

Early life manganese exposure and reported attention-related behaviors in Italian adolescents

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Background: Manganese (Mn) is an essential nutrient and neurotoxicant, and the neurodevelopmental effects of Mn may depend on exposure timing. Less research has quantitatively compared the impact of Mn exposure on neurodevelopment across exposure periods.

Methods: We used data from 125 Italian adolescents (10–14 years) from the Public Health Impact of Metals Exposure Study to estimate prospective associations of Mn in three early life exposure periods with adolescent attention-related behaviors. Mn was quantified in deciduous teeth using laser ablation-inductively coupled plasma-mass spectrometry to represent prenatal (2nd trimester–birth), postnatal (birth ~1.5 years), and childhood (~1.5–6 years) exposure. Attention-related behavior was evaluated using the Conners Behavior Rating Scales in adolescence. We used multivariable linear regression models to quantify associations between Mn in each exposure period, and multiple informant models to compare associations across exposure periods.

Results: Median tooth Mn levels (normalized to calcium) were 0.4 area under the curve (AUC) $^{55}\text{Mn}:$ $^{43}\text{Ca} \times 10^4$, 0.1 AUC $^{55}\text{Mn}:$ $^{43}\text{Ca} \times 10^4$, and 0.0006 $^{55}\text{Mn}:$ ^{43}Ca for the prenatal, postnatal, and childhood periods. A doubling in prenatal tooth Mn levels was associated with 5.3% (95% confidence intervals [CI] = –10.3%, 0.0%) lower (i.e., better) teacher-reported inattention scores, whereas a doubling in postnatal tooth Mn levels was associated with 4.5% (95% CI = –9.3%, 0.6%) and 4.6% (95% CI = –9.5%, 0.6%) lower parent-reported inattention and attention deficit/hyperactivity disorder index scores, respectively. Childhood Mn was not beneficially associated with reported attention-related behaviors.

Conclusion: Protective associations in the prenatal and postnatal periods suggest Mn is beneficial for attention-related behavior, but not in the childhood period.

Keywords: Attention; Critical periods; Manganese; Metals; Neurodevelopment; Teeth

Introduction

Manganese (Mn) is a metal required for normal biological functions in humans.¹ Mn is a component of various enzymes that are involved in neurodevelopment, making it an essential

nutrient for neurodevelopment.² However, overexposure to Mn, especially in populations exposed to anthropogenic sources like air pollution, has been associated with neurotoxicity in both human and animal models.^{3–5} Emerging epidemiological evidence suggests that the degree to which Mn acts beneficially versus as a neurotoxicant may depend not only on dose but also on the timing of exposure.⁶

Mn exposure in the prenatal period (~2nd trimester to birth) has been beneficially associated with cognition, including decreased scores for externalizing symptoms (e.g., aggression, hyperactivity) in some epidemiologic studies.^{6,7} This likely reflects the role of Mn as an essential nutrient during pregnancy.^{7,8} Fetal exposure to Mn is tightly regulated during gestation, and Mn is actively transported across the placenta to support the growth and development of the fetus.^{9,10} Further, Mn superoxide dismutase enzymes are critical for protecting the placenta and the developing fetus from oxidative stress.¹¹ However, other studies have reported nonlinear or adverse associations of prenatal Mn with neurodevelopment,^{12–15} suggesting the dose of environmental exposure during this period of development plays a critical role in cognitive outcomes in children.

Mn exposure in the early postnatal period and during childhood has been more consistently associated with worse

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What this study adds

This study is among the first to report manganese (Mn)-neurodevelopment associations in multiple exposure periods, where Mn exposure in the prenatal and postnatal periods was beneficially associated with adolescent attention-related behaviors. Conversely, Mn exposure in the childhood period was not beneficially associated with neurodevelopment. Our study supports the hypothesis that Mn neurotoxicity in early life is dependent on the timing of exposure.

attention-related behaviors, including attention deficit/hyperactivity disorder (ADHD), internalizing problems, and externalizing symptoms, such as hyperactivity and oppositional behavior.^{7,16–29} Neurotoxicity resulting from early life Mn exposure has been observed in animal models,^{30–33} and reflects the toxic mechanisms of Mn in the brain, including induction of oxidative stress and inflammation, mitochondrial disruption of neurons, and disruption of neurotransmission.³⁴ Greater evidence points to Mn toxicity in the postnatal and childhood periods, as compared with the prenatal period, which may be due to differences in dose, but also to differences in exposure sources and behavioral factors across developmental periods. For example, whereas fetal exposure to Mn in the prenatal period is regulated by the placenta,¹⁰ infants and children are exposed to environmental Mn through a variety of sources, including contaminated dust, air emissions, infant formula, and other dietary sources.³⁵ Hand-to-mouth behavior in early childhood may also increase Mn exposure from the environment via ingestion, and consequent gastrointestinal absorption of Mn tends to be higher in young children compared with adults.³⁵ The toxicity of Mn in early life may also reflect susceptibility to neurotoxic insults affecting the prefrontal cortex, which modulates attention, executive function, and emotion regulation during these developmental periods.³⁶ This hypothesis is supported by animal data, where early life Mn exposure in rats led to altered concentrations of the neurotransmitters serotonin, dopamine, and norepinephrine, and their receptors, in the prefrontal cortex.^{30,32,33}

Despite prior findings describing differential associations of Mn with neurodevelopment in the prenatal, postnatal, and childhood periods, less research has quantitatively compared Mn neurotoxicity across exposure periods, especially for attention-related behaviors. Attention, formally defined as the ability to process incoming information,³⁷ is critical for the overall cognitive performance of children: attentional deficits have been linked with worse scholastic performance, including reading speed and accuracy.^{38,39} Scholastic performance, in turn, plays a role in cognitive function later in life.^{40,41} Therefore, quantifying the impact of early life Mn on attention-related behavior is of public health interest. The goal of the current analysis was to identify critical periods of Mn exposure (prenatal, postnatal, early childhood) measured in deciduous teeth in relation to reported attention-related behaviors in adolescence. Given emerging evidence of sexual dimorphism in Mn-neurodevelopment associations,^{15,42–44} we also explored potential sex differences in associations between Mn and Conners scores in each early life period.

Methods

Study population

We used cross-sectional data from the Public Health Impact of Metals Exposure (PHIME) study, which was designed to

examine associations between metals exposure from ferroalloy industry emissions and neurodevelopment in early adolescence. A total of 721 adolescents (10–14 years) were recruited from three regions in the province of Brescia in northern Italy, including Bagnolo Mella (BM), Garda Lake (GL), and Valcamonica (VC). These three regions each had varied historical ferroalloy industry: BM has had active ferroalloy industry since 1974, GL had no ferroalloy industry, and VC had a ferroalloy industry that ceased in 2001.

Adolescents were recruited in two distinct phases that reflected two waves of funding for the study: the first phase (2007–2010) enrolled 311 adolescents and the second phase (2010–2014) enrolled 410 adolescents. All study protocols and questionnaires were consistent between the study phases. The second phase specifically recruited adolescents from the BM site and administered the Home Observation Measurement of the Environment (HOME) Short Form.⁴⁵ Participants were eligible for enrollment if they were (1) 10–14 years of age at the time of recruitment, (2) had lived in the study area since birth, and (3) were born into families who resided in the study region since the 1970s. Participants were excluded from the study if they (1) had a diagnosed neurologic, psychiatric, hepatic, endocrine, or metabolic disease, (2) had clinically relevant motor deficits that could have impacted testing, (3) used medication with any neurologic side effects, (4) had clinically diagnosed behavioral or cognitive impairments, (5) had vision deficits with no corrective measures, or (6) had ever received parenteral nutrition. PHIME study procedures have been described in depth previously in the literature.⁴⁶

Additional supplemental funding was received in 2013 to collect deciduous teeth and quantify Mn levels from a subset of the PHIME participants ($n = 195$). The current analytic sample comprised 125 adolescents who provided teeth and had complete outcome data (Figure 1).

Informed consent was given by all participants after receiving detailed study information. Study protocols were approved by human subjects review boards (Institutional Review Boards) at the Icahn School of Medicine at Mount Sinai, University of California Santa Cruz, and the Ethical Committee of Brescia.

Tooth collection and manganese measurement

We collected and measured Mn levels in one naturally shed deciduous tooth (incisor, canine, or molar) that was absent of obvious decay (i.e., caries) per child. Upon collection, each tooth was assessed for loss of dentin layers due to natural wear (i.e., attrition), which can affect the accuracy of Mn measurements.⁶ Tooth attrition was defined as an ordinal variable reflecting tooth tissue loss due to natural wear as follows: no tissue loss, less than one-third tissue loss, or one-third or more but less than two-thirds tissue loss. Teeth with two-thirds or more tissue loss were not analyzed.

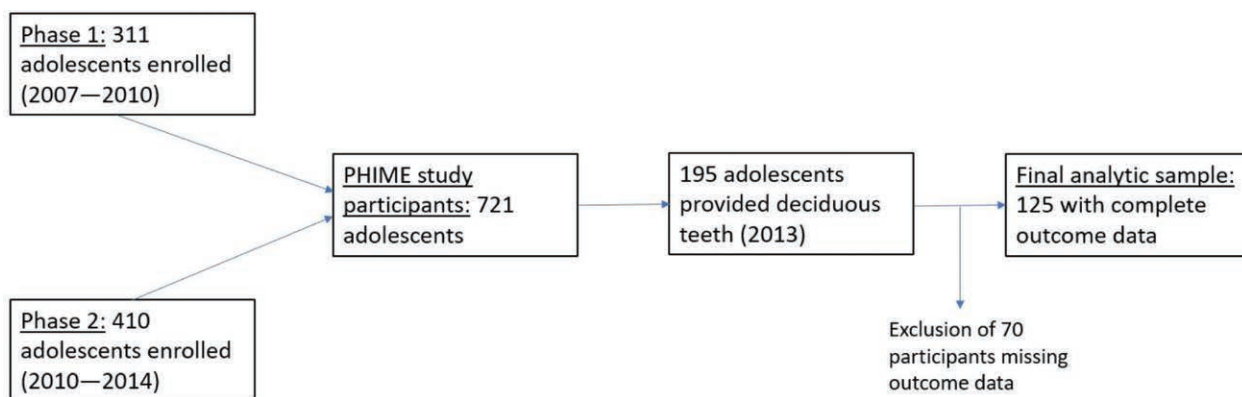


Figure 1. Schematic of the Public Health Impact of Metals Exposure (PHIME) study.

All tooth analysis protocols have been described in detail elsewhere.^{47,48} In brief, a full cross-section of dentin for each tooth was exposed by sectioning teeth vertically. The neonatal line (NL), a histological feature that forms within the tooth at birth, was identified for each tooth.⁴⁹ Using the NL as a temporal reference, a total of forty measurements were taken from the primary (n = 30) and secondary (n = 10) dentin to estimate Mn levels in three exposure periods: prenatal, measured in primary dentin and reflecting Mn exposure from the second trimester to birth; early postnatal, measured in primary dentin and reflecting Mn exposure from birth to ~1.5 years; and childhood, measured in secondary dentin and reflecting exposure from ~1.5 to 6 years or until the tooth is shed. Mn levels were quantified using laser ablation-inductively coupled plasma-mass spectrometry.^{47,48} Tooth calcium (Ca) levels were analyzed using the same methods as tooth Mn. All tooth Mn levels were normalized to tooth Ca levels as the ratio of Mn to Ca (⁵⁵Mn:⁴³Ca, unitless) to account for differences in mineral density of the tooth within and between participants.

To reflect cumulative exposure in each period, prenatal and postnatal Mn levels were estimated as the ⁵⁵Mn: ⁴³Ca area under the curve (AUC) × 10,000 across all primary dentin sampling points; childhood ⁵⁵Mn: ⁴³Ca levels were averaged across secondary dentin measurements. Only two values in the postnatal period were below the limit of detection (LOD) of 0.03 ⁵⁵Mn: ⁴³Ca × 10,000; these values were imputed as the LOD/2. Laboratory technicians were blinded to participants' neurodevelopmental outcomes.

Cognitive assessment

We used the Conners Rating Scales (CRS) to assess attention-related behavior. The CRS is a validated neurodevelopmental assessment tool developed to determine clinically relevant ADHD symptomology in children.^{50–52} It is a comprehensive, multi-informant assessment with three scales for parent-, teacher-, and self-reports: Conners–Wells' Adolescent Self-Report Scale-Long Form, S-Conners' Parent Rating Scales Revised-Short Form, and S-Conners' Teaching Rating Scale-Short Form. Scores from both the parent- and teacher-reports have been shown to have good internal consistency and long-term test-retest reliability;⁵³ therefore, we focused our primary analyses on scores from parent- and teacher-reports. The parent- and teacher-reports include scales for oppositional behavior, inattention/cognitive problems, hyperactivity, and an ADHD index.⁵⁰ The CRS was administered to participants in Italian by trained examiners who were blinded to the exposure status of participants.⁵³ Raw scores were converted into standardized T-scores (mean: 50, standard deviation: 10) that were adjusted for age and sex.

Measurement of confounders

Standardized questionnaires were administered either in person or via the phone by trained study staff. Information was collected on socioeconomic and demographic covariates, including age at enrollment (continuous, years), biological sex (male vs. female), area of residence (BM, GL, or VC), parental occupation, parental education, and tooth attrition. Nine items from the HOME Short Form were administered to participants, and scores ranged from 0 to 9.⁴⁵ We classified each participant's socioeconomic status (SES; low, medium, or high) based on parental education and occupation using a methodology developed for Italian populations, as described previously.^{54,55}

Tooth lead (Pb) levels, reflecting Pb exposure in the same exposure periods as Mn, were not quantified. However, we measured Pb concentrations in whole blood collected in adolescence at the time of neurodevelopmental assessment. Whole blood samples (4ml) were collected using 19-gauge butterfly catheters and stored in lithium-heparin Sarstedt Monovette Vacutainers. Concentrations of Pb were quantified using magnetic sector inductively coupled

plasma-mass spectrometry.^{46,56,57} All blood Pb measurements were above the LOD (0.03 ng/ml).

Statistical analysis

We first examined distributions of Conners scores, covariates, and tooth Mn levels in each of the three exposure periods. Conners scores, blood Pb, and tooth Mn levels were right-skewed; these variables were natural log (ln)-transformed to meet the assumption of normality of residuals and reduce the influence of extreme values. Summary statistics were calculated for all variables (Table 1) and we estimated Spearman correlation coefficients between Mn levels in the prenatal, postnatal, and childhood periods.

Covariates were chosen a priori based on directed acyclic graphs and prior literature (eFigure 1; <http://links.lww.com/EE/A243>).^{6,15,58} All models were adjusted for SES, HOME score, ln-transformed blood Pb, and tooth attrition. Pb is a known neurotoxicant and may be associated with Mn exposure; we therefore hypothesized Pb to be a potential confounder of Mn-neurodevelopment associations among children residing near the ferroalloy industry.⁵⁹ We did not include sex or age as covariates in statistical models because the Conners T-scores were age and sex adjusted. Additionally, all models were mutually adjusted for Mn levels in all three exposure periods (prenatal, postnatal, and early childhood).

Prior epidemiological evidence suggests that associations between Mn and neurodevelopment may be nonlinear because Mn is both an essential nutrient and a toxicant.^{12,15,26,58} We assessed the potential for nonlinear associations between Mn in each exposure period and Conners scores using covariate-adjusted generalized additive models with penalized splines (knots = 4). There was little evidence of nonlinearity based on visual inspection; therefore, Mn was modeled as a continuous variable in subsequent multivariable linear regression models.

There was little missing data (<7% missing for all variables; see eTable 1; <http://links.lww.com/EE/A243>), but to maximize the analytic sample and reduce potential bias,⁶⁰ we used Monte Carlo Markov Chain multiple imputations to impute missing covariate data using the mice package in R,^{61,62} where data were assumed to be missing at random. We generated 20 imputed datasets for the full PHIME cohort (n = 721) using all variables possibly related to the missing data, including Mn levels in each exposure period, concentrations of all metals (Mn, Pb, copper, and chromium) measured in other biomarkers (hair, nail, saliva, blood, and urine),⁶³ Mn concentrations in environmental samples (soil, air, water, and dust),⁶³ Conners scores, and confounder data.⁶ We restricted our final analytic sample to adolescents with complete exposure and outcome data (n = 125), but used imputed values for missing confounder information.

Multivariable linear regression models were fit for each imputed dataset to examine the association between tooth Mn levels in the prenatal, postnatal, and childhood periods with each of the Conners scales while adjusting for a priori-determined confounders. We used Rubin's rule to statistically combine findings from linear regression models across the 20 imputed datasets,⁶⁴ and generated pooled beta coefficients and 95% confidence intervals (CI). For ease of interpretation, we back-transformed the beta coefficients and 95% CI to represent a percent change in Conners T-scores for a doubling in tooth Mn levels using the following equations:

$$\% \text{ change in Conners scores} = \left(e^{(\ln(2) \times \beta)} \right) - 1 \times 100 \quad (1)$$

$$\% \text{ change in Conners scores, 95\% CI} = \left(e^{(\ln(2) \times (\beta \pm 1.96 \times \text{standard error}))} \right) - 1 \times 100 \quad (2)$$

Table 1.
Summary statistics for the PHIME study population

Characteristic	N (%) or mean (SD)	N (%) or mean (SD)	N (%) or mean (SD)
	Full cohort (n = 125)	Males (n = 57)	Females (n = 68)
Age (years)	11.9 (0.9)	11.8 (0.8)	12.0 (0.9)
Socioeconomic status index			
Low	26 (20.8%)	5 (8.8%)	21 (30.9%)
Medium	71 (56.8%)	40 (70.2%)	31 (45.6%)
High	28 (22.3%)	12 (21.0%)	16 (23.5%)
HOME score	6.3 (1.4)	6.3 (1.4)	6.3 (1.5)
Site			
Bagnolo Mella	74 (59.2%)	35 (61.4%)	39 (57.4%)
Garda Lake	28 (22.4%)	11 (19.3%)	17 (25.0%)
Valcamonica	23 (18.4%)	11 (19.3%)	12 (17.6%)
Tooth loss due to attrition			
None	68 (54.4%)	26 (45.6%)	42 (61.8%)
Less than one-third	46 (36.8%)	24 (42.1%)	22 (32.3%)
One-third or more	11 (8.8%)	7 (12.3%)	4 (5.9%)
but less than two-thirds			
Two-thirds or more	0 (0%)	0 (0%)	0 (0%)
Conners parent-reported T-scores			
ADHD index	50.1 (11.3)	48.7 (8.4)	51.3 (13.2)
Hyperactivity	48.1 (8.2)	47.3 (7.2)	48.8 (9.0)
Inattention	48.9 (10.8)	46.9 (8.5)	50.6 (12.3)
Oppositional behavior	49.1 (10.0)	48.9 (11.0)	49.1 (9.2)
Conners teacher-reported T-scores			
ADHD index	45.8 (6.4)	44.7 (7.7)	46.6 (5.1)
Hyperactivity	45.0 (5.1)	43.7 (6.3)	46.1 (3.4)
Inattention	47.5 (7.6)	46.7 (8.3)	48.1 (6.9)
Oppositional behavior	45.4 (4.3)	44.0 (4.0)	46.6 (4.2)
Metal biomarker: median (25th, 75th percentile)			
Tooth Mn, Prenatal (AUC ⁵⁵ Mn: ⁴³ Ca × 10 ⁴) ^a	0.4 (0.3, 0.5)	0.4 (0.3, 0.5)	0.4 (0.4, 0.5)
Tooth Mn, Postnatal (AUC ⁵⁵ Mn: ⁴³ Ca × 10 ⁴) ^a	0.1 (0.1, 0.2)	0.1 (0.1, 0.2)	0.1 (0.1, 0.2)
Tooth Mn, Childhood (average ⁵⁵ Mn: ⁴³ Ca) ^a	0.0006 (0.0005, 0.0009)	0.0006 (0.0004, 0.0009)	0.0007 (0.0005, 0.0009)
Blood Pb (µg/dl)	1.6 (1.0, 1.8)	1.3 (1.1, 1.9)	1.3 (0.9, 1.5)

^aprenatal period = 2nd trimester of gestation to birth, postnatal period = birth to ~1.5 years, childhood = ~1.5 years to 6 years.
ADHD indicates attention deficit hyperactivity disorder; AUC, area under curve; Ca, calcium; HOME, Home Observation Measurement of the Environment; Mn, manganese; Pb, lead.

Next, we fit multiple informant models to test whether associations between Mn and Conners scores differed across the prenatal, postnatal, and childhood periods.^{6,65} A generalized estimating equation was fit for each Conners scale. Generalized estimating equations were first fit for each of the 20 imputed datasets and then findings were statistically combined using Rubin's rule in SAS (for example code, see Bauer et al.⁶). Differences in associations of tooth Mn with Conners scales across exposure periods were considered significant based on a *P* < 0.10 from multiple informant models.

Emerging evidence suggests that associations between Mn and neurodevelopment may vary by biological sex.^{15,16,42,44,66,67} To explore potential sex differences in associations between Mn and Conners scores, we stratified our data by biological sex and fit multivariable linear regression and multiple informant models, as described above, in the stratified datasets.

All analyses were conducted in R version 3.6.1 and SAS version 9.4.

Results

About half of the participants were female (54%), and most were from families that were classified as medium SES (57%) and lived in Bagnolo Mella (59%, Table 1). The mean age of participants with an available tooth for analysis was 11.9 years (standard deviation [SD]: 0.9 years) and the mean HOME score was 6.3 (SD: 1.4). The median blood lead concentration was 1.6 µg/dl (25th–75th percentile: 1.0, 1.8 µg/dl). Median tooth Mn levels (reported as AUC ⁵⁵Mn: ⁴³Ca × 10⁴) were higher in the prenatal period (0.4; 25th–75th percentile: 0.3, 0.5) than in the postnatal period (0.1; 25th–75th percentile: 0.1, 0.2). Correlations between Mn concentrations across the exposure periods were weak: -0.06 (prenatal-childhood), 0.07 (postnatal-childhood), and 0.27 (prenatal-postnatal). Median levels of Mn in the prenatal, postnatal, and childhood periods were similar between males and females (Table 1). Average Conners scores on the parent-reported scales were consistently higher (indicating more reported problem behaviors) than scores on teacher-reported scales. Conners scores within the same respondent were moderately to highly correlated (range, parent-reported: 0.41–0.81; teacher-reported: 0.31–0.65), whereas correlations for scores across respondents were weaker (0.09–0.53). Scores for both the parent- and teacher-reported scales tended to be higher in females compared with males (Table 1). Summary statistics were similar between imputed and complete data (eTable 2; <http://links.lww.com/EE/A243>).

In adjusted linear regression models, a doubling of prenatal tooth Mn levels was associated with 5.3% (95% CI = -10.3%, 0.0%) lower teacher-reported inattention T-scores, suggesting beneficial effects of prenatal Mn on attention-related behavior. This association was attenuated in the postnatal period and null in childhood (Figure 2, eTable 3; <http://links.lww.com/EE/A243>), and was significantly different across the three exposure periods in multiple informant models (*P* = 0.01, eTable 4; <http://links.lww.com/EE/A243>). Similar to the beneficial association observed in the prenatal period, a doubling in postnatal Mn levels was associated with lower T-scores for parent-reported inattention (β = -4.5%; 95% CI = -9.3%, 0.6%), parent-reported ADHD index (β = -4.6%; 95% CI = -9.5%, 0.6%), and teacher-reported inattention (β = -2.4%; 95% CI = -6.0%, 1.4%). Conversely, a doubling in childhood tooth Mn levels was associated with 2.9% (95% CI = -1.5%, -1.0%), 3.6% (95% CI = -0.9%, 8.4%), and 3.5% (95% CI = -0.9%, 8.1%) higher parent-reported inattention, ADHD index, and oppositional behavior scores, respectively (Figure 2), suggesting that childhood Mn levels were adversely associated with parent-reported attention-related behaviors. There was some evidence that associations with parent-reported inattention and ADHD index differed across exposure periods in multiple informant models, though the *P* values were ≥ 0.10 (*P* = 0.15 and *P* = 0.10, respectively).

In exploratory sex-stratified models, a negative (i.e., beneficial) association between prenatal Mn and teacher-reported inattention was estimated in males (per doubling in Mn: β = -9.9%; 95% CI = -17.0%, -2.3%) but not in females (β = 4.8%; 95% CI = -3.5%, 13.9%, Figure 3). Based on multiple informant models, this beneficial association in the prenatal period among males differed significantly from the null associations in the postnatal and childhood periods (*P* < 0.01, eTable 5; <http://links.lww.com/EE/A243>).

Among females, negative (i.e., beneficial) associations between Mn and parent- and teacher-reported scales were also observed, but in the postnatal period: a doubling in postnatal Mn levels was associated with 12.3% (95% CI = -21.5%, -2.1%), 8.9% (95% CI = -19.2%, 2.8%), and 6.0% (95% CI = -13.2%, 1.9%) lower parent-reported T-scores

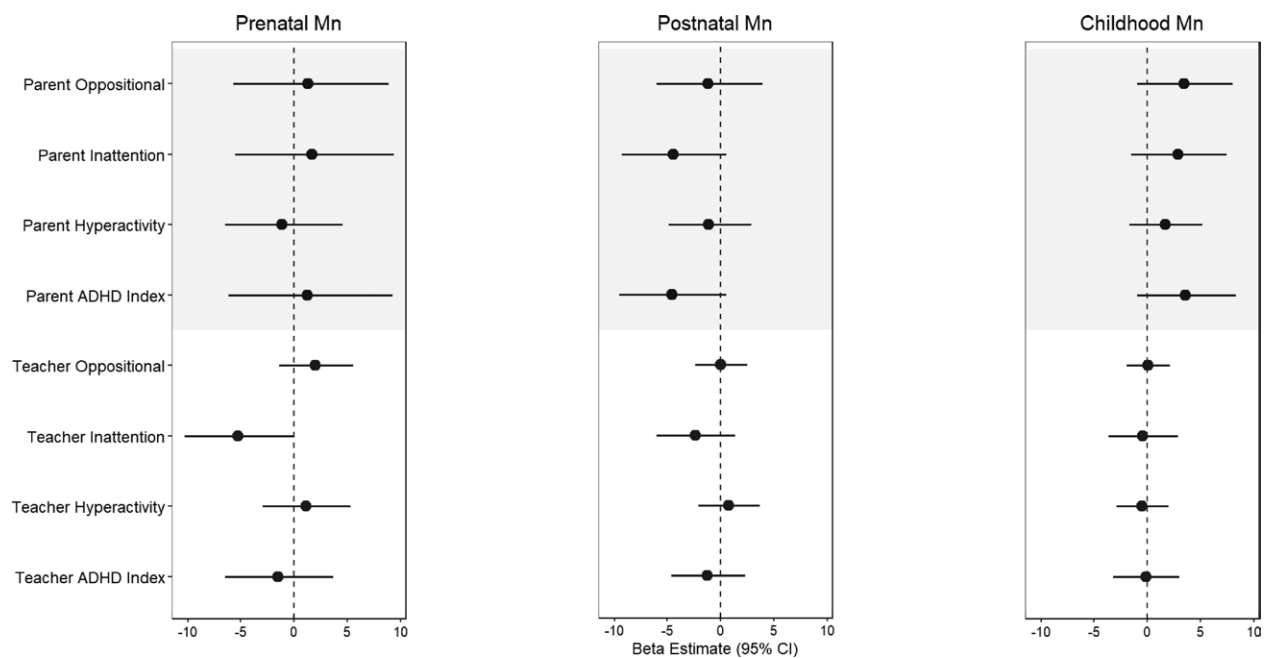


Figure 2. Adjusted beta (β) estimates and 95% CIs from multivariable linear regression models assessing associations between prenatal, postnatal, and childhood tooth Mn levels with parent- and teacher-reported scores from the Conners Rating Scales. Beta coefficients reflect the percent change in age- and sex-adjusted Conners T-scores for a doubling in tooth Mn levels. *Multivariable linear regression models were mutually adjusted for Mn in all exposure periods, and socioeconomic status, HOME score, tooth attrition, and ln-transformed blood Pb. **prenatal period = 2nd trimester of gestation to birth, postnatal period = birth to ~1.5 years, childhood = ~1.5 to 6 years.

for inattention, ADHD index, and hyperactivity, respectively, and 5.3% (95% CI = -11.2%, 1.1%) lower teacher-reported T-scores for inattention (Figure 3). Beneficial associations among females were not observed in the prenatal or childhood periods (Figure 3), although *P* values from multiple informant models comparing associations across exposure periods were not statistically significant (all *P* > 0.10, eTable 5; <http://links.lww.com/EE/A243>).

Discussion

In this study of Italian adolescents, we found that prenatal Mn was associated with decreased T-scores for teacher-reported inattention and that this association was significantly different across exposure periods. Postnatal Mn was similarly associated with decreased scores for inattention and the ADHD index on parent-reported scales, whereas childhood Mn was associated with modest increases in scores for parent-reported scores. Overall, these findings support a beneficial association of Mn with reported attention-related behaviors when exposure occurred in the prenatal and postnatal periods, but not in the childhood period.

Consistent with the findings of the current analysis, we previously found protective associations between prenatal tooth Mn and scores on the Weschler Intelligence Scale for Children in the PHIME cohort.⁶ Beneficial associations between prenatal Mn and neurodevelopment have been observed in at least one other cohort: in Mexican children, prenatal tooth Mn was associated with decreased externalizing symptoms, aggression, and hyperactivity.⁷ Protective associations of early life Mn with neurodevelopment likely reflect the known need for Mn during prenatal development: physiological requirements for Mn increase during pregnancy to support fetal growth, and Mn is actively transported across the placenta.⁶⁸ Specifically, Mn is essential for various enzymes used in neuronal tissues that mediate processes like protein synthesis and reactive oxygen species defense.² On the other hand, our findings conflict with those of two other studies: Mora et al.¹⁶ found that prenatal tooth Mn in Mexican–American children was associated with increased

internalizing problems, externalizing problems, and hyperactivity measured on the BASC at 10.5 years, and Skogheim et al.⁸ reported nonmonotonic dose responses for maternal (prenatal) blood Mn and attention-related behavior. These inconsistent findings may, in part, reflect differences in Mn exposure levels during pregnancy, the timing and test used to measure neurodevelopment, or SES across study populations. We were not able to directly compare the levels of tooth Mn in our study to those in other studies due to methodological differences in quantifying tooth Mn levels.¹⁶

Protective associations of tooth Mn with attention-related behaviors were also observed in the postnatal period, consistent with our prior work in PHIME.⁶ However, these findings conflict with findings from other cohorts, where postnatal tooth Mn was associated with increased internalizing problems in Mexican children,⁷ and with increased internalizing and externalizing problems among Mexican–American children.¹⁶ Evidence from animal models supports the notion that Mn exposure in the postnatal period may be neurotoxic, where early life Mn exposure in rats has been consistently associated with increased hyperactivity and attentional problems,^{30–33} and reductions in cortical norepinephrine, dopamine, and serotonin concentrations.³² Human brain maturation in the postnatal period is dynamic and occurs rapidly: in the first year of life, brain size increases from 36% (at birth) to 70% of the total adult brain size.⁶⁹ This growth includes several maturation processes, such as synaptic remodeling and pruning, myelination, synapse formation, and axonal and dendritic outgrowth and branching.⁶⁹ Thus, our findings may differ from prior studies in part because the exposure period that we captured for this highly dynamic and rapidly changing postnatal period (birth to ~1.5 years) was longer. This hypothesis is supported by findings from Horton et al.,⁷ where postnatal tooth Mn levels between 3 and 5 months were associated with reductions in externalizing symptoms in Mexican children, but this association was not present after 5 months. Future studies that examine postnatal Mn exposure with higher temporal resolution are warranted to identify possible critical periods of exposure within the early postnatal period.

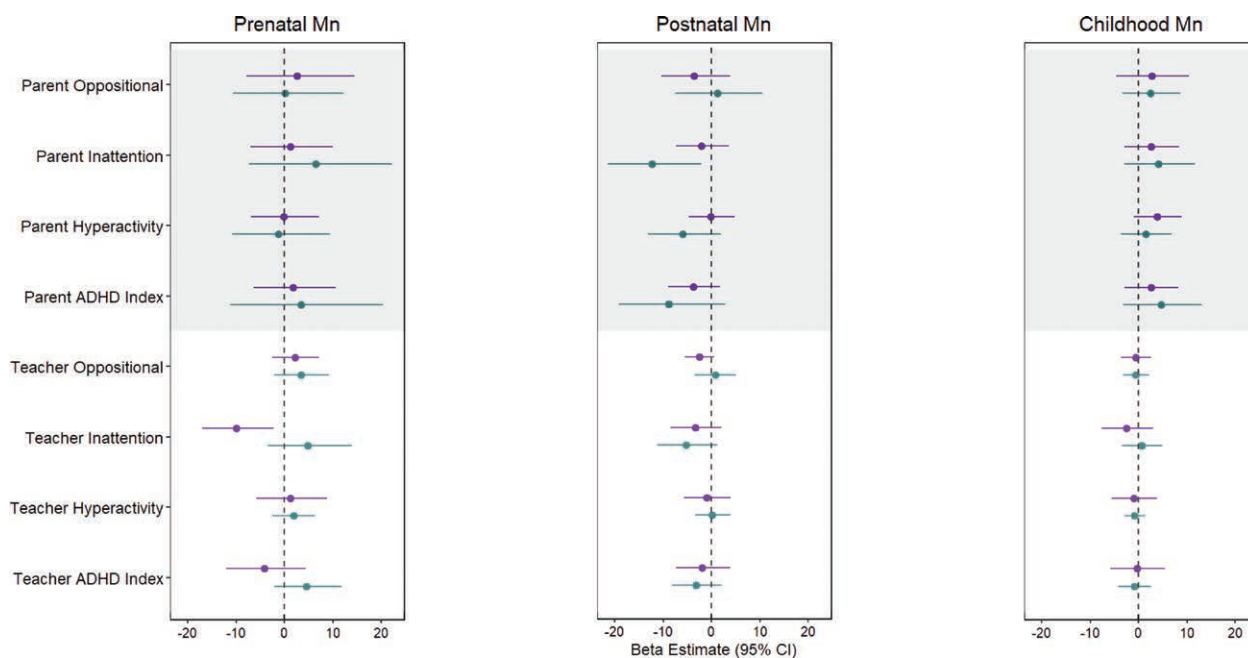


Figure 3. Adjusted beta (β) estimates and 95% CIs from sex-stratified multivariable linear regression models assessing associations between prenatal, postnatal, and childhood tooth Mn levels and change in age- and sex-adjusted Conners T-scores per doubling in tooth Mn levels. Females ($n = 68$) are shown in teal; males ($n = 57$) are shown in purple. *Multivariable linear regression models were mutually adjusted for Mn in all exposure periods, and socioeconomic status, HOME score, tooth attrition, and ln-transformed blood Pb. **prenatal period = 2nd trimester of gestation to birth, postnatal period = birth to ~1.5 years, childhood = ~1.5 to 6 years.

Similar to our findings, Mn exposure in childhood has been consistently associated with worse attention-related behaviors and ADHD diagnosis.^{21–23,28,70,71} It should be noted that the timing of Mn exposure in these prior studies varied, and many studies included participants from both childhood (1–9 years) and adolescence (10–16 years).^{17–26,28,70,71} Adolescence is characterized by a rapid maturation of the prefrontal cortex (e.g., pruning) of the brain,⁷² suggesting susceptibility to Mn neurotoxicity may differ for this age group. Therefore, prior epidemiological findings may not be directly comparable to our population. Nonetheless, studies of early childhood have similarly found associations between Mn and worse attention-related behaviors. Two studies reported associations between water Mn exposure in the first 5 years of life and increased odds of ADHD diagnosis in children from Bangladesh and Denmark,^{70,71} whereas hair Mn quantified at 6–9 years was associated with worse performance on the Trail Making Test among Indonesian children.²³ In a cross-sectional study of 5-year-old Chinese children, blood Mn was similarly associated with worse scores for hyperactivity, conduct problems, and the ADHD index on the CRS. This study also found that blood Mn was associated with decreased concentrations of the neurotransmitters glutamate and glycine, and that altered glycine concentrations mediated the association between Mn and increased Conners scores.²⁸ These findings support the hypothesis that Mn neurotoxicity in children is due, in part, to interference with neurotransmission.

In exploratory analyses, there was evidence of sex-specific associations in all three exposure periods. In the prenatal period, tooth Mn was protective of reported inattention only in males. This is similar to findings from the CHAMACOS study of Mexican–American children, in which prenatal Mn was associated with decreased scores on the ADHD Confidence Index of the continuous performance test-II in males only.¹⁶ In the postnatal period, we found that Mn was also protective of parent-reported attention and hyperactivity, but in females. This was in contrast to findings from the CHAMACOS study, in which worse attention scores on the BASC were associated with Mn among females.¹⁶ Consistent with our findings, most studies of childhood Mn exposure, with the exception of one,⁷¹ reported

no modification by sex.^{25,26,29} Sex-specific effects of Mn may be related to sex differences in the influence of Mn transporters on the regulation of Mn uptake across developmental periods.^{66,67}

Although findings were consistent across parent- and teacher-reports for several scales (e.g., inattention), the magnitude and variability of associations differed between parent- and teacher-reports for other scales (e.g., ADHD index), whereby associations were observed with parent-reported but not teacher-reported, scores. This might reflect differences in reporting from parents versus teachers. Although scores on parent- and teacher-reports are moderately correlated (e.g., 0.64 for inattention) in normative samples,⁷³ differences in parent- and teacher-reported scores have been reported in the literature.^{73,74} For example, one study in younger children reported higher (i.e., worse) average scores on the teacher than the parent report,⁷⁴ whereas another among adolescents found that parents reported higher mean scores,⁷³ which was the case in our study as well. Both prior studies concluded that differences in reported scores were likely the result of differing expectations in the home versus the school environment.^{73,74} In school, for example, children may be expected to have better attention and executive function to complete academic tasks. In adolescents, however, scores for teacher-report may be lower (i.e., better) than parental-report because individual teachers tend to spend less time with each student throughout the day than in childhood.⁷³ Differences in reporting likely explain some of the differences in our findings across respondent types, though they do not inherently suggest a lack of validity. Instead, the findings of this study may simply reflect variations in attention-related behaviors for different environments.

It should further be noted that our findings, particularly for the prenatal period, were not consistent across all parent- and teacher-reported scales. Although there is evidence in the literature supporting the associations observed in the current analysis, we cannot rule out the possibility that these findings were spurious given our limited sample size. This small sample size reduced the statistical power and precision of effect estimates, in particular for the sex-stratified models, which should be considered exploratory. This study has additional limitations. Given

the subjective nature of the CRS, it is likely that there is some degree of misclassification of the outcome metrics, though we would expect this error to be nondifferential with respect to exposure, and likely create a bias towards the null. We also had limited data on maternal characteristics in pregnancy and on participant characteristics in early life. For example, there may be residual confounding by maternal and early life iron status, given that iron is also an essential nutrient needed for neurodevelopment,⁷⁵ and altered iron status (e.g., deficiency) has been associated with increased Mn biomarker concentrations.⁷⁶ Finally, although we were able to adjust for adolescent blood Pb concentrations, we did not have early life measurements of Pb exposure or other toxic metals, which may have led to further residual confounding.

Our study also has several strengths. We used a tooth biomarker to retrospectively characterize Mn exposure in multiple developmental periods. Although the granularity with which we measured tooth Mn was lower than current capabilities for measuring high-resolution exposure data in tooth biomarkers,^{7,44} our use of the tooth biomarker nonetheless allowed us to quantify prospective associations of Mn exposure with neurodevelopment in three distinct exposure periods. Because Mn is both an essential nutrient and neurotoxicant, understanding how its toxicity may differ in the prenatal, postnatal, and childhood periods is essential for public health interventions. Further, teeth have other benefits over traditional exposure markers (e.g., blood) because they provide an objective, noninvasive, integrated measure of exposure across multiple exposure sources (e.g., air, water, and soil), which is particularly important for our study population, where children were likely exposed to Mn from multiple sources due to their residential proximity to ferroalloy industry.⁶³

Conclusion

In conclusion, we found suggestive evidence that prenatal and postnatal tooth Mn is protective for some attention-related behaviors in adolescence, particularly inattention, which was not observed for Mn levels in childhood. These findings suggest that the timing of exposure is critical when considering the neurotoxicity of Mn in children.

Conflicts of interest

The authors declare that they have no conflicts of interest with regard to the content of this report.

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