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Manganese in teeth and neurobehavior: Sex-specific windows of susceptibility

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ABSTRACT

Background: Manganese (Mn) is an essential element required for growth and development, but higher body burdens have been associated with neurobehavioral decrements in children.
Objectives: We examined whether prenatal or postnatal Mn measured in deciduous teeth was associated with scores on a test of visuospatial learning and memory.
Methods: Deciduous teeth were collected from 142 participants (ages 10–14 years) residing near varied ferro-manganese industry in Italy. Mn concentrations were measured in prenatal and postnatal tooth regions by laser ablation inductively coupled plasma mass spectrometry (ICP-MS). The Virtual Radial Arm Maze (VRAM), an animal-human analogue task, was used to assess visuospatial learning and memory. We used generalized additive, linear and zero-inflated Poisson mixed regression models to estimate associations between prenatal or postnatal Mn concentrations and repeated measures of all four VRAM outcomes: time, distance, working and reference memory errors. Effect measure modification by sex was examined in stratified models.
Results: U-shaped associations between prenatal Mn and VRAM outcomes were observed among girls only (p_{GAMM} = 0.001 to 0.02 in stratified models). Compared to the mid-tertile of prenatal Mn, girls in the highest tertile took 7.7 s [95% CI: − 6.1, 21.5] longer to complete the task, traveled 2.3 maze units [0.1, 4.4] farther, and committed more working and reference memory errors (β for count ratio = 1.33 [1.01, 1.83]; 1.10 [0.98, 1.24], respectively). This association was not observed among boys. In contrast, for postnatal Mn, no significant associations were found, and patterns were similar for boys and girls.
Conclusions: The prenatal period may be a critical window for the impact of environmental Mn on visuospatial ability and executive function, especially for females.

1. Introduction

Evidence for manganese (Mn) as a neurodevelopmental toxicant is mounting; yet our understanding of Mn susceptibility remains limited (Bellinger, 2013). Pediatric epidemiologic studies on health effects of environmental Mn exposure have reported adverse associations with cognition, memory, behavior and motor function (Bouchard et al., 2011; Khan et al., 2011; Lucchini et al., 2012a; Mora et al., 2015; Rahman et al., 2016). However, results have varied across studies, partly due to differences in exposure timing, as specific neurodevelopmental periods may be uniquely sensitive to Mn exposure (Grandjean and Landrigan, 2006). In particular, the prenatal and early postnatal periods may be unique windows of susceptibility for Mn overexposure (Sanders et al., 2015). During pregnancy, Mn absorption is up-regulated, and Mn is actively transported across the placenta from mother to fetus, as well as across the developing blood brain barrier (Aschner et al., 2005). In the postnatal period, infant homeostatic mechanisms of Mn absorption and excretion are underdeveloped to manage excess Mn exposure (Erikson et al., 2007). Although there is an increased nutritional need for Mn in early life (Mistry and Williams,

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2011; National Academy of Science, 2001), an ideal exposure range has not been defined and it is unclear at what level Mn becomes toxic rather than beneficial during this dynamic developmental period (Ljung and Vahter, 2007).

Several prospective studies have reported inverse or "U" shaped associations between Mn exposure in the prenatal or early postnatal period and performance on neurobehavioral tests in early childhood (Chung et al., 2015; Claus Henn et al., 2010; Lin et al., 2013; Yang et al., 2014; Yu et al., 2014). However, most studies assessing effects of prenatal Mn estimated fetal exposure using maternal biomarkers or umbilical cord blood (Gunier et al., 2014; Smargiassi et al., 2002; Zota et al., 2009); yet a single blood Mn spot measurement does not comprehensively capture fetal Mn exposure across the prenatal and postnatal periods. Dentin Mn is a non-invasive, validated biomarker that has been correlated with cord blood Mn (Arora et al., 2011, 2012; Gunier et al., 2014) and allows for retrospective exposure assessment in a cumulative manner. Because teeth accumulate metals and their mineralization forms a daily pattern similar to growth rings, measurement of dentin Mn provides precise exposure information from the beginning of the second trimester when deciduous teeth begin formation until approximately 1 year of age (Arora et al., 2012; Hillson, 1996).

Regions in the central nervous system such as the frontal cortex and extrapyramidal motor regions that subserve a number of cognitive processes, including executive function, visuospatial learning, and memory, are particularly sensitive to Mn homeostasis. With one exception (Mora et al., 2015), most epidemiologic studies that assessed childhood Mn exposure and learning and memory were cross-sectional in design and contained no prenatal or postnatal Mn measurements (Haynes et al., 2015; Hernández-Bonilla et al., 2016; Nascimento et al., 2016; Oulhote et al., 2014a; Torres-Agustín et al., 2013; Wasserman et al., 2011; Wright et al., 2006).

We examined early-life windows of Mn exposure measured in deciduous teeth in relation to a novel neurobehavioral task administered later in childhood among male and female adolescents living near varied ferro-manganese alloy industry. We used the Virtual Radial Arm Maze (VRAM) test, a neurobehavioral animal-human analogue test, to evaluate visuospatial learning and memory. Given that previous studies of associations between Mn and neurobehavior found interactions by sex (Gunier et al., 2015; Bouchard et al., 2011; Riojas-Rodríguez et al., 2010; Menezes-Filho et al., 2014; Torres-Agustín et al., 2013), we hypothesized that Mn associations would differ for girls and boys.

2. Methods

2.1. Study participants

Adolescent participants of the PHIME (Public Health Impact of Manganese Exposure in susceptible populations) study were the subjects for this analysis. Located in northern Italy, this study was designed to investigate associations between Mn exposure from anthropogenic emissions and neurodevelopmental outcomes. Details of the study have been described elsewhere (Lucas et al., 2015; Lucchini et al., 2012b, 2012a). Briefly, PHIME participants include children ages 10–14 years residing in three geographically distinct, but demographically similar, regions in the Province of Brescia, Italy: Bagnolo Mella (BM), an area with currently active ferro-manganese industry that has been in operation since 1970; Valcamonica (VC), where ferro-manganese plants were operating for approximately a century until 2001; and Garda Lake (GL), a tourist area with no history of ferroalloy industry.

We enrolled 720 children from these three regions via informational community and public school session recruitment. For funding reasons, participants were enrolled in two phases: 312 participants were enrolled in the first phase (2007–2010) and 408 were enrolled in the second phase (2010–2014). The inclusion criteria for participants of both enrollment phases were: (1) born to families residing in the study area since 1970; (2) living in the study area since birth; and (3) ages 10–14 years old at time of enrollment. Potential participants were excluded from the study if they (1) had any diagnosed neurological, metabolic, hepatic or endocrine diseases, or clinically evident hand/finger motor deficits; (2) were currently taking any prescription psychoactive drugs or had psychiatric disturbances; (3) had received total parenteral nutrition; or (4) had inadequately corrected visual deficits.

The Virtual Radial Arm Maze (VRAM) was incorporated into the neurobehavioral test battery as part of the second phase only. From among the 408 participants enrolled during the second phase, 402 (99%) completed the VRAM. A convenience sample of naturally shed teeth was collected from 142 (35%) of the 402 participants. Complete exposure and outcome data were available for 142 participants, which comprise the final sample for this analysis. Study protocols were approved by the Institutional Review Boards at the Ethical Committee of the Public Health Agency of Brescia, the University of California, Santa Cruz, and the Icahn School of Medicine at Mount Sinai.

2.2. Manganese exposure measurements

Prenatal and early postnatal Mn exposures in children were estimated using naturally shed deciduous teeth. We collected canines, incisors and molars (first and second) that were shed 6 months prior to or during the study. Analysis methods have been validated and described previously in detail (Arora et al., 2011, 2012; Gunier et al., 2014). Briefly, teeth were washed in an ultrasonic bath of ultrapure Milli-Q water (18.2 Mohm-cm^2) and dried before and after sectioning. Teeth were sectioned on a vertical plane using a fitted diamond-tipped stainless-steel blade. The neonatal line, a histological line delineating pre- and postnatally formed regions of enamel and dentin (Sabel et al., 2008), was identified using confocal scanning laser microscopy (CSLM). Using laser ablation inductively coupled mass spectrometry (LA-ICP-MS; Agilent QQQ 8800 coupled with ESI 193 nm laser ablation unit), thirty Mn measurements were taken throughout the pre and postnatal regions for each tooth and used to calculate an area under the curve (AUC) in order to estimate cumulative Mn exposure during the prenatal and postnatal periods, separately. Because mineral density varies within and between teeth, Mn concentrations were normalized to Ca concentrations (^{55}Mn:^{43}Ca ratio). We used Mn:Ca AUC for the prenatal and postnatal periods separately as our final exposure measurements. The limit of detection was 0.02 μg/g. Laboratory measurements were made by technicians who were blinded to the participants' outcome and demographic information.

2.3. VRAM test procedure

The Virtual Radial Arm Maze (VRAM), a computerized maze task, was administered to assess visuospatial learning, working memory and reference memory (Astur et al., 2004). The VRAM is an adapted version of the Radial Arm Maze (RAM), a task that has previously been successful in assessing Mn, lead (Pb) and chlorpyrifos effects on visuospatial learning and memory in rodents (Astur et al., 2004; Haider et al., 2005; Kern et al., 2010; Levin et al., 2001). This virtual task was recently developed, and to our knowledge, has thus far been used only in adults (Astur et al., 2004, 2005; Goodrich-Hunsaker and Hopkins, 2010). The VRAM and its administration in our population have been described previously (Braun et al., 2012). The prior analysis, conducted on a subset of participants in the present study, reported associations between better VRAM performance and higher scores on IQ subtests (Block Design, arithmetic, digit span and verbal comprehension) and on the California Verbal Learning Test (Braun et al., 2012). The VRAM was administered in a quiet room located in the participant's school using a laptop and a Microsoft Sidewinder joystick (Microsoft Corp, Seattle, WA). The task involves using the joystick to navigate a maze, which is pictured on the screen as a 3D maze situated in a room (Supplemental Fig. S1). The maze consists of eight arms of equal distance, four of which are baited with a visual auditory reward. Using visual cues in the