not observe non-linear associations or clear dose–response relationships in our analyses. A study of 230 children conducted in Taiwan reported an association of cord blood Mn levels above the 75th percentile (>59.3 μg/L) with poorer cognitive and language development at 2 years of age (Lin et al., 2013), whereas, in our study, we observed positive associations of prenatal and postnatal dentine Mn levels with cognitive abilities among boys. In addition, the French study observed that higher cord blood Mn levels were associated with lower non-verbal memory (in boys and girls combined) and hand skill scores (in boys only) at age 3 years (Takser et al., 2003); however, in our study, prenatal and postnatal dentine Mn levels were consistently associated with better memory and motor outcome scores in somewhat older boys. These inconsistent findings may be due to differences in the exposure matrix used to quantify Mn levels (blood and hair samples vs. dentine of deciduous teeth) or Mn exposure pathways [inhalation vs. dietary and non-dietary (i.e., hand-to-mouth) ingestion]. Discrepancies between previous studies and ours may also be due to the fact that dentine Mn levels in our study population could be within the range at which Mn acts as a nutrient in a beneficial capacity rather than a neurotoxicant, thus resulting in improved neurodevelopmental outcomes. Because no other studies have measured Mn levels in dentine using the same

analytical method, we were not able to compare our dentine Mn levels with those reported previously (Battistone et al., 1967; Lappalainen and Knuuttila, 1982). Nevertheless, based on a small number of samples that were analyzed for Mn (Gunier et al., 2014), it seems that maternal and cord blood Mn levels detected in our study population are comparable to those observed in other prospective studies (Chung et al., 2015; Lin et al., 2013; Takser et al., 2003).

Previous cross-sectional studies have reported stronger negative associations of Mn levels with behavior, cognitive, memory, and motor outcomes for girls than for boys (Bouchard et al., 2011a; Hernandez-Bonilla et al., 2011; Menezes-Filho et al., 2014; Riojas-Rodriguez et al., 2010; Torres-Agustin et al., 2013). Our study did not show negative associations between prenatal or postnatal dentine Mn levels and these outcomes for girls, but instead we observed several positive and significant associations for boys. Biological differences in response to Mn may explain differences between boys and girls. Animal studies have shown that Mn accumulation across body tissues (Dorman et al., 2004) and changes in striatal morphology (Madison et al., 2011) differ between male and female rodents. Further animal and epidemiologic studies are needed to elucidate possible biological differences between males and females.

Several epidemiologic studies have shown that lead can modify the association between Mn exposure and neurodevelopment (Claus Henn et al., 2012; Kim et al., 2009; Lin et al., 2013). For example, a study of 455 Mexican children observed greater deficits in both mental and psychomotor development at ages 12–36 months in children with the highest 12-month blood Mn and lead levels compared to those with lower levels of both metals (Claus Henn et al., 2012). Similarly, a recent study of 230 children in Taiwan reported significantly lower cognitive, language, and overall development quotients at 2 years of age in the group with the highest cord blood Mn and cord blood lead levels (≥75th percentile for both metals) compared to the group with the lowest cord blood Mn and lead levels (<25th percentile for both metals) (Lin et al., 2013). In the present study, we observed that higher prenatal Mn levels were associated with poorer visuospatial memory outcomes at 9 years and worse cognitive scores at 7 and 10.5 years in children with higher prenatal lead levels (≥0.8 μg/dL) but not in children with lower lead levels; however, these differences were not statistically significant and this lack of significance could be due to the low prenatal lead levels in our population (median = 0.8, range = 0–13.1 μg/dL).

This study has several limitations. First, the relatively small sample size, further reduced in the stratified analyses (i.e., child sex and prenatal lead exposure), limited our statistical power. Second, we conducted multiple comparisons and cannot rule out the possibility that some associations were due to chance. However, given that conventional approaches for correcting for multiple comparisons have low efficiency and poor accuracy (Rothman et al., 2008), we were careful to point out patterns in our results rather than highlighting isolated findings. Third, although we were able to control for numerous potential confounders, residual confounding in the relationships between exposure to Mn and neurodevelopmental outcomes could exist. Finally, unlike most previous studies that were cross-sectional, we did not have measurements of Mn levels that were concurrent with the neurodevelopmental assessments.

Despite its limitations, the present study also has considerable strengths including its longitudinal design, use of comprehensive neurodevelopmental assessments at different ages, information on a wide variety of potential confounders, and use of a novel matrix for Mn measurements. We measured Mn levels in dentine of deciduous teeth, a biological matrix that, unlike enamel (Ericson et al., 2007), can be directly linked to the developmental timing of exposure (Smith, 1998) and has been validated against other biomarkers of Mn exposure (Arora et al., 2012; Gunier et al., 2014). In contrast with other biological matrices that reflect short-term exposures (e.g., blood and urine reflect exposures of hours to days), dentine Mn measurements are useful in retrospectively discerning Mn levels in the developing fetus close to the time of birth and in estimating cumulative exposure over the perinatal period (Arora et al., 2012).

Overall, we found that higher Mn levels, as measured in deciduous teeth, were associated with poorer behavior in the CHAMACOS boys and girls, but better cognitive, visuospatial and verbal memory, and motor abilities in boys. These findings add to a growing literature addressing the potential developmental neurotoxicity of Mn exposure. However, additional research is needed to understand the inconsistencies in the neurodevelopmental findings across studies and the degree to which differences may be associated with Mn exposure pathways and biomarkers, child sex, and levels of other environmental toxicants such as lead.

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Conflict of interest statement

One of the authors (AB) has served as a consultant on cases unrelated to the issues covered in this paper and has participated as a member of the board for The Organic Center, a non-profit organization that provides information for scientific research about organic food and farming. The other authors declare that they have no actual or potential competing financial interests.

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Appendix A

Table A.1
Distribution of Mn levels in teeth dentine (^{55}Mn:^{43}Ca AUC × 10^4), CHAMACOS Study, Salinas, California.

Biomarkers	*n*	Mean ± SD	GM (GSD)	Min	Percentile			Max
					25th	50th	75th	
Prenatal Mn	248	0.50 ± 0.18	0.46 (1.48)	0.07	0.38	0.49	0.57	1.34
Postnatal Mn	244	0.19 ± 0.21	0.14 (2.47)	0.001	0.11	0.14	0.20	2.50

Abbreviations: AUC, area under the curve; SD, standard deviation; GM, geometric mean; GSD, geometric standard deviation.

Table A.2
Means and standard deviations for behavioral, cognitive, memory, and motor outcomes in children at 7, 9, and 10.5 years.

Outcome	n[a]	Mean ± SD
7-year assessment		
Behavioral outcomes		
CADS — maternal report (T-scores)		
ADHD index	198	49.5 ± 7.7
DSM-IV total scale	198	49.7 ± 8.0
Inattentive subscale	198	48.6 ± 7.5
Hyperactive/impulsive subscale	198	51.0 ± 7.8
BASC-2 - Maternal Report (T-scores)		
Internalizing problems	193	48.5 ± 9.7
Externalizing problems	193	44.0 ± 8.2
Attention problems	193	49.4 ± 10.6
Hyperactivity	193	44.9 ± 8.1
CADS — teacher report (T-scores)		
ADHD index	170	53.4 ± 11.6
DSM-IV total scale	170	52.0 ± 9.9
Inattentive subscale	173	48.3 ± 8.9
Hyperactive/impulsive subscale	173	52.0 ± 10.1
BASC-2 — teacher report (T-scores)		
Internalizing problems	173	50.2 ± 11.9
Externalizing problems	173	48.6 ± 9.2
Attention problems	173	51.0 ± 7.8
Hyperactivity	173	49.0 ± 10.0
Cognitive outcomes		
WISC-IV Full-Scale IQ (scaled scores)	175	104.1 ± 14.1
Verbal Comprehension IQ	193	106.8 ± 16.7
Perceptual Reasoning IQ	193	102.2 ± 16.8
Working Memory IQ	176	93.4 ± 13.0
Processing Speed IQ	176	109.0 ± 12.8
Motor outcomes		
WRAVMA Pegboard (scaled scores)		
Dominant hand	193	120.4 ± 17.0
Non-dominant hand	193	122.7 ± 17.5
Finger tap (raw scores)[b]		
Dominant hand	193	32.4 ± 6.2
Non-dominant hand	193	28.6 ± 5.7
9-year assessment		
Behavioral outcomes		
CADS — maternal report (T-scores)		
ADHD Index	243	51.1 ± 9.3
DSM-IV total scale	242	51.5 ± 9.6
Inattentive subscale	242	49.7 ± 9.0
Hyperactive/impulsive subscale	242	53.5 ± 10.5
CPT-II (T-scores)		
Errors of omission	238	58.1 ± 16.8
Errors of commission	238	49.7 ± 9.3
ADHD confidence index	238	52.2 ± 22.2
Memory outcomes		
NEPSY-II Memory for Designs (scaled scores)		
Immediate total	184	8.8 ± 3.3
Delayed total	185	9.5 ± 3.3
Motor outcomes		
Luria-Nebraska Motor Scale (raw scores)[b]		
All items	227	54.8 ± 9.0
5-item sum	227	41.6 ± 7.5
10.5-year assessment		
Behavioral outcomes		
BASC-2 — maternal report (T-scores)		
Internalizing problems	232	48.2 ± 8.7
Externalizing problems	227	45.7 ± 7.3
Attention problems	232	48.6 ± 10.6
Hyperactivity	232	46.2 ± 7.5
BASC-2 — self-report (sex-standardized T-scores)		
Attention problems	225	48.8 ± 9.1
Hyperactivity	228	46.8 ± 9.0
Cognitive outcomes		
WISC-IV Full-Scale IQ (scaled scores)	231	90.8 ± 10.5
Verbal comprehension IQ	233	84.9 ± 11.5
Perceptual reasoning IQ	233	93.3 ± 14.3
Working memory IQ	233	97.0 ± 10.3
Processing speed IQ	233	99.2 ± 12.1
Memory outcomes		
CAVLT-2 (standardized scores)		
Immediate recall	233	98.9 ± 16.6
Delayed recall	233	96.2 ± 15.9

Table A.2 (*continued*)

Outcome	n[a]	Mean ± SD
10.5-year assessment		
Memory outcomes		
CAVLT-standardized scores)		
Immediate memory span	233	89.6 ± 15.2
Level of learning	233	96.8 ± 14.6
Motor outcomes		
Luria-Nebraska Motor Scale (raw scores)[b]		
All items	233	48.3 ± 8.9
5-item sum	233	35.1 ± 7.1

Abbreviations: SD, standard deviation; CADS, Conners' ADHD/DSM-IV Scales; ADHD, Attention Deficit/Hyperactivity Disorder; BASC-2, Behavior Assessment System for Children 2nd edition; IQ, intellectual quotient; WRAVMA, Wide Range Assessment of Visual Motor Ability; CPT-II, continuous performance test 2nd edition; and CAVLT-2, Children's Auditory Verbal Learning Test 2nd edition.

[a] Children who completed the 7-, 9-, or 10.5-year neurobehavioral assessment and had dentine Mn levels measured in shed incisors.

[b] For statistical analysis, these scores were converted to z-scores for the CHAMACOS population.

Table A.3
Generalized estimating equation models for behavioral, cognitive, and motor outcomes in children at 7, 9, and 10.5 years, per two-fold increase in prenatal and postnatal dentine Mn (^{55}Mn:^{43}Ca AUC × 10^4).

Outcomes	Prenatal Mn			Postnatal Mn		
	n	*k*	β (95% CI)	*n*	*k*	β (95% CI)
Behavioral outcomes[a]						
CADS — maternal report (T-scores)[b,c]						
ADHD index	441	251	−0.2 (−1.7, 1.3)	433	247	0.2 (−0.5, 0.8)
DSM-IV total scale	440	251	−0.1 (−1.8, 1.6)	432	247	0.3 (−0.3, 0.9)
Inattentive subscale	440	251	−0.4 (−1.9, 1.1)	432	247	0.2 (−0.4, 0.7)
Hyperactive/impulsive subscale	440	251	0.1 (−1.9, 2.0)	432	247	0.4 (−0.2, 1.0)
BASC-2 — maternal report (T-scores)[b,d]						
Internalizing problems	425	245	0.8 (−0.9, 2.5)	417	241	0.6 (0.0, 1.2)
Externalizing problems	420	244	0.1 (−1.3, 1.6)	413	240	0.4 (−0.1, 0.8)
Attention problems	425	245	0.3 (−1.7, 2.4)	417	241	0.0 (−0.7, 0.8)
Hyperactivity	425	245	0.2 (−1.2, 1.6)	417	241	0.3 (−0.1, 0.8)
Cognitive outcomes						
WISC-IV Full-Scale IQ (Scaled scores)[e,d]	408	243	1.5 (−0.7, 3.6)	400	239	0.7 (−0.3, 1.6)
Verbal comprehension IQ	426	245	0.9 (−1.2, 2.9)	418	241	0.6 (−0.1, 1.4)
Perceptual reasoning IQ	426	245	2.5 (−1.0, 6.1)	418	241	0.9 (−0.5, 2.3)
Working memory IQ	409	243	1.0 (−1.1, 3.1)	401	239	0.6 (−0.4, 1.5)
Processing speed IQ	409	243	0.5 (−1.8, 2.8)	401	239	−0.2 (−1.1, 0.8)
Motor outcomes						
Luria-Nebraska Motor Scale (z-scores)[e,f]						
All items	460	239	0.1 (0.0, 0.2)	452	235	0.0 (−0.1, 0.0)
5-item sum	460	239	0.2 (0.0, 0.3)*	452	235	0.0 (−0.1, 0.0)

Abbreviations: *n*, number of observations; *k*, number of children; CI, confidence interval; CADS, Conners' ADHD/DSM-IV Scales; ADHD, Attention Deficit/Hyperactivity Disorder; BASC-2, Behavior Assessment System for Children 2nd edition; WISC-IV, Wechsler Intelligence Scale for Children 4th edition; and IQ, intellectual quotient.

[a] Higher scores indicate poorer performance or more symptomatic behavior.

[b] Adjusted for maternal education, intelligence (PPVT score), years in the US, and depression at time of assessment; child's sex and age at maternal interview; language of maternal interview; HOME z-score, household income, and number of children in the home at time of assessment.

[c] Outcomes measured at 7 and 9 years.

[d] Outcomes measured at 7 and 10.5 years.

[e] Adjusted for maternal education, intelligence (PPVT score), years in the US, and depression at time of assessment; child's sex, age at assessment, and language of assessment; HOME z-score, household income, number of children in the home at time of assessment, and psychometrician at time of assessment.

[f] Outcomes measured at 9 and 10.5 years.

* $p_{Linear} < 0.10$.

Table A.4
Adjusted linear models for behavioral outcomes in children at 7, 9, and 10.5 years, per two-fold increase in prenatal dentine Mn (^{55}Mn:^{43}Ca AUC × 10^4) stratified by prenatal lead exposure (maternal blood lead levels during pregnancy <0.8 vs. ≥0.8 μg/dL).

Outcomes[a]	Lower lead exposure		Higher lead exposure		p_{INT}
	n	β (95% CI)	*n*	β (95% CI)	
7-year assessment					
CADS — maternal report (T-scores)[b]					
ADHD index	85	−1.0 (−4.0, 2.0)	85	−0.5 (−3.2, 2.3)	0.96
DSM-IV total scale	85	−0.9 (−3.8, 1.9)	85	−1.1 (−3.8, 1.5)	0.95
Inattentive subscale	85	−2.5 (−4.9, −0.2)**	85	−0.9 (−3.4, 1.6)	0.47
Hyperactive/impulsive subscale	85	0.7 (−2.6, 3.9)	85	−1.3 (−4.0, 1.4)	0.39
BASC-2 — maternal report (T-scores)[b]					
Internalizing problems	83	3.9 (−0.5, 8.4)*	82	−0.7 (−3.6, 2.1)	0.08

(*continued on next page*)

Table A.4 (*continued*)

Outcomes[a]	Lower lead exposure		Higher lead exposure		p_{INT}
	n	β (95% CI)	*n*	β (95% CI)	
7-year assessment					
BASC-2 — maternal report (T-cores)[b]					
Externalizing problems	83	1.1 (−1.8, 4.0)	82	0.5 (−2.5, 3.4)	0.58
Attention problems	83	1.8 (−1.3, 5.0)	82	0.4 (−2.5, 3.2)	0.57
Hyperactivity	83	−2.3 (−6.0, 1.4)	82	0.4 (−4.3, 5.2)	0.47
CADS — teacher report (T-scores)[c]					
ADHD index	73	−4.6 (−10.7, 1.6)	71	1.1 (−2.5, 4.7)	0.12
DSM-IV total scale	73	−3.2 (−8.9, 2.5)	71	1.8 (−1.2, 4.8)	0.09
Inattentive subscale	74	−2.4 (−7.0, 2.1)	73	2.7 (0.4, 5.0)**	0.03
Hyperactive/impulsive subscale	74	−3.4 (−9.3, 2.5)	73	−0.2 (−3.5, 3.2)	0.33
BASC-2 — teacher report (T-scores)[c]					
Internalizing problems	74	−5.5 (−9.6, −1.3)**	73	−0.7 (−6.7, 5.3)	0.46
Externalizing problems	74	−1.4 (−6.2, 3.4)	73	1.5 (−1.3, 4.3)	0.25
Attention problems	74	−2.3 (−7.8, 3.2)	73	0.3 (−2.7, 3.3)	0.19
Hyperactivity	74	−2.5 (−6.3, 1.4)	73	0.3 (−1.9, 2.5)	0.29
9-year assessment					
CADS — maternal report (T-scores)[b]					
ADHD index	83	−0.8 (−6.6, 4.9)	83	−0.1 (−2.7, 2.6)	0.95
DSM-IV total scale	83	1.9 (−4.7, 8.4)	82	0.3 (−2.4, 3.0)	0.62
Inattentive subscale	83	−0.1 (−5.9, 5.6)	82	−1.0 (−3.5, 1.5)	0.92
Hyperactive/impulsive subscale	83	3.8 (−3.8, 11.5)	82	2.0 (−1.2, 5.2)	0.47
CPT-II (T-scores)[d]					
Errors of omission	80	−8.2 (−16.8, 0.5)*	81	−6.4 (−14.5, 1.7)	0.72
Errors of commission	80	−2.9 (−7.8, 2.0)	81	−1.4 (−5.6, 2.8)	0.68
ADHD Confidence index	80	−13.9 (−23.4, −4.4)**	81	−7.1 (−16.7, 2.5)	0.44
10.5-year assessment					
BASC-2 — maternal report (T-scores)[b]					
Internalizing problems	79	5.1 (1.9, 8.3)**	79	−0.2 (−2.7, 2.3)	0.01
Externalizing problems	77	1.7 (−2.1, 5.4)	78	1.8 (−0.5, 4.1)	0.87
Attention problems	79	−2.2 (−7.8, 3.4)	79	0.2 (−3.5, 3.9)	0.70
Hyperactivity	79	2.7 (−0.9, 6.3)	79	0.7 (−1.7, 3.1)	0.45
BASC-2 — self-report (T-scores)[d]					
Attention problems	75	2.0 (−2.8, 6.8)	77	−0.7 (−4.2, 2.7)	0.83
Hyperactivity	77	2.0 (−2.1, 6.2)	78	−2.2 (−7.5, 3.1)	0.42

Abbreviations: AUC, area under the curve; CADS, Conners' ADHD/DSM-IV Scales; ADHD, Attention Deficit/Hyperactivity Disorder; BASC-2, Behavior Assessment System for Children 2nd edition; and CPT-II, Continuous Performance Test 2nd edition.

[a] Higher scores indicate poorer performance or more symptomatic behavior.

[b] Adjusted for maternal education, intelligence (PPVT score), years in the US, and depression at time of assessment; child's sex and age at maternal interview; language of maternal interview; HOME z-score, household income, and number of children in the home at time of assessment.

[c] Adjusted for maternal education, intelligence (PPVT score), years in the US, and depression at time of assessment; child's sex and age at interview; HOME z-score, household income, and number of children in the home at time of assessment.

[d] Adjusted for maternal education, intelligence (PPVT score), years in the US, and depression at time of assessment; child's sex, age at assessment, and language of assessment; HOME z-score, household income, number of children in the home, and psychometrician at time of assessment (9-year and 10.5-year assessments).

* $p_{Linear} < 0.10$.

** $p_{Linear} < 0.05$.

Table A.5
Adjusted linear models for cognition, memory, and motor outcomes in children at 7, 9, and 10.5 years per two-fold increase in prenatal dentine Mn ($^{55}Mn:^{43}Ca$ AUC × 10^4) stratified by prenatal lead exposure (maternal blood lead levels during pregnancy <0.8 vs. ≥0.8 μg/dL).

Outcomes	Lower lead exposure		Higher lead exposure		p_{INT}
	n	β (95% CI)	*n*	β (95% CI)	
Cognition					
7-year assessment					
WISC-IV Full-Scale IQ (scaled scores)	80	3.7 (−2.8, 10.3)	78	−3.5 (−7.2, 0.2)*	0.08
Verbal comprehension IQ	83	1.3 (−4.3, 7.0)	82	−2.7 (−6.8, 1.3)	0.29
Perceptual reasoning IQ	83	3.0 (−5.0, 11.0)	82	−3.2 (−9.2, 2.8)	0.21
Working memory IQ	81	0.5 (−6.1, 7.2)	78	−1.4 (−5.1, 2.4)	0.54
Processing speed IQ	81	7.5 (1.8, 13.2)**	78	−1.4 (−5.5, 2.8)	0.10
10.5-year assessment					
WISC-IV Full-Scale IQ (scaled scores)	78	1.5 (−4.1, 7.1)	79	−3.7 (−7.7, 0.2)*	0.21
Verbal comprehension IQ	79	−3.5 (−7.9, 0.9)	80	−4.1 (−8.0, −0.3)**	0.97
Perceptual reasoning IQ	79	3.3 (−4.6, 11.1)	80	−3.3 (−9.1, 2.5)	0.20
Working memory IQ	79	3.9 (−1.1, 8.9)	80	−0.9 (−5.3, 3.6)	0.21
Processing speed IQ	79	2.9 (−2.5, 8.3)	80	−1.0 (−5.4, 3.4)	0.52
Memory					
9-year assessment					
NEPSY-II Memory for Designs (scaled scores)					
Immediate total	79	1.2 (−0.8, 3.2)	79	−0.1 (−1.7, 1.4)	0.40
Delayed total	79	1.9 (−0.2, 4.0)*	80	−1.2 (−2.4, 0.0)*	0.04

Table A.5 (*continued*)

Outcomes	Lower lead exposure		Higher lead exposure		p_{INT}
	n	β (95% CI)	*n*	β (95% CI)	
Memory					
10.5-year assessment					
CAVLT-2 (standardized scores)					
Immediate recall	79	−4.5 (−13.6, 4.7)	80	4.1 (−2.7, 11.0)	0.11
Delayed recall	79	1.3 (−8.0, 10.5)	80	4.3 (−2.1, 10.6)	0.61
Immediate memory span	79	2.7 (−4.1, 9.4)	80	−4.3 (−10.9, 2.2)	0.31
Level of learning	79	2.8 (−5.2, 10.8)	80	2.3 (−3.4, 8.1)	0.84
Motor function					
7-year assessment					
WRAVMA Pegboard (scaled scores)					
Dominant hand	83	5.3 (−2.6, 13.3)	82	−0.3 (−5.8, 5.1)	0.19
Non-dominant hand	83	4.8 (−3.2, 12.7)	82	0.4 (−6.2, 7.0)	0.45
Finger tap (z-scores)					
Dominant hand	83	0.4 (0.0, 0.9)*	82	−0.1 (−0.5, 0.2)	0.25
Non-dominant hand	83	0.2 (−0.3, 0.7)	82	−0.1 (−0.5, 0.2)	0.70
9-year assessment					
Luria-Nebraska Motor Scale (z-scores)					
All items	80	0.2 (−0.2, 0.7)	78	−0.2 (−0.6, 0.2)	0.20
5-item sum	80	0.3 (−0.2, 0.7)	78	−0.2 (−0.7, 0.3)	0.21
10.5-year assessment					
Luria-Nebraska Motor Scale (z-scores)					
All items	79	0.2 (−0.2, 0.5)	80	0.1 (−0.2, 0.3)	0.93
5-item sum	79	0.1 (−0.4, 0.6)	80	0.0 (−0.4, 0.4)	0.92

Abbreviations: AUC, area under the curve; CI, confidence interval; WISC-IV, Wechsler Intelligence Scale for Children 4th edition; IQ, intellectual quotient; CAVLT-2, Children's Auditory Verbal Learning Test 2nd edition; and WRAVMA, Wide Range Assessment of Visual Motor Ability.
Models adjusted for maternal education, intelligence (PPVT score), years in the US, and depression at time of assessment; child's sex, age at assessment, and language of assessment; HOME z-score, household income, number of children in the home, and psychometrician at time of assessment (9-year and 10.5-year assessments).

* $p_{Linear} < 0.10$.

** $p_{Linear} < 0.05$.

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