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Elevated manganese and cognitive performance in school-aged children and their mothers

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Abstract

Background—Growing evidence suggests that excess manganese (Mn) in children is associated with neurobehavioral impairments. In Brazil, elevated hair Mn concentrations were reported in children living near a ferro-manganese alloy plant.

Objectives—We investigated these children's and caregivers' cognitive function in relation to bioindicators of Mn exposure.

Methods—In this cross-sectional study, the WISC-III was administered to 83 children aged between 6 and 12 years; the Raven Progressive Matrix was administered to the primary caregivers (94% mothers), who likewise responded to a questionnaire on socio demographics and birth history. Mn in hair (MnH) and blood (MnB) and blood lead (PbB) were measured by graphite furnace atomic absorption spectrometry (GFAAS).

Results—Children's mean MnB and MnH were 8.2 µg/L (2.7 – 23.4) and 5.83 µg/g (0.1 – 86.68), respectively. Mean maternal MnH was 3.50 µg/g (0.10 – 77.45) and correlated to children's MnH ($\rho=0.294$, $p=0.010$). Children's MnH was negatively related to Full-Scale Intelligence Quotient (IQ) and Verbal IQ; β coefficients for MnH were -5.78 (95% CI -10.71 to -0.21) and -6.72 (-11.81 to -0.63), adjusted for maternal education and nutritional status. Maternal MnH was negatively associated with performance on the Raven's ($\beta = -2.69$, 95% CI -5.43 to 0.05), adjusted for education years, family income and age.

Conclusions—These findings confirm that high MnH in children is associated with poorer cognitive performance, especially in the verbal domain. Primary caregiver's IQ is likewise associated to Mn exposure, suggesting that, in this situation, children's cognition may be affected directly and indirectly by Mn exposure.

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Declaration: The authors declare that there are no conflicts of interest.

Keywords

Manganese; intelligence; children; neurobehavioral; alloy plant

Introduction

There is growing interest in environmental manganese exposure in children. Recent studies suggest that excess Mn may interfere with developing brain functions. In Bangladeshi children, Wasserman and associates (2004, 2006) observed a negative effect of high Mn levels in tube well water on cognitive capacities. In Quebec, Bouchard et al. (2007) reported a significant association between hair manganese (MnH) levels and hyperactive and oppositional behavior in children exposed to Mn through municipal well water. Decrements in IQ scores in Korean children were associated with elevated blood Mn levels in a population-based study, but Mn sources were not identified (Kim et al. 2009). Zoni et al. (2007), in a review of recent studies on neurobehavioral performance and manganese exposure across the lifespan, suggested that children's cognitive functions might be particularly vulnerable to manganese.

The developing nervous system is a prime target for the disrupting effects of toxic chemicals (Faustman et al. 2000; Landrigan et al. 2005; Weiss and Landrigan, 2000; Rice and Barone 2000; Bellinger 2009). In a birth cohort study in France, cord blood Mn was negatively associated with attention and non-verbal memory and boys' manual ability at 3 years, after adjusting for mother's educational level. (Takser et al. 2003). In a study in the United States, Mn was analyzed in the enamel of deciduous teeth. Its concentration in tissue formed during the intra-uterine phase, was significantly associated with disinhibitory behavior evaluated at 36 and 54 months of development (Ericson et al. 2007).

Among the many factors that influence children's neurodevelopment, mothers' education and/or IQ constitute a major determinant (To et al. 2004; Kordas et al. 2004; Andrade et al. 2005). In an exposure situation, parents may also be exposed and suffer some of the toxic effects of the polluting agent (Bellinger, 2009). In a non-occupational population, living in the vicinity of a ferro- and silico- manganese alloy plant, (Mergler et al. 1999) reported decreased memory and learning and poorer performance on motor tests among persons with elevated blood Mn levels. In Mexico, studies performed with communities exposed to dust from Mn mines and transformation plants showed an increased risk of deficient cognitive performance and an association between air Mn concentrations, but not MnB levels (Santos-Burgoa et al. 2001).

Children's exposure has been investigated primarily with respect to ingested Mn through diet: baby formulas (Collip et al. 1983), water (He et al. 1994; Zhang et al. 1995; Wasserman et al. 2006; Bouchard et al. 2007) and for children with elevated Mn from total parenteral nutrition (Alves et al. 1997). One recent study of Mexican children living in an area with manganese mines and small transformation plants showed an inverse association between hair Mn (MnH) concentrations and IQ, with the strongest associations with verbal IQ (Riojas et al. 2010). We recently reported elevated MnH in children living in the vicinity of a ferro-manganese alloy plant (Menezes-Filho et al. 2009); MnH concentrations were significantly associated to the distance and position of their houses relative to wind direction. Mean air Mn concentration in the respirable fraction (PM_{2.5}) sampled during the raining season was, on average, three times higher than the US EPA reference concentration (RfC 0.05 µg/m³) (US EPA, 1993).

The objectives of the present study were to investigate the associations between (i) biomarkers of Mn exposure (hair and blood) and neurobehavioral performance in children living in the vicinity of this ferro-manganese alloy plant; (ii) caregivers' hair Mn concentrations and performance on a test of intelligence.

Material and Methods

Exposure

Manganese exposure of children from the village of Cotegipe, a small community of 620 people in the municipality of Simões Filho, 30 km from the city of Salvador, State of Bahia, Brazil (Figure 1), was characterized in 2007 (Menezes-Filho et al. 2009). The community resides within a 2-km radius from a ferro-manganese alloy plant and mostly in a downwind direction. The plant was inaugurated in 1970 and after two expansions, three ovens are currently in operation, with an annual production of SiMn and FeMn alloys of 280,000 tons. For the children living in this area, there is a gradient of exposure in relation to the child's house distance and position with respect to the plant (Menezes-Filho et al. 2009).

Study design and population

A cross-sectional study design was used. Children aged 6 years to 11 years and 11 months, who attended the Cotegipe Elementary School and had lived in the community for at least one year, were invited to participate. The school principal provided us with a list of the 110 children, enrolled and regularly attending classes. We sent out invitations to the mothers or legal guardians of all of the children who were in the specified age range (N=80). All accepted to participate. Five other children, who lived on an isolated road on the northwest side and downwind of the plant, were also included because our previous study indicated that the children on this area presented the highest hair Mn concentrations. The area is separated from the village by the plant. The children attend an elementary school on the boundary of the town of Simões Filho. Thus, a total of 85 children were enrolled in the study. Two children were excluded from the study: one boy had a history of seizure and one girl had hearing problems and used a hearing aid. The final study group was comprised of 83 children.

The Simões Filho Education Department gave permission to use the school premises to set up the study and the school principal gave us full support and provided two rooms where we carried out the assessments activities.

The project was approved by the Federal University of Bahia Ethics Committee. All parents signed the informed consent forms.

Questionnaires—Three psychology students, with clinical and psycho-diagnostic experience, were trained and monitored by the lead psychologist (C.O.N.) to administer the caregivers' interview questionnaires on socio-economic characteristics, family structure, child development, behavior and illnesses. A second questionnaire, translated and adapted from the HOME Inventory (Home Observation for Measurement of the Environment Scale, NLSY79 Child HOME-SF), comprised 20 items and was used to assess the quality of family environment. It included indicators of cognitive stimulation, parent-child interaction and general interpersonal interactions. A simple score was derived by summing the number of positive answers obtained for the twenty questions.

Anthropometry—A single person performed all weight and height measurements. Children took off their shoes but kept their clothes for both weight and height assessments. Weight was measured using an upright scale (CATSYS 2000 System®, Snerkkersten,

Denmark) connected to a computer, with a capacity to weigh 150 kg in 100-g increments. Height was taken using a measuring board. Body mass index (BMI) was calculated by dividing the weight in kilograms by the square of the height in meters. The chronic nutritional index, height-for-age (HA) z-score, encompasses growth and stature, which is inversely related to protein, calcium and iron deficiency during early childhood (Abrams et al. 2005). It was calculated using the AnthroPlus software (WHO, 2009) based on the WHO reference population collected in 2007 for 5–19 years.

Blood measurements—Children's venous blood samples were collected from the cubital vein into sodium–EDTA vacuum tubes proper for metal analysis (Vacutainer, Bencton Dickinson, USA). Thirteen children (15.7%) refused or were not available to provide blood samples. For blood analyses, we adapted the method described by Montes et al., (2002). The blood sample was diluted 1:5 with matrix modifier (1% ammonium-dihydrogenphosphate in 0.1% Triton X-100 solution). After homogenization in a vortex, it was centrifuged for 10 minutes at 14,000 rpm. Quality control of blood Mn analysis was assured by measuring human blood reference materials QMEQAS07B-03 and QMEQAS07B-06 (Centre de Toxicologie/INSPQ, Canada). Samples were measured in duplicate; every measurement consisted of two injections into graphite furnace, in all cases standard deviation was lower than 10%; if otherwise, sample was reanalyzed.

As lead (Pb) is an ubiquitous contaminant and a recognized neurotoxin, associated with effects on cognition and behavior in children at low PbB, it was also measured by GFAAS with Zeeman background correction (GTA-120, Varian Inc.).

An additional blood sample, collected with a no additive in the vacuum tube (Vacutainer, Bencton Dickinson, USA) was obtained for serum iron (FeS) determination. After blood clotting, tubes were centrifuged 9000 rpm for twenty minutes. FeS was determined by automated method using a commercial kit (Roche Hitachi 747, Roche®) for 58 children; twelve samples were rejected due to some degree of hemolysis.

Hair measurements—For children, and caregivers, a tuft of hair of approximate 0.5 cm diameter was cut off as close as possible to the scalp in the occipital region, with a surgical stainless steel scissor. Detailed information on hair sampling, washing procedure and Mn determination by GFAAS are reported in Menezes-Filho et al (2009).

Caregivers of seventy-seven children provided hair samples. One mother refused for religious reasons, three mothers refused for personal reasons and a father of one child had hair too short to be sampled.

Psychological Measures

Children's cognition—The Wechsler Intelligence Scale for Children, version III -WISC-III (Wechsler, 1991), previously validated for Brazilian children (Figueiredo 2002) and suitable for children ≥ 6 years of age, was administered by the same psychologist (C.O.N.) over a period of four weeks in July 2008. Testing followed the procedure recommended in the WISC-III manual. Total testing time ranged between 50 to 80 minutes. The tester was unaware of children's degree of Mn exposure.

Maternal Cognition—The Raven's Standard Progressive Matrices (Raven et al. 1983) was used to assess caregiver's intelligence. This instrument is culture-free and has been validated for the Brazilian population. The test was applied individually to each caregiver following the procedure described in the manual. The test is composed of five sets of 12 item of increasing difficulty level. In each test item, the participant is asked to identify the

missing item that completes a pattern. It was abbreviated between series, at the examiner's discretion when it became evident that the response was purely made by guessing.

Statistical Analyses

Descriptive statistics were used to examine the distribution of socio-demographic information, bioindicators of Mn and Pb exposure and cognitive function scores. For normally distributed variables, the arithmetic mean (AM) was used, otherwise the geometric means (GM) are presented.

Pearson correlation tests were applied to evaluate bivariate correlations between co-variables and exposure bioindicators.

Since the distributions of hair and blood metal levels were skewed, data were log₁₀ transformed for further analyses. Backward stepwise regression models were used to identify variables that were potentially associated with intellectual quotients (0.100 to enter; >0.05 to exclude). These variables were then included in linear regression models. The significance level of $p=0.05$ was used and residual analysis was performed to verify the model's parameters. Possible associations of other potential confounders (gender, time in years mothers lived in the community prior to child birth, marital status, caregiver's age, family income, children's blood lead, serum iron and hemoglobin levels, growth index and nutritional status) of manganese biomarkers' levels and cognitive functions were examined.

The residuals from the models were assessed in standardized residual vs predicted plot for heteroscedasticity and non-linearity and in a half-normal plot for non-normality. All statistical analyses were performed using SPSS version 13 software.

Results

Sample Characteristics

Table 1 presents a summary of the demographic and anthropometric characteristics. The primary caregivers included mothers (94%), a father (1.2%) a grandmother (1.2%) and three stepmothers (3.6%). Children were from 55 families, and approximately half live in a structured family with mother and father. Eight families (9.8%) have only one child, 25.5% have two children, 27.7% have three children, 13.3% of the families have four children and 22.9% have five or more children. The ethnic composition is representative of the population around the Todos os Santos Bay area, comprising approximately 80% of Brazilian-Africans with black curly hair. The socio-economic status is very low; the main income source is from cultivating cassava and rudimentary processing and commercialization of manioc flour. The average monthly income is US\$168, ranging from 25 to 444 US dollars. Families who maintain children at school receive social monthly benefits. The low SES is reflected in the low nutritional status, the mean age-for-height z-score, which reflects chronic malnutrition, is -0.16 (range -2.39 to 2.54). Four boys and two girls (7.3%) could be classified as suffering from stunted growth, their HA z-scores were below -2.0 (WHO, 2006).

FeS concentration ($N=58$) is normally distributed with mean $65.6 \mu\text{g/dL}$ (range 11 – $165 \mu\text{g/dL}$), with 41.4% below the normal range (55 – $120 \mu\text{g/dL}$) and classified as iron deficient (Takemoto et al. 2004). Fe deficient children have a mean HA z-score of 0.13 versus -0.37 for those with normal FeS, but this difference does not reach statistical significance ($p=0.06$).

Children who provided blood samples ($n=70$) and those who were not available to donate blood ($n=13$) did not statistically differ in several aspects: age, stature, maternal education,

family income, MnH levels and performances in IQ tests. Among those who did not provide blood 20.5% (n=9) were boys and 10.3% (n=4) were girls.

Exposure Characteristics

Table 1 also presents the distribution of the biomarkers of metal exposure. A large proportion of MnH concentrations (77.1%) surpass 3.0 µg/g, the upper cutoff limit that has been associated with hyperactive behavior (Bouchard et al. 2007). Blood manganese levels observed in this study (mean 8.2±3.6 µg/L) are similar to the levels observed in other studies with children in the same age range (Rollin et al. 2004 and Kim et al. 2009). Only 4% of blood Mn are above 14 µg/L. PbB is above 2 µg/dL for 51% (n=36) and only one child had PbB slightly above 10 µg/dL. MnH levels are not significantly correlated with age.

Table 2 presents the correlations between biomarkers. No relation was observed between MnB, PbB and FeS. Children with iron deficiency present similar MnB compared to those with normal FeS (8.7±4.52 µg Mn/L vs 7.9±3.24 µgMn/L; p=0.50), however, those with iron deficiency have higher MnH (15.94±19.68 µg/g) compared to those with FeS in the normal range (8.69±8.23 µg Mn /g); difference reached borderline statistical significance (p = 0.059).

Children's intellectual function

Table 3 presents the summary of the WISC scores. No significant difference was observed between boys and girls. In bivariate analyses, maternal education is significantly correlated with children's Full-Scale IQ (r=0.300, p=0.006) and Performance IQ, (r=0.364, p=0.001), but not with Verbal IQ. Maternal score on the Raven's Progressive Matrix is significantly correlated with children's Full-Scale, Performance and Verbal IQ's (r=0.311 p=0.004; r=0.341 p=0.002; r=0.221 p=0.044, respectively). Height-for-age z-score is positively correlated with Verbal IQ (r=0.239, p=0.030) and a tendency is observed for Full-Scale IQ (r=0.209, p=0.068). Family income is positively correlated with Full-Scale IQ (r=0.232, p=0.045) and Performance IQ (r=0.229, p=0.044). None of the IQ scores are significantly related to the HOME inventory score.

Biomarkers of Mn exposure and children's IQ

In bivariate analyses MnB or MnH levels are not significantly correlated with any of the children's IQ scores. In multiple regression analyses, MnB does not enter significantly into any of the models that were tested. On the other hand, negative associations are observed with children's MnH (Table 4). MnH is inversely associated with adjusted Full-Scale and Verbal IQ. Figure 2 presents the adjusted IQ scores with respect to MnH levels. We also tested the association between MnH levels with cognitive indices only of those children who provided blood samples (n=70). There were no significant statistical changes in the coefficients. The beta coefficient of the Verbal IQ (n=70) was -6.65 and 95% CI (12.58 to -0.72). No confounding effects were observed for PbB or low serum iron levels.

MnH and caregivers' Intelligence

The caregivers' mean raw score on the Raven's Progressive Matrix was 15.9 (SD=9.1, range 5–46). Bivariate analyses show significantly and positive correlations with years of formal education (Pearson Coefficient r=0.540, p<0.001), family income (r=0.378, p=0.001) and the HOME score (r=0.245, p=0.026). On the other hand, it is negatively correlated with age (r=-0.358, p=0.001) and with the log of maternal MnH levels (rho=-0.288, p=0.011). Maternal MnH is also significantly associated with HOME score (Pearson Coefficient r=-0.273, p=0.022) (Table 2). In the multiple regression model, these variables explain 43.2% of the variance in the mother's score.

Discussion

The findings of this study indicate that for these children living in the vicinity of a manganese alloy production plant, there is a negative association between hair Mn and Full-Scale and Verbal IQ. In addition, primary caregivers cognitive performance was likewise inversely associated with Mn hair level, suggesting that in the vicinity of this plant, Mn exposure is impacting both children and adults.

The MnH concentrations observed here are considerably higher than those reported in other studies; 76.8% are above the 3 µg/g cut-off that was used by Bouchard et al (2007) in their study of children exposed to Mn through well water. MnH in the present study are, on average, six times higher than the levels reported for the general Brazilian population (0.25–1.15 µg/g) (Miekeley et al. 1998). They are also considerably higher than those observed in children living near a waste site in the USA (mean 0.47 µg/g, range 0.89–2.15 µg/g) (Wright et al. 2006). On the other hand, MnB levels are very comparable to levels observed in other studies with children in the same age range. In South African children, MnB levels were 6.7 (1.6 to 32.8) µg/L and 9.8 (3.6 to 26.5) µg/L in Cape Town and Johannesburg, respectively, the authors attribute this difference to a Mn based gasoline additive (Rollin et al. 2005). Nonetheless, MnB concentrations in the present study are lower than those reported by Wasserman et al. 2006 Mn (12.8±3.2 µg Mn/L) for children exposed to Mn drinking from well water in Bangladesh.

In the present study hair, but not blood Mn, was associated with IQ deficits. Riojas et al (2010) had similar findings for school-age children living in a manganese mining region in Mexico. Wasserman et al (2006) did not observe an association between MnB and cognitive function in Bangladeshi children; poorer performance was associated with Mn content in well water. Wright et al. (2006) reported decrements in verbal functions associated with MnH in children living near a toxic waste site and Bouchard et al. (2007) reported a positive relation between hair Mn and behavioral problems in children exposed to Mn through drinking water. There are however some studies that have reported relations between MnB and neurobehavioral performance in infants. Takser et al (2003) found that cord blood manganese in Parisian newborns was associated with lower attention and nonverbal memory at 3 years. Claus Henn et al (2010) observed an inverted U shape doseresponse curve between MnB levels at age of one year of age and a mental development index. Kim et al. (2009), who studied Korean children aged 8–11 years, reported that full-scale IQ and verbal IQ of the children showed significant associations with blood Pb, when blood Mn concentration was above 14 µg/L, but not when Mn was below 14 µg/L, suggesting an effect modification between Pb and Mn. It is difficult to generalize at this stage because blood Mn concentrations vary with age, decreasing from birth onwards (Kirchgessner et al., 1981).

In the present study, the two bioindicators of MnB and MnH were not correlated, which raises the question of what each represents in terms of Mn toxicity. It is possible that MnB may be subjected to greater homeostatic control, while MnH may represent excess Mn. In this region, dominated by a manganese alloy production plant, MnH varies with distance from the plant (Menezes-Filho et al. 2009a).

Similar to the present study, Wasserman et al. (2006), Wright et al. (2006) and Kim et al. (2009), reported more pronounced deficits in the verbal domain. In the present study, a ten-fold increase of Mn in children's hair is associated with a loss of 6.7 point loss in Verbal IQ score. Verbal IQ reflects crystallized intelligence, related to general knowledge, it demonstrates the extension and depth of information acquired normally through school. Generally it is used to solve problems similar to those experienced in the past (Primi et al. 2001). Cognitive deficits have been reported in adults exposed to airborne Mn (for review

see Zoni et al. 2007), but it is only recently that the mechanisms of action have been examined. In non-human primates exposed to Mn by intravenous injection and inhalation, Schneider et al. (2006) observed that chronic manganese exposure was associated with cognitive deficits, such as impaired spatial working memory and behavioral alterations consisting of compulsive-like behaviors. Burton and Guilarte (2009), in a review article of their non-human primate studies suggest that changes in gene expression (i.e. tumor suppressor p53, amyloid beta precursor-like protein (1A β LP1)) and markers of neurodegeneration in the frontal cortex (i.e. copper homeostasis dysregulation and extracellular accumulation of toxic peptide beta amyloid (A β)) may explain subtle cognitive deficits and other early manifestations of Mn neurotoxicity in humans related to working memory and neuropsychiatric behaviors.

Children's development is strongly influenced by their families and by the social forces and cultural values in the society they live. Early maternal as well as paternal influences are crucial in children's development (Barros et al. 2009). In our study the quality of family environment was not associated with children's cognition, even though it was significantly correlated with maternal intelligence score. In other studies on Mn exposure in children, maternal education was an important covariable in the association between children's exposure to Mn and neuro-psychological outcomes in France (Takser et al. 2003), in the USA (Wright et al. 2006) and in Bangladesh (Wassermann et al. 2006). In an investigation carried out in the outskirts of Salvador, Brazil on the association between social factors and intelligence among children from low income Families, Andrade et al. (2005) reported a positive association between the quality of family stimulation and children's cognitive performance. They concluded that a better pattern of stimulation was observed among children who live with their parents and whose mothers have better education, a job and a partner involved in the family environment.

In the present study, cognitive function was markedly affected by the nutritional status of the child. Malnutrition is a well recognized risk factor for intellectual deficit. Niehaus et al. (2002) observed that height-for-weight z-score was positively correlated with the TONI-III (Test of Non-verbal Intelligence) score; Wasserman et al. (2006) reported that anthropometric parameters, such as stature and head circumference, which likewise reflect nutritional status, explained 17.7% of the Verbal IQ in Mn exposed children. Fonseca et al. (2008) also reported significant correlations between height-for-age z-scores and several WISC-III subtests in Amazonian children exposed to methyl mercury. The association between height and cognitive outcomes was also observed in a study with Pb exposed Mexican children (Kordas et al. 2004). The authors commented that the association of height-for-age z-score with cognitive performance might be explained to some extent by parent and teacher perceptions of ability in taller children.

This is the first investigation to demonstrate not only that children's intellectual impairment, but also mothers' cognition can be affected by Mn exposure. About 40% of the mothers lived in the community since childhood and the alloy plant has been emitting high levels of Mn in the air for almost four decades. Women's environmental exposure to Mn in reproductive age has been little investigated. Mn blood levels increase during pregnancy, independently of iron status, peaking in the third trimester (Tholin et al. 1995). High MnB levels at delivery were reported by Takser et al. (2003) in pregnant Quebecoise women living near farms where pesticides were sprayed. Ljung et al. (2009) observed high MnB levels, similar to occupationally exposed groups, in pregnant Bangladeshi women exposed to Mn from tube well water.

There are few reports of the effects of environmental Mn on adults. In Mexico, in a pilot study of 72 persons, the majority women (86%), living in a community exposed to high air

levels of Mn from Mn mining and transformation activities, the authors reported a twelvefold increase in risk for deficient cognitive performance (cutoff <17) in the Minimental Test score (Santos-Burgoa et al. 2001). A recent study in the same region detected a significant association between elevated Mn air levels and attention deficit in adults of the region; no gender differences were observed (Sollis et al. 2009). In Quebec, Canada, persons living in the vicinity of a manganese alloy plant had higher blood Mn than those living elsewhere and blood Mn was associated with several neurobehavioral deficits, particularly among the older residents (Mergler et al. 1999).

Although it is not possible to determine the direction of the associations between mother's cognitive capacities, her educational level and the home environment, the results of our study suggest that there is an association between mothers' exposure and her performance of the Raven's. Thus, the children from Cotegipe village may be affected not only through the direct effects of Mn on their own brain, but also, possibly indirectly, as consequence of Mn on their caregiver's cognition, resulting in a diminished ability to provide better stimulating environment.

The IQ levels observed in Cotegipe's children are very low, in the range of one SD lower than normal (Figueiredo, 2002). Walker et al. (2007) reviewed the proximal risk factors for child development in the developing countries. Among the most consistent in the literature, inadequate cognitive stimulation was the most important psychosocial determinant along with maternal depression and exposure to violence. The authors also mention other risk factors for impaired child development are biological (stunting, iodine and iron deficiencies, malaria, intrauterine growth retardation) and exposure to metals. The Cotegipe community has a very low socio-economic status and is needy of attention for sanitary and education interventions. There is a high unemployment index and many families depend on governmental social assistance. It is well known that cognitive deficit has multi factorial causation, and yet, despite many adverse factors we were able to observe associations with MnH.

This study bears the limitations of a cross-sectional study and causal inferences cannot be made on the relationship of Mn exposure and cognitive deficits. Moreover the HOME inventory adaptation has not been validated previously and FeS measurements were not available for all of the children. Only bioindicators of manganese and lead exposure were measured, it is possible that other neurotoxic chemicals are present in this environment. On the other hand, we were able to test all of the children in the desired age range attending the only local elementary school, thus avoiding selection bias.

In the face of the evidence presented here, we strongly recommend cognitive strategy interventions. These interventions are designed to improve performance through compensatory procedures or through more efficient functioning of weak or deficient processes (Morris and Mather, 2008). An intervention program with mothers in the Northeastern, Brazil, observed positive associations in cognitive and motor development in children whose mothers were included in the intervention group (Eickmann et al. 2003). Barros et al. (2009) demonstrated a clear interaction between stimulation and maternal schooling, indicating not only that stimulation has a stronger effect in children of lesseducated mothers, but also that by effectively stimulating these children they can achieve a very similar result to those who are more stimulated with better-schooled mothers.

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Figure 1.
Schematic map of the Cotegipe village in Simões Filho town in the metropolitan area of Salvador, Bahia, Brazil.

Figure 2. Scatter plot of children's Full-Scale (a), Verbal (b) IQ scores versus Mn hair levels, adjusted for maternal education and nutrition status.

Table 1
Socio-demographic characteristics and bioindicator levels in the study participants.

	N	Mean	SD	Min	Max
Age (months)	83	106.1	19.8	74	147
Height (cm)	83	130.2	10.8	108	155
Weight (Kg)	83	26.8	6.9	16.2	49.9
BMI (kg/m²)	83	15.6	2.0	11.8	22.9
HA z-score	83	-0.16	1.21	-2.39	2.54
Boys	44 (53.0%)				
Ethnicity					
African-Brazilian	62 (78.1%)				
Non African-Brazilian	21 (21.9%)				
Parents living together	47 (56.6%)				
Years of maternal education	82	6.9	4.0	0	14
Maternal age at birth	82	24.5	6.4	14.3	46.1
Mother's Raven score	82	15.9	9.1	5	46
Family income (US/month)	75	168	103	21	444
HOME Inventory (%)	82	51.8	15.2	6.7	80
Number of children in the home	82	3.6	2.3	1	15
Fe Serum	58	65.7	31.8	11	164
Mn hair (µg/g)	83	5.83 ^a	11.5	0.10	86.68
Mn blood (µg/L)	70	8.2 ^b	3.6	2.7	23.4
PbB (µg/dL)	70	1.43 ^a	1.90	0.5	10.35
Mother's MnH (µg/g)	77	3.50 ^a	12.76	0.10	77.45

US\$ exchange rate=1.868 BRR (Brazilian Reais) on Aug.02.2009

^a geometric mean;

^b arithmetic mean

Pearson correlation coefficient matrix among metal biomarkers in children and caregivers along with cognitive functions. Data are coefficients, p-values and N.

Table 2

	Log MnH	LogMnB	PbB	Maternal LogMnH	FeS	HOME	Raven Score	FS-Q	Verbal IQ	Perform. IQ
LogMnH	1.00	0.053	0.205	0.202	-0.142	-0.020	-0.153	-0.184	-0.210	-0.054
		0.660	0.089	0.079	0.289	0.862	0.167	0.096	0.057	0.625
		70	70	77	58	82	83	83	83	83
LogMnB	1.00	0.219	0.307*	0.056	-0.273*	-0.190	0.072	0.139	-0.052	
		0.072	0.012	0.688	0.022	0.114	0.555	0.252	0.672	
		68	66	54	70	70	70	70	70	
PbB	1.00	-0.077	0.028	0.213	-0.213	-0.130	-0.103	-0.173		
		0.540	0.845	0.076	0.077	0.284	0.397	0.151		
		52	70	70	70	70	70	70		
Maternal MnH	1.00	-0.140	-0.274*	-0.205	-0.144	-0.150	-0.113			
		0.300	0.016	0.073	0.210	0.194	0.329			
		77	77	77	77	77	77			
FeS	1.00	0.009	0.009	0.008	-0.006	-0.014				
		0.945	0.947	0.955	0.964	0.919				
		58	58	58	58	58				
HOME	1.00	0.245*	0.074	0.024	0.141					
		0.026	0.507	0.833	0.206					
		82	82	82	82					
Raven Score	1.00	0.288**	0.218*	0.342**						
		0.008	0.048	0.002						
		83	83	83						
FS-IQ	1.00	0.889**	0.872**							
		0.000	0.000							

	Log MnH	LogMnB	PbB	Maternal LogMnH	FeS	HOME	Raven Score	FS-Q	Verbal IQ	Perform. IQ
									83	83
Verbal IQ									1.00	0.598**
										0.000
										83
Performance IQ										1.00

**
p = 0.01 (2-tailed)

*
p = 0.05 (2-tailed).

Table 3

Summary of the Wechsler test scores of all 83 children according to gender

IQ scores	Gender	Mean	SD	Min	Max
Full-Scale	Boys	84.8	13.16	60	121
	Girls	85.3	14.10	50	115
Verbal	Boys	89.3	12.97	57	120
	Girls	90.6	15.63	55	127
Performance	Boys	82.6	13.83	57	117
	Girls	80.4	12.74	45	106

Table 4

Summary of the linear multiple regression models for children's IQ (n=83)

Intelligence Quotient	Crude Coefficients (95% CI)	Adjusted Coefficients (95% CI)
Full-scale IQ		
Intercept	88.60 (83.46 to 93.73)	82.80 (76.34 to 89.75)
LogMnH	-4.66 (-10.18 to 0.85)	-5.78 (-10.71 to -0.21)
Maternal Education (Years)		0.992 (0.23 to 1.64)
Height-for-age z-score		2.47 (-0.53 to 5.48)
Verbal IQ		
Intercept	94.03 (88.67 to 99.38)	90.10 (82.90 to 97.18)
LogMnH	-5.58 (-11.34 to 0.19)	-6.72 (-11.81 to -0.63)
Maternal Education (Years)		0.75 (-0.06 to 1.45)
Height-for-age z-score		2.45 (-0.39 to 6.00)
Performance IQ		
Intercept	82.56 (77.43 to 87.69)	74.90 (69.37 to 82.32)
LogMnH	-1.36 (-6.87 to 4.16)	-2.41 (-7.39 to 2.75)
Maternal Education (Years)		1.23 (0.45 to 1.81)
Height-for-age z-score		3.04 (0.15 to 5.95)

Table 5

Linear regression model for caregivers' cognition (n=70).

Variable	Crude Coefficients (95% CI)	Adjusted Coefficients (95% CI)
Intercept	17.1 (14.5 to 19.6)	12.7 (3.8 to 21.5)
LogMnH	-2.73 (-5.72 to 0.27)	-2.69 (-5.43 to 0.05)
Education Years		0.970 (0.50 to 1.44)
Age		-0.176 (-0.40 to 0.05)
Family Income		0.02 (0.01 to 0.04)