University of Cincinnati

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I, Adriana Reedy, hereby submit this original work as part of the requirements for the degree of Master of Science in Epidemiology (Environmental Health).

It is entitled:
Effects of Manganese Exposure on Cardiovascular Health in Children

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This work and its defense approved by:

Committee chair: Erin Nicole Haynes, Dr.P.H.
Effects of Manganese Exposure on Cardiovascular Health in Children

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of the College of Medicine

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By

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Abstract

Background

Manganese (Mn) is a trace element found naturally in the environment, and is essential for normal growth and development. Mn is necessary for metabolic functions including those involved in energy metabolism, activation of certain enzymes, nervous system function, bone and connective tissue development, immunological system function, reproductive hormone function, cardiac function, and is an antioxidant that provides cellular protection from damaging free radicals. Previous research has demonstrated the dual role of Mn as an essential nutrient and neurotoxicant. This dual role of Mn on cardiovascular function has not yet been evaluated. The studies that have investigated the impact of Mn on cardiovascular health often focused on adult occupational exposures, where the directionality of effects on the cardiovascular system varied from study to study. At present, there is an absence of literature that describes the influence of Mn on cardiovascular function in children.

Objective

The purpose of this pilot study is to evaluate the relationship between Mn exposure, as measured by blood and hair manganese (Mn) concentration, and cardiovascular function in children ages 7-9 years.

Design and Methods

The Communities Actively Researching Exposure Study (CARES) is a community-based participatory research study that was undertaken to address community concern about Mn
exposure in children residing near a ferromanganese refinery. Children ages 7-9 years were enrolled in CARES if they resided in Marietta or Cambridge, Ohio and their surrounding communities while their mother was pregnant with the child and remained in the area since their birth. Blood and hair Mn concentrations, blood lead (Pb) levels, serum ferritin, and serum cotinine were analyzed. Cardiovascular function was determined by the child’s systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate. Multivariable linear regression analyses were conducted to evaluate the association between Mn and cardiovascular outcomes.

Results

Of the 84 participants within this substudy, 36 were female and 48 male. Mean blood Mn was 9.78 µg/L (SD=2.28) and the geometric mean of hair Mn was 402.26 µg/g (SD=2.34). Mean heart rate was 80.55 beats per minute (SD=9.57), the mean of SBP was 93.57 mm/Hg (SD=6.72), and the mean of DBP was 59.50 mm/Hg (SD=4.82). In multiple regression analysis, it was found that blood Mn was significantly (p=0.02) and negatively associated with SBP after adjusting for age and Body Mass Index (BMI). There was a non-linear association that was approaching statistical significance (p=0.09) between blood Mn and DBP after adjusting for age, BMI, and serum ferritin.

Conclusions

The negative association found between blood Mn and SBP may indicate that with increasing Mn exposure there is a decline in SBP; however, the nonlinear relationship that was
found in blood Mn levels and DBP may demonstrate that extreme low or high doses of Mn exposure may interrupt and perhaps suppress the natural rhythm of DBP. Further research to assess the effects of chronic environmental Mn exposure on cardiovascular function in children and adults is warranted.
Acknowledgments

I would first like to acknowledge and thank all my committee members for providing insight, guidance, support, and for being patient with me throughout this endeavor. Thank you to Heidi Sucharew for being tolerant of my obsession over the smallest statistical details, for helping me understand the methodologies and statistical tactics used in the analysis, and for helping me to accurately report my findings. Thank you to Nick Newman for making me take a step back from the numbers to ensure I understood the biological plausibility of the associations I was seeing and for explaining in layman’s terms how the cardiovascular system works in the pediatric population. Furthermore, a special thank you to Erin Haynes, my advisor and committee chair, for letting me use the rich CARES data for my thesis, for keeping me on track throughout this process, and for her tremendous support and guidance in my transition into Epidemiology.

I would also like to thank Pierce Kuhnell for all his help in answering my annoying detailed SAS questions, assisting me in understanding the CARES data set, and for letting me vent to him about my latest arguments I was having with the SAS program. Thank you to my parents for their constant encouragement and for listening to me rant about the most recent dreams I was having in R or SAS code. Lastly, I would like to thank my sister and nieces for helping me maintain a healthy prospective on life, and for bringing a smile to my face after a long night in front of a computer.
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1) Introduction

Manganese (Mn) is a trace element found naturally in the environment, and is essential for normal development and maintaining proper function and regulation of numerous biochemical and cellular reactions. Mn is necessary for metabolic functions including those involved in protein and energy metabolism, activation of certain enzymes, nervous system function, immunological system function, reproductive hormone function, cardiac function, and is an antioxidant that provides cellular protection from damaging free radicals. Furthermore, Mn plays a part in the formation of bone and connective tissue development, the regulation of blood sugars, absorption of calcium, blood clotting factors, and metabolism of fats and carbohydrates. While ingested Mn is under strong homeostatic regulation, inhaled Mn can bypass the biliary excretion mechanism, is capable of crossing the blood brain barrier via several pathways including facilitated diffusion and active transport, and is able to directly enter the systemic circulation.

Overexposure to Mn that exceeds the homeostatic range may lead to various toxicities. To date, the studies of Mn exposure in children have focused mainly on how it may lead to deficits in cognitive function, motor impairment, and its impact on behavioral functions. High-level exposures to Mn can result in Manganism, an extrapyramidal movement disorder that is similar to Parkinson’s disease. In comparison, low-level exposures to Mn in children have been found to be associated with unfavorable neurodevelopmental outcomes in intelligence and memory and have also been correlated with hyperactive behavior and adverse motor function. While Mn neurotoxicity has been well recognized and documented, the effect of Mn on the cardiovascular system has received less attention.
Previous studies that have investigated the effects of Mn on heart function have focused on adult occupational exposures \(^{18-22}\). Therefore, this study was undertaken to address the research gap regarding the cardiovascular impact of Mn exposure in children.

Cardiovascular function may be determined by arterial blood pressure, which can be measured using systolic blood pressure (SBP) and diastolic blood pressure (DBP). The literature is divided regarding the effect of Mn exposure on blood pressure. Some studies have shown that there is a decrease in DBP with increasing levels of Mn concentrations while other studies have indicated that there is significant decrease in SBP with increasing Mn exposure \(^{18,21}\). On the contrary, there are a few studies that demonstrate an increase in blood pressure \(^{19,22}\). For example, researchers studying hypertension observed that with the doubling of blood Mn, there was increased risk of hypertension that was 1.83 fold in women, and 1.57 fold in men and 1.57 fold in all participants \(^{22}\). One theory proposed regarding Mn and its effect on the cardiovascular system is that Mn may selectively interfere with parasympathetic nerve function \(^{23}\).

In summary, although many studies demonstrate an effect of Mn exposure on cardiovascular function, the directionality of this effect is somewhat inconclusive. In addition, since existing research on the effects of Mn exposure on cardiovascular function was conducted in adult occupational exposures, further investigation of the possible cardiovascular effects in children is warranted.

The Mid-Ohio River Valley harbors many industrial corporations, including the longest running Mn refinery in America, Eramet Marietta Industries (EMI), Inc. The EMI Mn refinery
is located in Marietta, Ohio and is the world’s leading producer of Mn alloys for the steel industry and other Mn-based products like batteries, fertilizers and animal feeds \(^24\). This more than 50-year old plant leads the nation in fugitive airborne Mn emissions, and it has been reported that on average, there is 450,000 lbs./year of Mn fugitive air emissions since 1998 \(^25\). Due to the community’s growing concern of the possible neurological effects of ambient Mn exposure in children, a community based participatory research study was undertaken\(^24\). The Marietta Community Actively Researching Exposure Study (CARES) aim was to examine the neurological effects of chronic manganese (Mn) exposure in children who live in a rural Appalachian community that is home to a ferromanganese refinery \(^24\). Thus far, the Marietta CARES has provided insight into the effects of Mn exposure on postural balance and on the assessment of personal Mn exposure. In addition, CARES investigators have also published findings on the development of a bidirectional academic-community partnership in an environmental health research project \(^24,26–29\).

As part of this larger cross sectional study on Mn exposure, this pilot study was employed to investigate whether chronic Mn exposure in children has any effects on cardiovascular function. It was hypothesized that children who exhibited elevated manganese levels outside the homeostatic range would display early signs of abnormal cardiovascular function as measured by changes in heart rate and blood pressure, and that this association would remain after controlling for other covariates that have been shown to influence heart function or measurements of Mn concentrations. More specifically, based on the literature that has demonstrated a possible effect of Mn on the parasympathetic system, it was postulated that increased Mn exposure would be associated with a decrease in both systolic
blood pressure (SBP) and diastolic blood pressure (DBP) and that this association would remain after controlling for such as age, sex, Body Mass Index (BMI) as well as other exposures such as lead, cotinine, and ferritin levels.

2) Methods

2.1 Obtainment of Data and Study Design

Children ages 7-9 years were enrolled in larger cross-sectional study CARES if they resided in Marietta or Cambridge, Ohio and their surrounding communities while their mother was pregnant with the child and remained in the area since their birth. A small subgroup of participants (n=84) that participated in the larger CARES (N=407) had their blood pressure and heart rate measured. All participants who had data on blood pressure and heart rate were used for this analysis. In order to determine if Mn exposure influenced blood pressure in children of this sample, an evaluation of the association between Mn exposure, as measured by hair and blood Mn, and blood pressure was conducted while controlling for other environmental exposures such as secondhand tobacco smoke and blood lead level. An additional investigation was conducted to assess whether increased manganese exposure was associated with heart rate irregularities in children, while also controlling for secondhand tobacco smoke and blood lead level.
2.2 Cardiovascular Health Assessment

Cardiovascular function was measured using each child’s blood pressure and heart rate. Heart rate was determined by using a stethoscope to listen to the child’s pulse to count the number of heartbeats and was reported in beats per minute (bpm). Blood pressure was measured using a computerized blood pressure cuff and was expressed as a ratio between systolic blood pressure (mmHg) over diastolic blood pressure (mmHg). Systolic blood pressure (SBP) measures the pressure in the arteries when the muscle contracts (heart beat), diastolic heart pressure (DBP) measures the arterial pressure when the heart relaxes between beats.

In children, blood pressure norms vary according to age, sex and height. Blood pressure percentile is determined in order to establish levels of normotensive, pre-hypertension, and hypertension (stages I and II) \(^{30}\). Blood pressure percentiles for this study were calculated using the child’s age, height percentile, and gender. Reference tables created from normative blood pressure data obtained in the NHANES 1999–2000 that were stated in “The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents,” were utilized to determine blood pressure percentiles for this sample \(^{30}\). More specifically, the regression coefficients, intercepts, and standard deviations for both boys and girls that were used to determine blood pressure percentiles for these reference tables were also provided in the report and were used to calculate SBP and DBP z-scores for this data set. The calculation of height percentiles were also needed in order to further compute the SBP and DBP percentiles for this data set. Height percentiles were obtained using the age and height growth charts for girls and boys that are recommended by the Center for Disease Control (CDC) for this age group.
The classification of hypertension status is often determined by SBP alone. Therefore, only the calculated SBP percentiles were used to designate each participant as either hypotensive, normotensive, pre-hypertensive, or hypertensive.

2.3 Specimen Collection and Analysis

**Hair:** Approximately 20 strands of hair were collected from the occipital region, cut with ceramic scissors as close to the scalp as possible. The hair was at least 1 cm in length for analysis. Long hair was trimmed to 6 cm and taped towards the non-scalp-side end of the hair shaft onto an index card with an arrow pointing in the direction of the scalp end on the index card. The card with the taped hair sample was placed into a pre-labeled envelope and stored at room temperature until shipped to the Channing Trace Metals Laboratory, Brigham and Women’s Hospital, Harvard School of Public Health for analysis in Boston, Massachusetts. The samples were first washed in a 1% Triton™ X-100 solution and then digested using concentrated HNO₃. Acid digestates were then analyzed by ICP-MS. The method detection limit (MDL) for Mn in hair was <2 ng/g.

**Blood metals:** A trained phlebotomist collected whole blood from each child following proper preparation and collection protocols that were enlisted to safeguard the samples from contamination. Venous whole-blood specimens were collected from the antecubital vein in 3-mL purple top (K₂EDTA) tubes certified by the analyzing laboratory for trace element analysis. Specimens were refrigerated at 5°C until they were shipped to the Laboratory of Inorganic and Nuclear Chemistry at the New York State Department of Health’s (NYS DOH) Wadsworth Center for analysis in Albany, New York. Blood specimens were analyzed for Mn using graphite furnace
atomic absorption spectrometry (GFAAS, PerkinElmer® Model 4100 ZL) equipped with a transversely-heated graphite atomizer and a longitudinal Zeeman background correction system (PerkinElmer® Life and Analytical Sciences, Shelton, CT) using previously described method and quality-control measures (Praamsma et al., 2012) 32. The MDL for Mn in blood was 1.5 µg/L. Blood Pb was determined by inductively coupled plasma-mass spectrometry (ICP-MS) 33 using a method optimized and validated for biomonitoring purposes as described elsewhere 34,35. A PerkinElmer® Sciex ELAN DRC Plus ICP-MS instrument equipped with a Burgener Teflon MiraMist® nebulizer (Burgener Research Inc., Mississauga, ON, Canada) and a Cinnabar spray-chamber (Glass Expansion, West Melbourne, VIC, Australia), and operated in standard mode was used for all blood Pb measurements. The between-run precision for blood Mn based on IQC data was 7.0% RSD at 8.5 µg/L, and 2.7 % RSD at 23.1 µg/L. The between-run precision for blood Pb was 2.8% at 3.5 µg/dL. The MDL for blood Pb was 0.04 µg/dL.

**Serum:** The serum separated from the child’s blood sample provided data on ferritin levels which was obtained to control for any possible influence iron may have on blood Mn concentrations that may be due to their shared transport and metabolic pathways and similarities in systemic absorption 36–38. The serum was further analyzed for cotinine concentrations. Serum cotinine is a reliable measure of exposure to secondhand tobacco smoke considering that it has a longer half-life than blood nicotine, it does not require adjustment for hydration differences among individuals, and it provides a more uniform matrix measurement than urine 39–41. Serum cotinine levels were also measured at the Wadsworth Center, using a high throughput 96-well plate format sample preparation, then analyzed using an isotope dilution, liquid chromatography/tandem mass spectrometry (LC/MS/MS) method.
The method used is a modification of techniques used by the Centers for Disease Control and Prevention for the National Health and Nutrition Examination Survey (NHANES) \(^{42}\) and New York State Wadsworth Laboratories for the NYC Health and Nutrition Examination Survey studies \(^{43}\). Each serum specimen was equilibrated with a trideuterated cotinine internal standard solution and extracted using a 96-well Bond Elut Plexa Solid Phase Extraction (SPE) plate (Varian, Palo Alto, CA). The acetonitrile sample extract was taken to dryness, reconstituted in 96%/4% acetonitrile/water solution, and analyzed by LC/MS/MS using electrospray ionization. The instrumental systems comprised a Shimadzu Prominence LC with a Phenomenex Luna Hilic (100 x 2.00 mm) column and an AB Sciex API 4000 triple quadrupole mass spectrometer operated in ESI positive ion mode using multiple reaction monitoring (MRM) detection. Three QC pools were used at low, medium and high target cotinine concentrations of 0.173, 1.61 and 15.7 ng/mL. Final results were blank corrected using the mean batch blank value. The MDL for this method was 0.05 ng/mL cotinine in serum.

**2.4 Child Characteristics and Demographics**

The child’s height and weight were measured during their visit to the study site and additional information on gender and medication use was collected at the time of the heart function assessment. BMI was calculated from weight (kg) and height (cm) measurements (weight/(height)\(^2\)) and the percentiles were calculated using SAS code provided by CDC website that was based upon growth chart data for this age group \(^{31}\).

Furthermore, any data on common children’s medications that were taken chronically and that have been shown in the literature to influence blood pressure measurements (such as
stimulants used to treat Attention Deficit Hyperactive Disorder or asthma medications) were collected at the time of assessment.

2.5 Data Analysis

For continuous measurements, data distributions were assessed for normality and appropriate transformations were applied to highly skewed distributions. The natural log transformations of hair Mn, blood Pb and cotinine were applied due to the data’s deviations from normality and their corresponding geometric means were calculated. Also, a correlation matrix was created to look for any possible associations between the covariates and the outcome variables. T-tests were conducted to determine if there was a significant mean difference of medication usage (yes or no) for each heart function variable. The medication variable was only introduced in further regression modeling if the t-test was statistically significant for that specific measure of cardiovascular function.

Previous studies both by CARES investigators and using other datasets have indicated an inverted U-shaped exposure/response relationship for Mn and multiple other adverse health outcomes and is best described using polynomial regression models\(^5,44-47\). Scatterplots and unadjusted polynomial regression models were used to assess the need for quadratic terms in evaluating the association between Mn levels and outcome measures. Any significant quadratic term found in the unadjusted models was later used in the multivariable linear regression models.

Next, multivariable regression analysis was performed to evaluate the association between Mn and cardiovascular function. The covariates and potential confounders that were entered in the adjusted models were: blood lead, cotinine, and serum ferritin levels, as well as,
age, sex, and BMI. A stepwise elimination approach was employed in order to ascertain a parsimonious model. Variables were retained in the model if they were found to be significant at p<0.05 or if elimination from the model changed the beta coefficient for the Mn effect by more than 10%.

3) Results

3.1 Cardiovascular health assessment results

Mean heart rate was 80.55 bpm (SD=9.57), the mean SBP was 93.57 mm/Hg (SD=6.72), and the mean DBP was 59.50 mm/Hg (SD=4.82). Based upon SBP percentiles, none of the participants were within the prehypertension percentile range (90th to <95th) nor in the hypertension percentile range (>95th) based on their age, sex, and height percentile 30. Moreover, 96.43% of the sample was in the normotensive percentile range (5th-90th) and only 3.57% were in the hypotensive range (<5%) 30. Furthermore, 79.76% of the subjects were below the midpoint of normal (50th).

3.2 Child Demographics and Exposure Results

Demographic characteristics of the subjects included in the analysis are summarized in Table 1. Of the 84 participants within this substudy, 36 were female and 48 male. Age and sex adjusted BMI percentiles indicated that 10.71% of the sample were categorized as overweight (85th to <95th percentile) and 20.24% of the participants were considered obese (>95th percentile) 48.
The mean of blood Mn for this substudy was 9.78 µg/L (SD=2.28) and the geometric mean of hair Mn was calculated to be 402.26 µg/g (SD=2.34). Mean serum ferritin was 10.10 ng/mL (SD=19.13) and the geometric means of blood lead and cotinine were 0.78 µg/L (SD=1.50) and 0.14 ng/mL (SD=6.65), respectively.

Chronic use of stimulants or asthma medications was categorized as either a “yes” or “no” for the medication variable. A statistically significant difference in the mean “yes” and mean of “no” categories was found in the medication variable for the outcome variable heart rate. Therefore, the medication variable was only taken into consideration in the heart rate models for the multivariable linear regression analysis. Since only 14 participants reported the chronic use of these medications, a sensitivity analysis was conducted by removing these 14 children from the analytic dataset and rerunning the heart rate model. As a result, the overall model results did not change and the effects of hair Mn was still not significant.
Table 1. Descriptive Statistics of the Study Sample (*n* = 84)

<table>
<thead>
<tr>
<th>Variables</th>
<th>n</th>
<th>Mean ± SD</th>
<th>Range</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dependent variables</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic Blood Pressure (mm/Hg)</td>
<td>84</td>
<td>93.57 ± 6.72</td>
<td>80.00 – 108.00</td>
<td></td>
</tr>
<tr>
<td>&lt;5&lt;sup&gt;th&lt;/sup&gt; Percentile (n %) (Hypotensive)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3</td>
<td>3.57</td>
<td></td>
<td>96.43</td>
</tr>
<tr>
<td>5&lt;sup&gt;th&lt;/sup&gt;-90&lt;sup&gt;th&lt;/sup&gt; Percentile (n %) (Normotensive)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>81</td>
<td>96.43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diastolic Blood Pressure (mm/Hg)</td>
<td>84</td>
<td>59.50 ± 4.82</td>
<td>48.00 – 70.00</td>
<td></td>
</tr>
<tr>
<td>Heart rate (Beats Per Minute)</td>
<td>84</td>
<td>80.55 ± 9.57</td>
<td>63.00 – 111.00</td>
<td></td>
</tr>
<tr>
<td><strong>Demographic characteristics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (Years)</td>
<td>84</td>
<td>8.32 ± 0.99</td>
<td>7.00 – 10.00</td>
<td></td>
</tr>
<tr>
<td>Sex (% Girls)</td>
<td>84</td>
<td>42.86</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height (Centimeters)</td>
<td>84</td>
<td>130.58 ± 8.05</td>
<td>116.21– 152.72</td>
<td></td>
</tr>
<tr>
<td>Weight (Kilograms)</td>
<td>84</td>
<td>31.06 ± 9.5</td>
<td>17.69 – 64.23</td>
<td></td>
</tr>
<tr>
<td>Body Mass Index (Weight/ Height&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>84</td>
<td>17.96 ± 3.99</td>
<td>11.60 – 35.30</td>
<td></td>
</tr>
<tr>
<td>85&lt;sup&gt;th&lt;/sup&gt; to &lt;95&lt;sup&gt;th&lt;/sup&gt; Percentile (n %) (Overweight)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9</td>
<td>10.71</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;95&lt;sup&gt;th&lt;/sup&gt; Percentile (n %) (Obese)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17</td>
<td>20.24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic Use of Stimulant or Asthma Medications (N=yes, % yes)</td>
<td>14</td>
<td>16.67</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Biomarkers</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood manganese (µg/L)</td>
<td>63</td>
<td>9.78 ± 2.38</td>
<td>5.70 – 16.50</td>
<td></td>
</tr>
<tr>
<td>Serum ferritin (ng/mL)</td>
<td>59</td>
<td>40.10 ± 19.13</td>
<td>9.00 – 83.00</td>
<td></td>
</tr>
<tr>
<td><strong>Geometric mean of biomarkers</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hair manganese (µg/g)</td>
<td>80</td>
<td>402.26 ± 2.34</td>
<td>60.65 – 3679.04</td>
<td></td>
</tr>
<tr>
<td>Blood lead (µg/dL)</td>
<td>63</td>
<td>0.78 ± 1.50</td>
<td>0.36 – 2.03</td>
<td></td>
</tr>
<tr>
<td>Cotinine (ng/mL)</td>
<td>63</td>
<td>0.14 ± 6.65</td>
<td>0.008 – 10.10</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> SBP percentiles categories were based upon the National Heart, Lung, and Blood Institute’s Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents.<sup>30</sup>

<sup>b</sup> BMI categories were based upon the Barlow (2007)<sup>48</sup>
3.4 Unadjusted Regression Models

The representation of blood Mn as a quadric term was entered into the unadjusted regression models for the heart function variables. Table 2 shows the unadjusted models for each heart function variable and Mn exposure. The only model that showed any significant associations was the one containing DBP with the quadratic term of blood Mn and the linear term of blood Mn. The quadratic term of blood Mn showed a significant negative association ($\beta = -0.17$, $SE=0.08$, $p=0.04$) with DBP and a significant positive association with the linear term for blood Mn ($\beta= 3.68$, $SE=1.74$, $p=0.04$) with DBP. Furthermore, the unadjusted model of SBP showed that blood Mn had a negative regression coefficient of -0.67 ($SE=0.36$), and was approaching statistical significance ($p=0.07$). The $R^2$ value for the SBP and DBP models with blood Mn was only 0.05 and 0.07, respectively. The heart rate model showed no association with blood Mn ($\beta=0.17$, $SE=0.49$, $p=0.74$). None of the models incorporating Ln (hair Mn) as the exposure variable were significantly associated with any cardiovascular function outcomes.
Table 2. Unadjusted Regression Models

<table>
<thead>
<tr>
<th>Unadjusted Model:</th>
<th>B(SE)</th>
<th>95% Confidence Interval</th>
<th>P-Value</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP (n=63)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood Mn</td>
<td>-0.67(0.36)</td>
<td>-1.40 to 0.05</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>Model 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP (n=80)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ln(Hair Mn)</td>
<td>1.44(0.88)</td>
<td>-0.31 to 3.19</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>Model 4&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DBP (n=63)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood Mn</td>
<td>3.68 (1.74)</td>
<td>0.21 to 7.16</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Blood Mn * Blood Mn</td>
<td>-0.17 (0.08)</td>
<td>-0.33 to -0.01</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Model 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DBP (n=80)</td>
<td></td>
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</tr>
<tr>
<td>Ln(Hair Mn)</td>
<td>0.53 (0.64)</td>
<td>-0.75 to 1.81</td>
<td>0.41</td>
<td></td>
</tr>
<tr>
<td>Model 6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart Rate (n=63)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Blood Mn</td>
<td>0.17 (0.49)</td>
<td>-0.82 to 1.15</td>
<td>0.74</td>
<td></td>
</tr>
<tr>
<td>Model 7</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Heart Rate (n=80)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ln(Hair Mn)</td>
<td>0.13 (1.26)</td>
<td>-2.37 to 2.64</td>
<td>0.92</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Only Model 4 contained a quadratic term of blood Mn.

3.4 Adjusted Regression Models

Table 3 displays the multivariable linear regression models. In Model 1, blood Mn was found to have a significant negative association (p=0.02) with SBP (β= -0.77 SE=0.31), after controlling for age and BMI. The model with blood Mn and SBP had the highest adjusted R² of all models with a value of 0.30. Model 3 of DBP with the quadratic term of blood Mn resulted in serum ferritin (p=0.05) and BMI (p=0.002) being statistically significant predictors of DBP. Additionally, the quadratic term of blood Mn was found to be approaching significance in its
association with DBP (p=0.09) with a negative coefficient of -0.14 (SE=0.08) and a positive linear coefficient of 3.00 (SE=1.76, p=0.09), with an adjusted R² value of 0.25.

In Model 2, Ln(hair Mn) had a non-significant association with SBP (β=1.60, SE=1.01, p=0.12), and the model displayed an adjusted R² value of 0.27. Additionally, Models 4 and 6 show that Ln(Hair Mn) had non-significant associations with DBP (β=0.97, SE=0.80, p=0.23), and heart rate (β=0.34, SE=1.63, p=0.84). Furthermore, the models with Ln(hair Mn) for DBP and heart rate had small adjusted R² values of 0.15 and 0.02, respectively.

In conjunction with the results found above, it should be noted that both BMI and age had a positive significant association in both SBP models containing blood Mn and Ln(hair Mn). Also, BMI alone had a positive significant association in the DBP models containing blood Mn and Ln(hair Mn). On the contrary, BMI had a non-significant negative association (p=0.07) in the model of heart rate containing blood Mn.
Table 3. Adjusted Regression models

<table>
<thead>
<tr>
<th>Adjusted Model:</th>
<th>B(SE)</th>
<th>95% Confidence</th>
<th>P-Value</th>
<th>Adjusted R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td></td>
<td></td>
<td></td>
<td>0.30</td>
</tr>
<tr>
<td>SBP (n=63)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood Mn</td>
<td>-0.77 (0.31)</td>
<td>-1.39 to -0.15</td>
<td>0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>0.86 (0.23)</td>
<td>0.39 to 1.32</td>
<td>0.0005&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>1.97 (0.74)</td>
<td>0.49 to 3.45</td>
<td>0.0099&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Model 2</td>
<td></td>
<td></td>
<td></td>
<td>0.27</td>
</tr>
<tr>
<td>SBP (n=80 )</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ln(Hair Mn)</td>
<td>1.60 (1.01)</td>
<td>-0.41 to 3.62</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>0.84 (0.25)</td>
<td>0.35 to 1.33</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>1.91 (0.77)</td>
<td>0.36 to 3.46</td>
<td>0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Ln(Blood Pb)</td>
<td>1.72 (1.99)</td>
<td>-2.26 to 5.70</td>
<td>0.39</td>
<td></td>
</tr>
<tr>
<td>Model 3&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td>0.25</td>
</tr>
<tr>
<td>DBP (n= 59)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Blood Mn</td>
<td>3.00 (1.76)</td>
<td>-0.53 to 6.53</td>
<td>0.094&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Blood Mn * Blood Mn</td>
<td>-0.14 (0.08)</td>
<td>-0.31 to 0.02</td>
<td>0.088&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>0.55 (0.16)</td>
<td>0.22 to 0.88</td>
<td>0.002&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.86 (0.55)</td>
<td>-0.25 to 1.96</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>Serum Ferritin</td>
<td>0.06(0.03)</td>
<td>0.000021 to 0.12</td>
<td>0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Model 4</td>
<td></td>
<td></td>
<td></td>
<td>0.15</td>
</tr>
<tr>
<td>DBP (n=61)&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ln(Hair Mn)</td>
<td>0.97 (0.80)</td>
<td>-0.63 to 2.56</td>
<td>0.23</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>0.53 (0.17)</td>
<td>0.18 to 0.88</td>
<td>0.004&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>1.11 (0.57)</td>
<td>-0.03 to 2.24</td>
<td>0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Ln(Cotinine)</td>
<td>-0.28 (0.32)</td>
<td>-0.91 to 0.36</td>
<td>0.39</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>-0.74 (1.21)</td>
<td>-3.16 to 1.68</td>
<td>0.54</td>
<td></td>
</tr>
<tr>
<td>Model 5</td>
<td></td>
<td></td>
<td></td>
<td>0.03</td>
</tr>
<tr>
<td>Heart Rate (n=63 )</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood Mn</td>
<td>0.21 (0.48)</td>
<td>-0.76 to 1.18</td>
<td>0.66</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>-0.66 (0.37)</td>
<td>-1.40 to 0.07</td>
<td>0.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Ln(Blood Pb)</td>
<td>-2.09 (2.86)</td>
<td>-7.82 to 3.65</td>
<td>0.47</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>-1.44 (1.15)</td>
<td>-3.73 to 0.86</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td>Model 6</td>
<td></td>
<td></td>
<td></td>
<td>0.02</td>
</tr>
<tr>
<td>Heart Rate (n=61)&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ln(Hair Mn)</td>
<td>0.34 (1.63)</td>
<td>-2.93 to 3.60</td>
<td>0.84</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>-1.76 (1.19)</td>
<td>-4.13 to 0.62</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>-0.58 (0.38)</td>
<td>-1.34 to 0.18</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>Ln(Blood Pb)</td>
<td>-1.34 (3.06)</td>
<td>-7.47 to 4.78</td>
<td>0.66</td>
<td></td>
</tr>
<tr>
<td>Ln(Cotinine)</td>
<td>-0.23 (0.68)</td>
<td>-1.59 to 1.12</td>
<td>0.73</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>p<0.05

<sup>b</sup>p<0.10

<sup>c</sup>Only Model 3 had a significant quadratic term

<sup>d</sup>N=61 when both Ln(Hair Mn) and Ln(Cotinine) were both used in the final model.
4) **Discussion**

Previous research on blood Mn has shown that deleterious health effects of Mn may manifest at either deficient levels of Mn or at elevated levels of Mn \(^{49}\). Prior studies have demonstrated an inverted U-shaped association for maternal blood Mn levels with infant birth weight, and in fetal enzyme activity \(^{45,47}\). More specifically, these studies described how at the extreme ends of maternal Mn concentration, Mn may have inhibited birth weight. Other studies have described a nonlinear association between Mn concentrations and neurodevelopment in infants and children \(^{5,46}\). It was further suggested that both low and high Mn levels may have adverse neurological effects and that children may be more sensitive to both low and high Mn levels during this developmental stage \(^5\). Based upon studies demonstrating a nonlinear association between exposure to Mn and various outcomes, it is speculated that similar nonlinear relationships may hold for cardiovascular outcomes. In the context of this study, it is surmised that the inverted U-shaped association that was approaching significance (p=0.09) for blood Mn levels and DBP may provide some support that low or excessive Mn exposure could possibly interrupt the natural rhythm of DBP \(^2-6\). It is further speculated that DBP may be suppressed at these extreme low and high doses of Mn. There is lack of statistical significance in support of this non-linear association in this study. In addition, there is insufficient evidence in the literature indicating that such a relationship has been investigated for Mn and cardiovascular outcomes. This implies that more research is needed to elucidate whether this inverse parabolic relationship exists for Mn exposure and cardiovascular outcomes.
The other significant finding in this study demonstrated that an increase in 1 ug/L Mn concentration in the blood was significantly associated with a decrease in SBP by about -0.77 mm/Hg, after adjusting for BMI and age. In a study of male workers exposed to airborne manganese at varying levels, it was found that the lowest mean values of SBP were discovered in the men with the highest exposure to manganese, however they were also the oldest workers in the cohort. Other studies have shown that there is a decrease in DBP with increasing level of Mn exposure in which the proposed mechanism to explain this phenomena suggests that that Mn exposure may cause vasodilatation, leading to a decline in DBP and even possibly hypotension. In a comprehensive review of studies on Mn’s effects on the cardiovascular system, authors found that the incidence of diastolic hypotension was significantly higher in the 20-30 year old age group as compared to other age groups. There were a few studies that demonstrate an increase in both DBP and SBP. For example, one study on Mn inhalation exposure among smelter workers supported a reverse trend where overall blood pressure was significantly higher in Mn exposed groups than controls and that SBP and DBP increased approximately 10% in both the high Mn and low Mn exposure groups. Importantly, there may be unmeasured confounding of the many other chemicals that smelter workers are exposed to, in particular chemicals such as polycyclic aromatic hydrocarbons and volatile organic compounds that have been associated with negative cardiovascular outcomes.

It is unclear why blood Mn would show different relationships with SBP and DBP. One study speculated that higher Mn exposure may lead to a higher reduction in both SBP and DBP, however the observed drop in SBP may be faster. Another possible explanation for the
different relationship between blood Mn with DBP as compared to blood Mn with SBP could be in part due to serum ferritin’s significant positive association with DBP that was not found for SBP. Serum ferritin and Mn have been shown to share transport and metabolic pathways and various studies have indicated that there is an inverse relationship between serum ferritin and manganese concentrations in the blood. Although further investigation would be needed to elucidate why the iron-Mn interaction would only be seen in DBP regulation and not for SBP.

Heart rate and heart rate variability (HRV) has also been used to help measure heart function. Overall, the heart rate models in this study lacked any significant variables and the nature of the positive relationship seen in the models for blood Mn and hair Mn with heart rate has only been somewhat supported in the literature. Some clinical and epidemiological studies have shown that abnormal ECG findings was significantly higher in Mn exposed workers and that the accelerated heart beat was mainly manifested in female workers as compared to males. In general, most studies have indicated a decline in heart rate or heart rate variability. In a study on occupational exposure to particulate matter, the largest decline in HRV was seen for manganese, as expressed per increase in interquartile range of exposure. In addition, Mn exposure was shown to produce abnormal ambulatory electrocardiogram (ECG) in adult occupations such as smelting, welding, and boilermakers. Further analysis of a 24-hour, non-resting ECG in Mn-poisoned alloy workers showed a reduced heart rate reaction in response to parasympathetic nerve activity.

The conflicting results about the directionality of the relationship between Mn and cardiovascular function outcomes, may be in part due to the varying exposure status of the
participants being assessed, how concentrations were measured, the differences in the types of worker occupations and the results being confounded by the presence of many other chemical compounds in the workplace. Furthermore, the majority of the studies were conducted on a small scale and not necessarily representative of the overall general population. There was also a lack of consistency from study to study in controlling for various covariates affecting BP and heart rate outcomes.

Apart from the aforementioned findings of the relationships of blood Mn with cardiovascular outcomes, ancillary results indicated that BMI was also significantly associated with SBP and DBP. There have been multiple studies that have supported this relationship between BMI and blood pressure. Furthermore, significant effects of both age and BMI on blood pressure were observed and there was no difference in these effects between obese and non-obese groups or based upon sex. In addition, studies have showed that age is significantly associated with an increase in BP outcomes as well as negatively associated with heart rate. Interestingly, in this study, age had a significant or slightly significant positive association in all of the SBP and DBP models and it had a negative association with heart rate, although not statistically significant.

Although many studies have demonstrated that Mn may significantly alter cardiovascular function, a well-defined characterization of the biological mechanism to support this phenomenon is still lacking. A cardiovascular toxicant such as Mn can directly affect the intrinsic control of cardiac tissues. It can also indirectly alter the extrinsic control of the autonomic cardiovascular system. The autonomic nervous system strictly regulates the
cardiovascular functions in the body by maintaining continuous rhythmic contractions\textsuperscript{65}. Furthermore, any slight changes that may occur in the autonomic function could lead to significant negative effects in both cardiac and vascular performance\textsuperscript{18,65}. The stimulation of the parasympathetic division of the autonomic nervous system leads to a decrease cardiac output (the volume of blood being pumped by the heart), a lowered heart rate, vasodilatation (widening of blood vessels), a decreased force of contraction of the atrial cardiac muscle, and an inhibition of atrioventricular conduction\textsuperscript{66}. It is possible that any deleterious effect of Mn on heart function may be indirectly due to Mn’s effect on the general autonomic nervous system\textsuperscript{18}. For instance, Mn exposed workers have been shown to have a disrupted autonomic nervous system function and this disturbance may influence an alteration in cardiac rhythm and blood pressure that is observed in clinic settings\textsuperscript{18,23,54,67}. Intrinsic control of the heart is provided by the sinoatrial (SA) node which acts as pacemaker by sending action potentials to atrial muscle cells to initiate their contraction\textsuperscript{65}. An action potential in the SA node can be triggered by a change in cell membrane permeability to elements such as calcium\textsuperscript{65}. Mn appears to alter heart function by blocking the calcium channel in a way that the excitation phase is separated from the contraction phase in the myocardium, which eventually leads to a decrease in the strength of the heart contraction and may possibly change the rhythm of contraction\textsuperscript{18,65}. This mechanism may only be relevant to acute toxicity caused by high exposures\textsuperscript{18,65}. Additional research on animals, have shown that the heart is one of the most mitochondrial-rich tissues in the body, and that the accumulation of Mn in heart tissue can occur rapidly and may result in acute cardio depression and hypotension\textsuperscript{18,68}. It is speculated that the deleterious cardiovascular outcomes observed in these animal studies may be partially
due to the interaction of Mn with the calcium channel and Mn’s instigation of mitochondrial damage in the cardiovascular system\textsuperscript{18,68}.

Given that the children in CARES were enrolled on the basis that their mothers were pregnant with them while living near the ferromanganese plant, it is important that we understand the possible windows of susceptibility to Mn exposure during their heart development. In the fetus, the first organ to develop is the cardiovascular system since it is needed for the delivery of oxygen and nutrients to the quickly developing cells of the embryo\textsuperscript{69}. The most vulnerable period of prenatal heart development occurs between weeks two and eight and the heart begins to beat at three weeks of embryonic age\textsuperscript{69}. Although the research is inconsistent, manganese has been shown to cross the placental barrier in humans and animals and could possibly affect heart development in the fetus\textsuperscript{2,70}. Neonates have a larger respiratory ventilation rate relative to lung surface as compared with adults and this could potentially lead to a greater uptake of airborne compounds based on body weight\textsuperscript{71}. In addition, the newborn’s heart rate is greater than in older children, and the heart’s rate of pumping oxygen-rich flood to other organs also changes with age\textsuperscript{72}. Furthermore, Mn’s role in the regulation of metabolism may impact the fact that most metabolizing enzyme systems develop from the middle of gestation until a few months after birth\textsuperscript{73}. Also, the metabolic capacity in neonates and young children may handle toxic levels of Mn differently as compared to adults\textsuperscript{73}.
The main limitation of this pilot study was its small sample size. The data was also taken from a larger cross sectional study; confounders or covariates specific to heart function (such as serum creatine or hemoglobin) were not available.

5) Final Conclusions and Future Implications

The negative association found between blood Mn and SBP may indicate that with increasing Mn exposure there is a decline in SBP. The nonlinear relationship between blood Mn levels and DBP provides some support for the premise that extreme low or high doses of Mn exposure may interrupt the natural rhythm of DBP.

The few studies that have assessed the cardiovascular effects of Mn exposure have mainly focused their investigation on adult occupational exposures, providing significant findings of adverse effects of Mn on heart function in adults. Additionally, the literature is somewhat inconsistent on the directionality of Mn’s effect on cardiovascular outcomes. The findings in this study of a small sample of children, and the lack of pediatric focused literature on Mn exposure and heart function shows that further investigation of Mn’s effect on cardiovascular health in the pediatric population may be warranted. In addition, since children have different windows of susceptibility as they age, it is important to elucidate at what time in development Mn exposure could affect children’s heart function.
6) References


