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Sex differences in sensitivity to prenatal and early childhood manganese exposure on neuromotor function in adolescents

Yueh-Hsiu Mathilda Chiu[1,2,1], **Birgit Claus Henn**[3], **Hsiao-Hsien Leon Hsu**[1], **Mathew P. Pendo**[4], **Brent A. Coull**[5,6], **Christine Austin**[1], **Giuseppa Cagna**[7], **Chiara Fedrighi**[7], **Donatella Placidi**[7], **Donald R. Smith**[8], **Robert O. Wright**[1,2], **Roberto G. Lucchini**[1,7], and **Manish Arora**[1]

[1]Department of Environmental Medicine and Public Health, Icahn School of Medicine at Mount Sinai, New York, NY, USA

[2]Department of Pediatrics, Icahn School of Medicine at Mount Sinai, New York, NY, USA

[3]Department of Environmental Health, Boston University School of Public Health, Boston, MA, USA

[4]Center for Medicine, Health and Society, Vanderbilt University College of Arts and Science, Nashville, TN, USA

[5]Department of Biostatistics, Harvard T.H. Chan School of Public Health, Boston, MA, USA

[6]Department of Environmental Health, Harvard T.H. Chan School of Public Health, Boston, MA, USA

[7]Department of Medical and Surgical Specialties, Radiological Sciences and Public Health, Section of Occupational Medicine, University of Brescia, Brescia, Italy

[8]Department of Microbiology and Environmental Toxicology, University of California, Santa Cruz, USA

Abstract

Introduction—While studies have suggested that exposure to manganese (Mn) may be associated with neurodevelopment in school-age children, there is limited information on prenatal and postnatal Mn exposures and tremor or motor function in children.

Methods—We measured Mn levels in dentine of shed teeth, representing prenatal, early postnatal, and cumulative childhood exposure windows, from 195 children in Italy. Pursuit Aiming, Luria Nebraska Motor Battery, as well as Tremor and Sway system from Computerized Adaptive Testing System (CATSYS) were administered at 11–14 years old. We examined the relationships of tooth Mn (ln-transformed) with motor function using generalized linear models

[1]Communicating Address. ScD. Department of Environmental Medicine and Public Health, Icahn School of Medicine at Mount Sinai, One Gustave L. Levy Place, Box 1057, New York, NY 10029

and generalized additive models, adjusting for age, sex, and socioeconomic status index. Effect modification by sex was also examined.

Results—We found that higher prenatal Mn was associated with better body stability in boys in a number of sway tests (including mean sway, transversal sway, sagittal sway, sway area, and sway intensity), while Mn was associated with poorer performance in girls on all of these metrics (all p for Mn × sex interaction <0.05). Higher prenatal Mn was also modestly associated with better hand/finger and eye-hand coordination in boys compared to girls in sex-stratified analyses, although interaction models did not reach statistical significance. For tremor, on the other hand, higher early postnatal Mn was associated with increased center frequency in girls (p for interaction<0.01), but increased Mn level at the later postnatal period was associated with increased center frequency in boys (p for interaction=0.01).

Conclusions—This study, which used a direct measure of prenatal and childhood Mn exposure, suggested sex-specific critical windows of early life Mn exposure in relation to neuromotor function in adolescents. The sex-specific associations might be strongest with measures of whole body stability, for which the critical exposure window was during the prenatal period.

Keywords

manganese; tooth biomarker; motor function; prenatal and postnatal exposure; adolescents; sex difference

1. INTRODUCTION

Manganese (Mn) is an essential element necessary for a number of biological processes. It plays crucial roles in healthy body growth, immune system function, and the regulation of metabolism and bone growth (ATSDR 2012; Yoon et al., 2011). However, Mn has also been recognized as a potential neurotoxin when present in excess levels (Kern et al., 2010). The most widely studied neurological health outcome related to Mn exposure is termed manganism, a Parkinson’s-like condition observed in adults with high occupational exposure to Mn (Lucchini et al., 2009; O'Neal and Zheng 2015). The negative neurological health outcomes correlated with Mn overexposure in children include behavioral disinhibition (Ericson et al., 2007), olfactory and motor function (Lucchini et al., 2012), and hyperactivity (Mora et al., 2015). However, investigation of the pediatric health outcomes of Mn effects has also led to inconsistent findings. For example, Ode et al. (2015) found no significant relationship between umbilical cord Mn levels and hyperactivity (Ode et al., 2015). Additionally, there is a lack of knowledge specifically on tremor and motor development in children with prenatal and postnatal Mn exposures. Most previous studies on tremor and neuromotor functions focused on elderly or adult populations.

Much of the inconsistency in findings among studies of Mn as a pediatric neurotoxin might be due in part to the lack of an accepted standard biomarker for Mn levels as well as measurements taken at different exposure time points. However, in recent years dentine in the shed teeth of children has emerged as a novel validated biomarker that has the ability to accurately measure Mn exposure in more precise periods of development (Arora et al., 2012; Arora et al., 2011; Gunier et al., 2015; Gunier et al., 2013). The primary benefit of this novel

biomarker is the ability to determine a timeline of exposure that can be differentiated based on developmental periods.

Further, animal studies have shown potential sex differences in the association between Mn and neurodevelopmental outcomes. Behavioral damage associated with Mn exposure in rats showed a sex-specific response where male rats were more severely impacted, and female rats did not experience as severe motor damage until the dose was increased (Yamagata et al., 2016). However, to date, limited human research has studied the potential for effect modification by sex on the associations between perinatal Mn exposure and neuromotor functions.

To address these research gaps, the goal of this study was to investigate the relationships of early life exposure to Mn, measured in dentine of shed teeth, with motor function in adolescents. Specifically, we considered tooth Mn levels in different exposure windows, including prenatal, early postnatal, and childhood cumulative periods. The major focus was to examine effect modification by sex, and to disentangle the effects on whole body stability (e.g. sway) from finer/local motor skills.

2. MATERIALS AND METHODS

2.1 Study Participants

Participants of the Public Health Impact of Manganese Exposure (PHIME) Study were enrolled from a total of 20 junior high schools through the local public school system in Italy. These schools were located in three communities with different levels of Mn exposure (historical, current and reference areas). Details about the study sites and the recruitment process were described previously (Lucchini et al., 2012). In brief, teachers, parents and children were informed with ad hoc meetings and brochures. Subjects who agreed to participate filled in a screening questionnaire for the evaluation of inclusion and exclusion criteria. The inclusion criteria were: (i) to be born in the study area from resident families living in the study area since the 1970s; (ii) to live in the study area since birth; (iii) to be aged 11–14 years. The exclusion criteria included: (i) pathological conditions potentially affecting neuro-development, including neurological, hepatic, metabolic, endocrine and psychiatric diseases; (ii) consumption of medications with known neuropsychological side-effects; (iii) clinically diagnosed motor deficits of hand and fingers; (iv) clinically diagnosed cognitive impairment and behavioral manifestations; (v) visual deficits not adequately corrected. A total of 720 adolescents were enrolled into the study. In 2013, supplemental funding was obtained to conduct an initiative to assess tooth biomarkers in a subset of these participants. The study design, study aims, and the forms for informed consent had been reviewed and approved by the ethical committees of the local Public Health agencies of the study sites, University of Brescia, and Icahn School of Medicine at Mount Sinai.

2.2 Tooth Collection and Measurement

For this initiative, we contacted children within tooth shedding age to request that they provide their shed deciduous teeth, and a total of n=195 participants were able to provide at least one shed tooth that was suitable for analysis at the time of this study. Incisors that were

free of obvious defects such as caries and extensive tooth wear were analyzed. Detailed analytical methods have been described elsewhere (Arora et al., 2012; Arora et al., 2011). In brief, we first identified the neonatal line (NL), a histological feature formed in deciduous teeth at birth, using light microscopy. With the NL as a reference point, the concentrations and spatial distribution of Mn in different developmental windows were determined using laser ablation inductively coupled plasma mass spectroscopy (LA-ICP-MS) as detailed previously (Arora et al., 2012; Arora et al., 2011). Prenatally formed primary dentine adjacent to the enamel-dentine junction was sampled to obtain *prenatal Mn exposure* information; postnatally formed primary dentine after the NL was sampled to obtain *early postnatal Mn exposure* information, which reflects the exposure at approximately 0–1 years old. Measurements in secondary dentine, formation of which starts after the completion of the tooth root and proceeds at a slower rate as long as the tooth remains vital, were used to estimate *childhood cumulative Mn exposure* from root completion to the time the tooth was shed. All tooth Mn levels were normalized to measured tooth calcium levels (^{55}Mn:^{43}Ca ratio) to provide a measure independent of variations in mineral density. Multiple measurements were taken in prenatal and postnatal dentin, and thus the area under the curve (AUC) of Mn levels across all sampling points was calculated to estimate cumulative Mn exposure during each developmental period. Final Mn exposure values are (^{55}Mn:^{43}Ca AUC) x 10,000 for prenatal and early childhood exposures, and average ^{55}Mn:^{43}Ca for cumulative childhood levels. Values below the detection limit (n=2 for early postnatal and childhood cumulative samples) were assigned half of the lowest value among the samples above the detection limit.

2.3 Motor Function Assessment

The neuromotor test battery was designed based on the review of the tests reported in the Mn neurotoxicity literature (Zoni et al., 2007; Zoni and Lucchini 2013). We assessed two major domains–whole body related postural measurements and hand related motor measurements. Whole body postural sway/stability was assessed using the force plate test of the CATSYS 2000 system (Danish Product Development, Ltd) (Despres et al., 2000). The participants were asked to stand erect on a force platform with eyes opened and with eyes closed, each for 75 seconds. The force plate produces signals from three sensors to provide a map of the position of the force center during the test period, and the change in position of the force center can be observed in a X-Y coordinate system. Hand/finger motor coordination was assessed with the 5 subtasks from the Luria Nebraska Neuropsychological Battery (Golden et al., 1980), which consists of dominant hand clenching, non-dominant hand clenching, alternative hands clenching, thumb-touching of dominant hand, and thumb-touching of non-dominant hand, each lasting 10 seconds. We then calculated the sum and the mean score for each participant based on the scores of these subtasks. Hand dexterity and eye-hand coordination was assessed using the Pursuit Aiming test (Fleishman 1954), in which the participants were asked to use a pencil to place one dot inside each circle with 2 mm diameter on a test sheet that consists of a series of circles following a pattern as quickly as possible for two sessions of 60 seconds each. The number of correct dots and error dots were recorded. Finally, hand resting tremor was assessed using the tremor pen test in the CATSYS 2000 system, in which each participant was asked to hold a light stylus at approximately 10 cm in front of the navel as they would hold an ordinary pen without any

body contact or obstacles. The test was done for 10 seconds with the right hand and then repeated with the left hand. The hand vibrations were recorded and displayed in a time axis plot, and the accelerations were analyzed by methods drawn from vibrations measurements. The main indices considered included tremor intensity (the root mean square of accelerations, recorded in the 0.9 Hz to 15.0 Hz band during the test period; unit is in m/s^2) and center frequency (the median frequency of the acceleration in the 0.9–15 Hz band; unit is in Hz or s^{-1}) (Despres et al., 2000).

2.4 Covariates

Information on the participants' sex, age, dominantly used hand, height, weight, smoking status, frequency of video game play, alcohol consumption, parental education level, and parental occupation was collected by questionnaire administered at the time of neuromotor tests. Each participant's family socioeconomic status (SES) level was calculated using parental education level and parental occupational level as previously suggested by Cesana et al (Cesana et al., 1995). In brief, education was divided in three levels: low (elementary and junior high school), medium (senior high school) and high (degree and post-degree). Occupations were also grouped into three ordinal categories that considered criteria of the International Classification, SES situation of Italy, and variables such as decision latitude and job demand. As the model by Cesana et al was developed in the 1990's, we updated the three occupational levels according to the current Italian socioeconomic, cultural and work conditions. Criteria followed indications from ISTAT (The Italian National Institute for Statistics) and an agreement between five independent social epidemiology researchers. The three categories were: low (e.g., housewife, skilled/unskilled worker, hospital ancillaries, etc.), middle (e.g., clerical workers, teachers, educators, nurses, shop assistant, etc.), and high (e.g., engineer, entrepreneur, tradesman, craftsman, etc.) (Supplemental Material, Table S1). To obtain the final SES index, we combined the level of education and occupation (the higher level between mother and father) to obtain three levels of the SES index: low, medium, and high (Supplemental Material, Table S2) (Cesana et al., 1995). Body mass index (BMI) was calculated by dividing the weight (in kg) by the square of the height (in meter). We also measured the participant's concurrent Mn exposure at the time of neuromotor testing with blood level of Mn. Venous whole blood samples (4 mL) were collected using a 19-gauge butterfly catheter into a Li-Heparin Sarstedt Monovette Vacutainer. All samples were stored at 4 ^{o}C until analyzed at the laboratory facility of the University of Brescia. Manganese measurements in blood were performed by Zeeman graphite furnace atomic absorption spectrometry (Varian SpectrAA) in the Industrial Hygiene laboratory at the University of Brescia, Italy, using the methods previously reported in the first round of the recruitment and afterwards by magnetic sector inductively coupled mass spectrometry (Thermo Element XR ICP-MS) with rhodium and thallium as internal standards (Apostoli et al., 2000; Lucas et al., 2015).

2.5 Statistical Analysis

Among 195 participants who provided their shed teeth, the tooth condition for some participants were not optimal (e.g., caries, attrition, cracks, discoloration), and thus Mn levels for these participants were not available for some developmental periods (n=3, 8, and 10 for prenatal, early postnatal, and childhood cumulative measurements, respectively). We

natural-log transformed the Mn teeth levels (ln-Mn). Extreme values, defined by >(mean +3*SD) of ln-Mn, were excluded from the analyses (n=3 for prenatal, n=2 for early postnatal and childhood cumulative levels). Subsequently, the analyses were conducted in a total of n=194 participants (n=189, 185, and 183 for prenatal Mn, early postnatal Mn, and childhood cumulative Mn, respectively).

Univariate and multivariable-adjusted regression analyses were conducted to examine the associations between the Mn exposure in different developmental time points and child's motor functions. Covariates considered for the main analyses included child's age, sex, SES index, and tooth attrition. Effect modification by sex was examined in sex-stratified analysis as well as in interaction models that included the Mn x sex interaction term. Further, sensitivity analyses were conducted by including additional covariates or potential pathway variables, such as dominant hand used, video game play, BMI, and blood Mn at the time of neuromotor tests. Of note, the frequency of smoking and alcohol consumption was extremely low for our participants (n=0 and n=3, respectively) and thus these two variables were not considered as covariates in our analysis. In addition, we conducted sensitivity analyses by including tooth Mn levels from other developmental time points into the same model among the participants with data on Mn levels from all three periods (n=176). To examine the potential non-linear associations, we also explored the exposure-response relationships using generalized additive models (GAMs) with smooth penalized spline terms for the Mn effects. Most analyses were performed using SAS (version 9.4, Cary, NC); GAMs were implemented in the mgcv package in R (version 3.2.3, Vienna, Austria).

3. RESULTS

Characteristics of the participants included in analyses are summarized in Table 1. Among 194 children included in the analysis, 89 (46%) were boys. The mean age at neuromotor testing was 12±0.9 years old. Children were primarily from middle SES class families (56%), right-handed (92%), non-smokers (100%), and did not consume alcohol (98%). No statistically significant difference was found in any of these variables between boys and girls. The levels of Mn at different developmental periods were also similar between boys and girls (Table 2). Overall, there were no significant correlations among Mn at different periods (i.e., prenatal, early postnatal, cumulative childhood tooth Mn, and blood Mn at adolescence), except that prenatal Mn was marginally correlated with early postnatal Mn (r=0.17, p=0.02).

For analyses on the overall sample that combined boys and girls, we did not find a consistent pattern or statistically significant associations between Mn levels in any of the perinatal periods and measurements of neuromotor functions. However, when examining effect modifications by sex, we found that the associations were significantly different between boys and girls in several motor function domains, as described below.

3.1 Whole Body Postural Sway

In general, we found a clear sex difference in the association corresponding to the prenatal developmental window, which was significant for 5 of the 6 measures of whole body sway with open-eyes (Table 3). We observed significant associations between higher prenatal Mn

exposure and increased opened-eye body sway in girls; on the contrary, higher prenatal Mn levels were associated with decreased opened-eye body sway (protective associations) in boys (Table 3). The interaction between prenatal Mn and sex was statistically significant for all but one index, including mean sway ($p_{interaction}$=0.01), transversal sway ($p_{interaction}$=0.003), sagittal sway ($p_{interaction}$=0.05), sway area ($p_{interaction}$=0.01), and sway intensity ($p_{interaction}$=0.04). The suggestive protective associations for boys were also seen at later postnatal period, althoughless statistically significant than the prenatal period. Of note, no significant sex-specific associations were found for closed-eye sway indices.

3.2 Hand-Related Motor Coordination and Tremor

Table 4 shows the results on the associations between perinatal Mn exposure and hand-related motor measurements, including hand dexterity and eye-hand coordination assessed by Pursuit Aiming test scores, hand/finger coordination assessed by Luria-Nebraska Motor Scale, and hand resting tremor assessed by tremor pen test. Unlike the whole body postural sway, in general the pattern of the sex-specific associations on hand-related motor measurements was less clear and generally non-significant for most indices. Nonetheless, the effect estimates generally more likely to suggest beneficial associations in boys, compared to girls, for Pursuit Aiming test and Luria-Nebraska Motor Scale with prenatal and childhood cumulative Mn levels, whereas there was no clear pattern of sex-specific association with hand tremor across different time points.

Pursuit Aiming test—Higher Mn exposure in prenatal and postnatal periods was associated with more correct dots in boys, compared to girls, in stratified analyses. However, the interaction between Mn exposure and sex was only marginally significant for the childhood cumulative exposure period and number of correct dots ($p_{interaction}$=0.05). On the other hand, higher exposure was associated with more error dots in girls, compared to boys, although none of the interaction models were statistically significant.

Luria-Nebraska Motor Scale—Sex-stratified analyses in general suggested that higher prenatal Mn and later childhood cumulative Mn levels were associated with better performance in boys, compared to girls. Higher prenatal Mn level and childhood cumulative Mn was associated with higher sum score of the 5 subtasks in Luria-Nebraska Motor Scale, especially in boys (β=3.85 for boys v.s. β=1.88 for girls for prenatal Mn; β=4.22 for boys v.s. β=−0.65 for girls for childhood cumulative Mn), while higher early postnatal Mn was associated with worse sum score in girls (β=−0.14 for boys v.s. β=−3.58 for girls), albeit none of these were statistically significant.

Hand Resting Tremor Indices—While no significant sex-specific associations were found for prenatal Mn exposure, early postnatal Mn exposure was associated with increased tremor center frequency in girls, compared to boys ($p_{interaction}$<0.01 for center frequency of right hand tremor). On the contrary, Mn exposure at the later postnatal childhood period was associated with increased center frequency in boys ($p_{interaction}$<0.01 for center frequency of right hand tremor). Of note, the sex-stratified effect estimates of postnatal Mn and center frequency of left hand tremor were generally in the same direction as the effect estimates for right hand tremor (i.e., early postnatal Mn was associated with increased frequency in girls

but childhood cumulative Mn was associated with increased frequency in boys); however, the Mn x sex interactions were not statistically significant.

3.3 Sensitivity Analysis

Sensitivity analysis additionally including Mn exposure from prior time points in the same model, for early postnatal and childhood cumulative models, generally yielded similar findings (Supplemental Material, Table S3). The models additionally adjusted for dominant hand, video game play, and concurrent blood Mn level (which, of note, overall no significant independent associations with neuromotor outcomes nor sex differences were found; Supplemental Material, Table S4) also did not materially change the results. Further, the results from GAMs did not suggest statistically significant non-linear relationships (all the p-values of the spline terms were >0.2).

4. DISCUSSION

To our knowledge, this is the first study to examine sex-specific associations between perinatal Mn exposure, measured by the novel tooth-matrix Mn biomarker to assess exposure at different time points, and comprehensively measured neuromotor functions in adolescents aged 11–14 years old. We observed stark sex differences in the association of Mn with whole body stability, and this difference was primarily linked with prenatal exposure, suggesting that this period might be a critical window of susceptibility. Prenatal Mn exposure was generally associated with better body stability (i.e., less body sway) in boys, whereas prenatal Mn was associated with poorer body stability in girls. For hand resting tremor, early postnatal Mn was associated with increased center frequency in girls, whereas childhood cumulative Mn was associated with increased center frequency in boys. These findings potentially support the sex-specific associations and existence of critical windows depending on the measured domains.

While previous studies have examined the effects of prenatal and/or postnatal Mn on neurodevelopmental outcomes such as behavioral, cognitive and memory functions in children, only few studies have evaluated motor functions. In addition, to our knowledge, no study to date has examined the association between prenatal or early postnatal Mn exposure and whole body stability. In this study, we found that prenatal Mn was statistically significantly associated with better opened-eye body stability among boys, but was associated with increased instability among girls. One previous study by Rugless et al. (2014) has measured Mn levels and body posture at the same time in children aged 7–9 years. They found that higher concurrent hair Mn level was associated with poorer body postural balance for four test indices assessed with several different test conditions; on the other hand, higher concurrent blood Mn level was adversely associated with only one of the four indices assessed, under only one particular condition (closed eye) among the six conditions tested (Rugless et al., 2014). In our study, we also did not find significant associations between concurrent blood Mn level and body sway indices. Although blood biomarker has been widely used in previous exposure studies, it is possible that one-time blood Mn measurement may not reflect the concurrent exposure accurately as some recent data suggested that the half-life of Mn in blood may be relatively short, potentially could be

< 2 hours, with large variations (O'Neal and Zheng 2015). More future studies are warranted to better understand the potential beneficial and/or harmful windows of Mn exposure on body stability during the life course of children beginning *in utero*.

Among the few studies that assessed perinatal Mn exposure and motor functions in children, hand coordination and dexterity were the most commonly measured outcomes, yet the findings varied by study design, measures of outcomes, and age of children. Mora et al. (2015) measured Mn levels in prenatal and early postnatal dentine of shed teeth in children living near agricultural fields treated with Mn-containing fungicides in the Center for the Health Assessment of Mother and Children of Salinas (CHAMACOS) cohort in California and found that higher concentrations of prenatal Mn was associated with improved motor outcomes assessed by Finger tapping test at age 7 years and Luria-Nebraska Motor Scale at age 9 and 10.5 years, but only in boys (Mora et al., 2015). They also found sex differences in the associations between early postnatal Mn and these motor function tests, where in general beneficial associations were found in boys but adverse associations were found in girls. These findings are similar to the results in our study of 11–14 years old adolescents in terms of the direction of sex-specific effect estimates, albeit we did not find statistically significant Mn × sex interaction terms. Other studies that examined the effects of prenatal Mn exposure were mostly in infants or toddlers, in which Bayley Scales of Infant Development (BSID) were most frequently used to measure psychomotor development. Gunier et al. (2015) measured tooth dentine Mn levels and found an inverted U-shaped relationship where both lower and higher concentrations of postnatal Mn were associated with psychomotor development index (PDI) measured by BSID in 6 months old infants, but no associations were found for prenatal Mn or PDI measured at 12 or 24 months of age (Gunier et al., 2015). Chung et al. (2015) reported an inverted U-shaped relationship where both lower and higher concentrations of maternal blood Mn measured at delivery were associated with poorer PDI scores in 6 month old infants in Korea (Chung et al., 2015). On the other hand, Claus Henn et al. (2010) measured early postnatal blood Mn at 12 and 24 months of age in Mexican children and did not find an association with PDI scores assessed contemporaneous with exposure (Claus Henn et al., 2010).

Other cross-sectional studies that measured postnatal Mn exposure and neuromotor functions, other than whole body stability, at the same time in school-age children generally suggested an inverse association or no association. Oulhote et al. (2014) reported a significant linear association between intake of Mn in drinking water and poorer motor function index, derived by a latent variable representing hand dexterity and coordination, in 6–13 year-old children in Canada (Oulhote et al., 2014a). Hernández-Bonilla et al. found suggestive associations between higher blood Mn with poorer motor speed and coordination in children aged 7–11 years in Mexico, although not for hair Mn (Hernández-Bonilla et al., 2011). Our group previously reported that higher concurrent Mn levels measured in soil were significantly associated with poorer motor coordination and hand dexterity in Italian adolescents, although no associations were found with Mn levels measured in blood, hair, air or water. Additionally, increased Mn levels in hair and blood, but not in other exposure matrices, were associated with increased tremor intensity (Lucchini et al., 2012). In contrast to the above studies, Parvez et al. (2011) did not find any associations between blood Mn and motor function in children at 8–11 years of age in Bangladesh (Parvez et al., 2011).

The inconsistency among previous studies may partly be explained by the potential existence of critical exposure windows of Mn on the development of neuromotor functions, given that the studies have measured exposures at different time points and used different exposure matrices that reflect different exposure timings. The use of tooth biomarkers in this PHIME study and the CHAMACOS study conducted by Mora et al. (Mora et al., 2015) and Gunier et al. (Gunier et al., 2015) has enabled the estimation of effects in different exposure windows in prenatal and early childhood periods, in order to ascertain a more complete perspective of the impact of Mn during early life. It has been suggested that Mn levels in pregnant women are significantly higher than that in non-pregnant women, likely due to the essentiality of Mn for neurodevelopment (Oulhote et al., 2014b). On the other hand, chronic exposure to Mn has been linked to the development of a neurological disorder known as manganism characterized by neuromotor deficits resembling that seen in Parkinsonism (Aschner et al., 2009). Therefore, it is possible that Mn exposure during the prenatal period may be beneficial to neuromotor functions in school-age children, as seen in the Mora et al. (2015) and in our study, but for exposure at later childhood the associations may be attenuated or become adverse. Importantly, previous studies have used different matrices to quantify Mn levels (e.g., environmental assessment of Mn in different media or different biomarkers), which may be measuring different exposure pathways and underlying biological uptake.

Further, growing literature has suggested that these associations may be sex dependent. In addition to this study and the study by Mora et al. (2015) that generally found associations between prenatal Mn and improved motor outcomes in older children, as described above, other studies also suggested significant interactions between Mn and sex on neuromotor functions. A significant interaction between early postnatal Mn exposure and sex on poorer PDI scores measured at 6 months old was reported by Gunier et al., in which a significant adverse linear relationship was seen only in girls but not in boys (Gunier et al., 2015). On the other hand, a study in France observed that higher cord blood Mn levels were associated with poorer hand skill scores only in boys, at age 3 years (Takser et al., 2003). Although limited previous studies have measured resting tremor in children, our findings also suggest sex differences such that early postnatal Mn was associated with increased center frequency in girls while the association with later postnatal childhood cumulative Mn was only found in boys. Biological differences in response to Mn may explain differences between boys and girls (Oulhote et al., 2014b). Animal studies have shown that Mn accumulation across body tissues and changes in striatal morphology differ between male and female rodents (Dorman et al., 2004; Madison et al., 2011). Of note, to our knowledge, no studies to date have evaluated the interaction between perinatal Mn exposure and sex on the context of whole body stability, and thus our findings of significant sex differences in the associations between prenatal Mn and body sway indices warrant replication. Moreover, integrating epidemiological findings using analytic methods to more objectively identify sex-specific vulnerable early life windows with knowledge regarding brain and nervous system developmental pathways as well as hormonal and micronutrient milieu involved in perinatal programming of neuromotor functions that may be disrupted in these time periods as summarized in recent reviews (Golding et al., 2014; Pitcher et al., 2006; Sandman et al., 2011) may better inform future mechanistic research in this area. Of note, literature on other

more widely studied neurodevelopmental domains, such as cognitive and behavioral outcomes, has also suggested sex differences in relation to early life Mn exposure, where stronger adverse associations seemed to be more likely to be found in girls, although some studies did not find evidence of effect modification by sex (Coetzee et al., 2016).

Discrepancies among studies may also be partly due to different ranges of exposure levels in different populations, in addition to the potential critical developmental windows as discussed above. It is possible that dentine Mn levels in our study population might be within the range at which Mn acts in a beneficial capacity as a nutrient rather than a neurotoxicant, thus resulting in overall improved outcomes particularly for boys in our study. Of note, both prenatal and early postnatal Mn levels in our study (AUC ^{55}Mn:^{43}Ca $\times 10^4$ IQR=0.34–0.53 for prenatal, and 0.10–0.17 for postnatal) covered a similar range as the range reported in the CHAMACOS study (AUC ^{55}Mn:^{43}Ca $\times 10^4$ IQR=0.38–0.57 for prenatal, and 0.11–0.20 for postnatal), which used the comparable analytic method to quantify Mn levels and also found sex-specific associations on motor functions at a younger age (7–10.5 years old) (Mora et al., 2015).

While this study leveraged the tooth biomarker to measure Mn exposure at different time points and is the first to focus on sex-specific associations with neuromotor functions, we acknowledge several limitations. First, the sample size was relatively small, and thus may have limited statistical power to examine the sex-specific associations. However, we were still able to identify significant interactions between Mn levels and sex especially in body stability. In addition, we conducted multiple comparisons and cannot rule out the possibility that some associations may be due to chance. However, we were careful to point out the patterns in both the magnitude and strength of the associations and effect modification of the measured indices in an overall domain, rather than focusing on isolated effect estimates. Of note, the fact that all the sex-specific associations were in the same pattern when comparing boys to girls for the whole body stability indices in relation to prenatal Mn exposure indicates that this finding of sex difference is not likely prone to a multiple comparison issue. Further, there might be residual or unmeasured confounding in the relationships between Mn exposure and neuromotor outcomes. For example, although none of these adolescents were smokers themselves, comprehensive information on their secondhand smoke exposure (which may be associated with neuromotor function (Yeramaneni et al., 2015)) was not available. Nonetheless, while it is not clear how secondhand smoke may also be associated with our exposure of interest (i.e., prenatal and childhood Mn exposure), which is another criterion for a variable to be a confounder, our analysis did control for SES index (which had previously been linked to secondhand smoke exposure (Orton et al., 2014)) and may in part account for the potential confounding even if it exists. Of note, our study population was fairly homogeneous in terms of age, race, smoking status, alcohol drinking, and dominantly used hand, which reduces the likelihood that observed associations are explained by confounding.

In summary, we found that higher prenatal Mn levels, as measured in deciduous teeth, were associated with better body stability in boys but increased instability in girls. The association may not be as strong when considering early postnatal or childhood cumulative Mn exposures. These findings suggest that there may be time-dependent and sex-specific

associations, and warrants additional research to understand the potential mechanism underlying these complex associations. Future studies examining the sex-specific effects of Mn exposure experienced at different time points on brain development of whole body versus local (e.g., hand) neuromotor functions or voluntary versus involuntary movements may provide insight into this context.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AUC	area under the curve
BMI	body mass index
BSID	Bayley Scales of Infant Development
CATSYS	Computerized Adaptive Testing System
CHAMACOS	Center for the Health Assessment of Mother and Children of Salinas
GAMs	generalized additive models
LA-ICP-MS	laser ablation inductively coupled plasma mass spectroscopy
Mn	manganese
NL	neonatal line
PDI	psychomotor development index
PHIME study	Public Health Impact of Manganese Exposure study
SES	socioeconomic status

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Highlights

- Literature on perinatal Mn exposure and motor function in adolescents is limited.
- We used a novel tooth biomarker to reconstruct prenatal and childhood Mn exposure.
- Sex-specific association between prenatal Mn and neuromotor functions was observed.
- Prenatal Mn was associated with better body stability in boys but instability in girls.

Table 1

Participant characteristics

	All (n=194)		Boys (n=89)		Girls (n=105)	
	n	%	n	%	n	%
Family SES index						
Low	43	22.2	13	14.6	30	28.6
Middle	108	55.7	55	61.8	53	50.5
High	43	22.2	21	23.6	22	21.0
Right hand as dominant hand	178	91.8	80	89.9	98	93.3
Plays video game >1 hour/day	37	19.1	19	21.4	18	17.1
Smoking	0	0	0	0	0	0
Alcohol consumption	3	1.6	2	2.3	1	1.0
Age at neuromotor test (years; mean, SD)	12.0	±0.9	11.9	±0.9	12.1	±0.9
BMI (kg/m^2; mean, SD)	20.4	±3.8	20.2	±3.9	20.5	±3.8

Table 2

Distribution of Mn levels during different developmental periods

	All		Boys		Girls	
	median	**IQR**	**median**	**IQR**	**median**	**IQR**
Prenatal Mn (AUC ^{55}Mn:^{43}Ca ×10^4)	0.43	0.34–0.53	0.45	0.33–0.57	0.42	0.34–0.51
Early postnatal Mn (AUC ^{55}Mn:^{43}Ca ×10^4)	0.13	0.10–0.17	0.13	0.08–0.16	0.13	0.11–0.17
Childhood cumulative Mn (^{55}Mn:^{43}Ca) ×100	0.07	0.06–0.09	0.07	0.06–0.09	0.07	0.06–0.09
Blood Mn at neuromotor test (μg/L)	11.0	9.1–13.0	10.9	8.9–12.4	11.1	9.4–13.6

Table 3

Sex-specific associations between perinatal Mn exposure and opened-eye body sway[a]

	Boys			Girls			*p* for interaction [b]
	β	s.e.	p-value	β	s.e.	p-value	
Prenatal Mn							
Mean sway	−0.80	0.54	0.14	1.06	0.46	0.02	**0.01**
Transversal sway	−0.48	0.31	0.13	0.70	0.26	0.01	**0.003**
Sagittal sway	−0.54	0.46	0.25	0.71	0.41	0.09	**0.05**
Sway area [c]	−0.49	0.19	0.01	0.17	0.15	0.27	**0.01**
Sway velocity	−1.39	0.80	0.09	−0.24	0.63	0.71	0.23
Sway intensity	−0.60	0.40	0.14	0.40	0.29	0.17	**0.04**
Early postnatal Mn							
Mean sway	−0.65	0.46	0.17	−0.09	0.41	0.83	0.45
Transversal sway	−0.30	0.27	0.28	−0.04	0.25	0.88	0.55
Sagittal sway	−0.53	0.40	0.19	−0.03	0.36	0.94	0.44
Sway area [c]	−0.16	0.17	0.35	−0.10	0.14	0.50	0.81
Sway velocity	−0.75	0.68	0.27	−0.28	0.62	0.66	0.56
Sway intensity	−0.30	0.34	0.38	−0.10	0.28	0.72	0.73
Childhood cumulative Mn							
Mean sway	−0.64	0.45	0.16	0.25	0.32	0.44	0.12
Transversal sway	−0.26	0.26	0.32	−0.15	0.20	0.43	0.86
Sagittal sway	−0.53	0.39	0.17	0.45	0.28	0.11	**0.04**
Sway area [c]	−0.11	0.15	0.46	0.02	0.11	0.83	0.53
Sway velocity	−0.19	0.64	0.77	0.35	0.44	0.43	0.58
Sway intensity	−0.32	0.33	0.34	−0.01	0.20	0.97	0.52

[a] All tooth Mn levels were ln-transformed. Multivariable linear regressions adjusted for children's age, SES index, tooth attrition; children's sex was also adjusted in the interaction models.

[b] p-value for ln-Mn × sex interaction term in the interaction models.

[c] Sway area was ln-transformed to reduce heteroskedasticity

Table 4

Sex-specific associations between perinatal Mn exposure and hand-related motor outcomes[a]

	Boys			Girls			
	β	s.e.	p-value	β	s.e.	p-value	*p* for interaction [b]
Prenatal Mn							
Pursuit Aiming Test							
total correct dots	12.53	6.96	0.08	3.65	7.29	0.62	0.33
total error dots	0.41	7.30	0.96	7.75	11.18	0.49	0.57
Luria-Nebraska Motor Scale							
sum score of 5 subtasks	3.85	3.45	0.27	1.88	3.47	0.59	0.60
mean score of 5 subtasks	0.77	0.69	0.27	0.38	0.69	0.58	0.60
Hand Resting Tremor							
Tremor intensity (R)	−0.04	0.11	0.70	−0.06	0.14	0.65	0.95
Tremor intensity (L)	−0.09	0.11	0.39	−0.15	0.12	0.23	0.83
Center frequency (R)	0.23	0.51	0.65	0.38	0.62	0.54	0.74
Center frequency (L)	0.13	0.42	0.76	−0.03	0.44	0.95	0.94
Early postnatal Mn							
Pursuit Aiming Test							
total correct dots	2.64	6.15	0.67	−2.33	6.97	0.74	0.53
total error dots	6.31	6.05	0.30	14.84	10.65	0.17	0.41
Luria-Nebraska Motor Scale							
sum score of 5 subtasks	−0.14	2.84	0.96	−3.58	3.34	0.29	0.35
mean score of 5 subtasks	−0.03	0.57	0.96	−0.69	0.66	0.30	0.36
Hand Resting Tremor							
Tremor intensity (R)	0.01	0.10	0.94	0.16	0.13	0.22	0.40
Tremor intensity (L)	−0.08	0.09	0.38	0.13	0.12	0.27	0.16
Center frequency (R)	−0.56	0.44	0.21	1.51	0.58	0.01	**0.003**
Center frequency (L)	−0.01	0.36	0.98	0.60	0.43	0.16	0.17
Childhood cumulative Mn							
Pursuit Aiming Test							
total correct dots	9.01	5.97	0.14	−7.12	5.55	0.20	**0.05**

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	Boys			Girls			
	β	s.e.	p-value	β	s.e.	p-value	*p* for interaction [b]
total error dots	7.60	6.23	0.23	11.65	8.49	0.17	0.65
Luria-Nebraska Motor Scale							
sum score of 5 subtasks	4.42	2.82	0.12	−0.65	2.66	0.81	0.21
mean score of 5 subtasks	0.88	0.56	0.12	−0.06	0.53	0.91	0.25
Hand Resting Tremor							
Tremor intensity (R)	−0.07	0.10	0.47	−0.11	0.10	0.29	0.70
Tremor intensity (L)	−0.13	0.09	0.18	−0.02	0.09	0.80	0.58
Center frequency (R)	1.11	0.43	0.01	−0.50	0.48	0.29	**0.01**
Center frequency (L)	0.59	0.36	0.10	0.04	0.34	0.91	0.28

[a] All tooth Mn levels were ln-transformed. Multivariable linear regressions adjusted for children's age, SES index, tooth attrition; children's sex was also adjusted in the interaction models.

[b] p-value for ln-Mn × sex interaction term in the interaction models.