Blood manganese and cognitive and motor skills at age 6-7 in Canadian cohort GESTE

Luc Staedelin
Universite de Sherbrooke Faculte de medecine et des sciences de la sante https://orcid.org/0000-0003-2582-2257

Abdelouahab Nadia
Universite de Sherbrooke

Anne-Sandrine Desautels
Universite de Sherbrooke

Virginie Gillet
Universite de Sherbrooke Faculte de medecine et des sciences de la sante

Amélie Boivin
Universite de Sherbrooke Faculte de medecine et des sciences de la sante

Erin Wilkie
Universite de Sherbrooke

Marie Farmer
Universite de Sherbrooke Faculte de medecine et des sciences de la sante

Kasey J.M Brennan
Columbia University

Hannah E. Laue
Dartmouth College Geisel School of Medicine

Christian Bocti
Universite de Sherbrooke Faculte de medecine et des sciences de la sante

Andrea A. Baccarelli
Columbia University

Larissa Takser (larissa.takser@usherbrooke.ca)
https://orcid.org/0000-0001-9262-1362

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Abstract

Background: Increasing evidence suggests that high exposure to manganese (Mn) may impair brain development. In Canada, Mn exposure comes from several environmental sources and the most common is drinking water. Our objective was to examine the relationship between blood Mn and psychomotor skills in school-aged children from the Eastern Townships, Qc, Canada. In addition, we examined the association of Mn with Attention-Deficit Hyperactivity Disorder (ADHD) diagnosis in our study group.

Methods: Children were recruited at birth (through their mother) and followed prospectively. At 6-7 years of age, 210 children provided a blood sample for manganese testing and underwent a battery of neuropsychological tests. This battery assessed several major cognitive domains including general intelligence, attention and others via subtests from the Wechsler Intelligence Scale for Children – IV (WISC-IV), the Developmental NEuroPSYchological Assessment-II (NEPSYII) and the Test of Everyday Attention for Children (TEACH). Parents were asked if their child had ever received a physician diagnosis of ADHD. Blood was analysed by inductively coupled plasma mass spectrometry. Multivariate statistic modelling was used to control for potential confounding factors, including blood lead.

Results: Median blood Mn was 9.9 µg/L (range 4.7 – 21.4 µg/L). The Design Copying – Fine motor score of the NEPSYII was positively associated with blood Mn (linear model β: 0.17 [95% Confidence Interval: 0.03 to 0.32]; adjusted model β: 0.16 [95% Confidence Interval 0.01 to 0.30]). Blood Mn was not associated with diagnosis of ADHD. Sex-stratified analyses indicated potential effect modification by child sex such that manganese had a beneficial association on the Score DT test (a measure of sustained attention), but only among boys (β: 0.29, [95% confidence interval: 0.098 to 0.49])

Conclusions: In agreement with studies from areas with similar environmental Mn levels, our study suggests that blood Mn level does not have wide-ranging associations with cognitive functions, psychomotor skills, or a diagnosis/suspicion of ADHD in school-aged children. To resolve the controversy about toxicity of environmental Mn on the developing brain, further studies should simultaneously focus on several biomarkers of Mn exposure, potential lifestyle protective factors, as well as brain imaging.

Introduction

Manganese (Mn) naturally constitutes approximately 0.1% of the earth's crust, and low levels of Mn in water, food, and air are ubiquitous. Certain geologic regions in Quebec have enriched Mn bedrock, thus the surrounding groundwater in these regions have higher Mn levels (1, 2).

Health-based guidelines for the maximum level of manganese in drinking water are set at 300 µg/L by the U.S. Environmental Protection Agency (EPA) (2004) (3) and at 400 µg/L by the World Health Organization (WHO) (2008) (4). However, as of 2019 Health Canada (5) has a much stricter maximum allowable manganese concentration of 120 µg/L (with an acceptable taste level of 20 µg/L). Current WHO (4) and Health Canada (2) guidelines for water are not based on toxicological evidence in children (6). Thus, some Canadian clinicians and researchers argue that even these regulations do not go far

enough to protect children, they consider the enforcement not strict enough and that the regulation should apply to private wells (7). In the Eastern Townships, a southeast region of Quebec, private or city wells often have a Mn concentration exceeding the maximum acceptable level for drinking water as stated by the health guidelines (8, 9).

Mn is considered to be an essential microelement, however, in high concentrations Mn can have neurotoxic effects on the brain (10). In adults, workplace Mn exposure through inhalation has been associated with parkinsonism (manganism), which manifests in motor symptoms such as bradykinesia and cognitive symptoms such as decreased memory and attention (11). Mn is also toxic for children with long term parenteral nutrition, because of the way they are getting manganese and the concentrations too high in what they are being given – because the optimal and safe concentration for children is not known (11, 12). Moreover, in individuals exposed to high amount of Mn, MRI studies show the accumulation of Mn in the brain, and mostly in the *globus pallidus* which regulates voluntary movement (13).

Disagreement among optimal daily intake for healthy children is common as Mn is also an essential element that plays a fundamental role in child growth and development (14). Fifteen studies have focused on associations between exposure to Mn and neurodevelopmental problems in children; 4 of them found no association (Table 1) while other studies showed a toxic effect of Mn on Children. Blood Mn is the best proxy for the pallidal index – a measure of Mn accumulation in the brain (15, 16). Thus, blood Mn is thought to be a better indicator of the Mn body burden (15, 16) than other biomarkers of exposure such as hairs or teeth.

Table 1
Summary of studies on manganese toxicity. * Arithmetic mean (standard deviation). ** Geometric mean (geometric standard deviation). Blood-Mn: BMn, Hair-Mn: HMn, Water-Mn: WMn

Study	Country	Study population	Mn measurement	Association with Neurodevelopment
Bhang *et al*, 2013(35)	South-Korea	Children 8–11 y	Blood: 4.25–31.50 µg/L	⇒ blood-Mn (BMn) ◊ ◊ scores in thinking, reading, calculation, and learning scores and ⇒ cognitive inhibition test scores.
Bouchard *et al*, 2006(54)	Quebec, Canada	Children 6–15 y	Hair: 0.28–20.0 µg/g	⇒ hair-Mn (HMn) ◊ ⇒ hyperactive and oppositional behavior was associated.
Carvalho *et al*, 2018(31)	Bahia, Brazil	Children 7–12	Hair: 0.52–55.74 µg/g	⇒ HMn ◊ ⇒ hyperactivity and a ◊ of verbal performances.
Chung *et al*, 2015 (55)	South-Korea	Mother-infant pairs recruited prenatally	Maternal blood: 22.5 ± 6.5 µg/L *	◊ and ⇒ levels of BMn ◊ ◊ psychomotor development scores in infants at 6 months.
Haynes *et al*, 2015 (36)	Ohio, U. S	Children 7–9 y	Hair: 416.51 (2.44) ng/g ** Blood: 9.67 (1.27) µg/L **	BMn and HMn ◊ ◊ full scale IQ, perceptual reasoning, ◊ processing speed.
Henn *et al*, 2010 (56)	Mexico City, Mexico	Children enrolled before birth and followed until 3 y	Blood: 24.3 (4.5) µg/L* at 12 months, 21.1 (6.2) * µg/L at 24 months	BMn ◊ Ø with the Bayley Scales of Infant Development-II.

Study	Country	Study population	Mn measurement	Association with Neurodevelopment
Hernández-Bonilla *et al*, 2011 (57)	Hidalgo, Mexico	Children 7–11 y	Exposed: Hair: 4.2–48 µg/g Blood: 5.5–18 µg/L Non-exposed: Hair: 0.06–3.6 µg/g Blood: 5.0–14 µg/g	HMn ◊ Ø neuromotor outcomes (grooved pegboard, finger tapping and Santa Anna test in children. ⇒ BMn ◊ ◊ finger tapping. BMn ◊ Ø Other motor function measures.
Khan *et al*, 2011 (39)	Bangladesh	Children 8–11 y	Water: 40.0–3442.5 µg/L Blood: 6.3–33.9 µg/L	BMn ◊ Ø externalizing (attention problems and aggression) and internalizing (anxiety) behaviors and a total behavioral score. ⇒ Water Mn (WMn) was associated with ⇒ externalizing and internalizing behaviors.
Kim *et al*, 2009 (58)	South-Korea	Children 8–11 y	Blood: 5.30–29.02 µg/g	⇒ BMn ◊ ◊ overall and verbal IQ.
Lucchini *et al*, 2012a (34)	Valamonica et Garda lake, Italia	Children 11–14 y	Hair: 0.024–3.45 µg/g Blood: 4–24.1 µg/L	⇒ WMn and BMn ◊ ⇒ Tremor intensity in dominant hand.
Lucchini *et al*, 2012b (28)	Valamonica et Garda lake, Italia	Children 11–14 y	Hair: 0.024–3.45 µg/g Blood: 4–24.1 µg/L	Mn ◊ Ø IQ (full scale, verbal and performance) or behavioral (hyperactivity, attention deficit) scores in adjusted analyses.
Menezes-Filho *et al*, 2011 (32)	Salvador, Brazil	Children 7–12 y	Hair: 0.10–86.68 µg/g Blood: 2.7 – 23.4 µg/L	⇒ HMn ◊ ⇒ Externalizing behaviors and attention problems on the Child Behavior Checklist for girls, but not boys. BMn ◊ Ø IQ scores.
Oulhote *et al*, 2014 (43)	Quebec, Canada	Children 6–13 y	Hair: 0.1–20.7 µg/g Water: 1–2,701 µg/L	Mn ◊ significant ◊ in memory (hair and water), attention (hair), and motor function (water).

Study	Country	Study population	Mn measurement	Association with Neurodevelopment
Riojas-Rodríguez *et al*, 2010 (59)	Hidalgo, Mexico	Children 7–11 y	Exposed: Hair: 4.20–48 µg/g Blood: 5.5–18 µg/L Non-exposed: Hair: 0.06–3.64 µg/g Blood: 5.0–13 µg/g	⇒ HMn ◊ ◊ full scale, verbal and performance IQ scores in children. Ø associations ◊ BMn with ◊ full scale, verbal and performance IQ scores.
Takser *et al*, 2003 (60)	Paris, France	Mother-infant pairs followed until 6y	Hair: 0.05–13.33 µg/g Cord blood: 14.9–92.9 µg/L Mother's blood: 6.3–151.2 µg/L Placenta: 0.01–0.49 µg/g	⇒ cord BMn at birth ◊ ◊ attention and non-verbal memory in three-year olds. Placental, mother's BMn and Child's HMn ◊ Ø general psychomotor developmental indices.
Wasserman *et al*, 2005 (18)	Bangladesh	Children 9.5–10.5 y	Blood: 12.8 (3.2) µg/L* Water: 4–3,908 µg/L.	WMn and BMn concentrations ◊ Ø overall, verbal and performance IQ scores.

In Bangladesh, a country well known for high Mn and arsenic (As) levels in drinking water from the bedrock, blood Mn showed no correlation with psychomotor outcomes after adjusting for As levels. These non-significant findings may be explained by the high concentration of As in Bangladeshi's water (*i.e.*, the harmful effects of manganese may be undetectable compared to the effects of As). Mn levels found in well water in some Quebec communities - including the Eastern Townships - are similar to those reported in Bangladesh, while concentrations of arsenic are nowhere near as high here as in Bangladesh (17, 18). Bouchard *et al* (2010) studied the relations between drinking water Mn, hair Mn and the cognition of children aged 6–13 from the Eastern Townships (19). They found a negative correlation between drinking water Mn level and cognition in children aged 6–13, but not with hair Mn.

The objective of this study was to examine the association between blood Mn and psychomotor skills, in a sample of children aged 6–7 years. We also explored the risk of ADHD and other neurodevelopmental comorbidities potentially related to Mn exposure. In addition, we included lead exposure as a covariate, given the known neurotoxic effect of lead in children and the strong correlation between lead and Mn (6).

Insert Table 1

Material And Methods

Study population

Participant's mothers were recruited between 2007 and 2009 during pregnancy (n = 761) in a prospective cohort (GESTE: GESTation and Environment) from the Eastern Townships region, Québec, Canada (20). Eligible participants were women from the Eastern Townships, Quebec region, aged > 18 y, and able to give informed consent. For this analysis, we excluded women illicit substance users, severely preterm births (< 33 weeks), resuscitated infants, and congenital malformations and families which were not certain to stay in the area for the follow up. The majority of GESTE families are caucasian French-Canadians. At 6–7 years of age, a total of 358 children completed a series of neuropsychological tests. The current analyses were conducted on a subsample 210 children who also provided a blood sample at age 6–7. Children using medication (including psychotropic drugs) were tested without any changes in posology. Test administration was done by two qualified neuropsychologists (ASD, SG) and one trained graduate student (YSG) following a standardized procedure. Five mock administrations were filmed and evaluated to increase interrater reliability. Children with known neurobehavioral disorders were evaluated only by certified neuropsychologists. Final results were validated by one senior neuropsychologist (ASD).

The Institutional Review Board of the CHUS (Centre Hospitalier Universitaire de Sherbrooke) approved the study protocol (05-057-S1, 2008 – 103).

Mn and lead (Pb) analysis

Blood was sampled after the psychomotor testing, collected in a BD Vacutainer®, K2EDTA certified metal-free tube (Becton-Dickinson, San Jose, California), and kept at – 20 °C until analysis which were done by the CTQ (Centre de Toxicologie du Québec, Qc, Canada, ISO/CEI 17043). Levels of Mn and Pb were measured using Induced Coupled Plasma Mass Spectrometry (ICP-MS). The limits of detection were at 2.07 µg/L for Pb and 4.4 µg/L for Mn. No participant was under the LOD for blood Mn or Pb.

Outcomes:

1) Psychomotor Testing

Children completed subtests from the WISC-IV (Wechsler Intelligence Scale for Children - IV) (21) including; Block Design, Coding, Digit Span (forward and reverse), Information, and Vocabulary. Age-specific normalized scores for each subtest were used in our analysis [mean (SD)]. In addition to the WISC-IV, participants completed NEPSY II (Design Copying and Visuomotor Precision subtests), a tool used by clinicians to asses 6 domains of child functioning (22). The current analyses focused on two domains, sensorimotor functioning and visuospatial processing, measured by tests such as design copying and visuomotor precision. Three subtests from the TEA-Ch (Test of Everyday Attention for

Children) (23) were implemented: Sky search, Score! and Score DT. Sky search is used to measure selective attention (B: number of target correctly circle, C: time per target, G: Attentional score), this subtest also includes one motor score (F: time per target of the motor control). Score! and Score DT are used to measure sustained attention (24). Children unable to count to 15 were excluded from Score! and Score DT subtests (n = 10).

2) ADHD

Information on ADHD diagnosis and medication were obtained from caregivers during the visit. Given that some children were currently being evaluated for suspected ADHD diagnosis, we divided our study group into two categories: ADHD (including both suspected – reported by the caregiver - n = 10 and diagnosed ADHD n = 8 ; 7 children with ADHD have a comorbid developmental disorder, i.e. language disorder) and no ADHD [all other children: neurotypical (n = 169) and children with neurodevelopmental disorder other than ADHD (n = 23)].

3) Developmental Coordination Disorder

The Developmental Coordination Disorder Questionnaire - French Canadian (DCDQ-FC) (25) was also completed by the caregiver, the questionnaire is subdivided in 3 sections: control during movement, fine motor skills/ writing and global coordination. Children with DCDQ-FC score greater than 45 points are considered to be at risk of having a Developmental Coordination Disorder (DCD). In addition to the cutpoint, the continuous scores were also considered in this study.

Covariate Data:

To estimate parental cognitive function, caregivers were evaluated by self-administered Raven matrices (26). If both caregivers were evaluated, we used maternal Raven score in our analyses. We found a strong correlation (r = 0.6) between mothers and fathers (n = 82), who were present and evaluated at the same time. A socio-demographic questionnaire was administered to obtain the information about parental education level, marital status, family income and other relevant information (like life stress events, industrial neighbourhood, …). Information about pregnancy was obtained from questionnaires and medical records at enrolment (20). The information on other neurodevelopmental disorders such as autistic spectrum disorder, language delay, motor coordination deficit or any pervasive developmental disorder was obtained from the caregiver.

Statistical analysis:

Statistical analyses were conducted using SAS Software version 9.4 (SAS Institute Inc., Cary, NC, USA). To assess the risk of potential selection bias, we compared data from children included in this analysis with those from children whose parents did not consent to blood sampling (n = 148). Continuous variables were assessed using Wilcoxon-Mann-Whitney test because of a non-normal distribution and categorical data were compared using Chi^2 test.

Blood Mn and Pb and outcome variables distributions were close to normality, except Visuomotor Precision – Total Error score. Visuomotor Precision – Total Error score was Log10 transformed to achieve normal distribution. In addition, given that for Visuomotor Precision – Total Error score and Sky Search scores there is no age-normalized scale, we corrected it for children age using a regression to the mean of residues, a method previously described (27).

There was one missing value for the WISC-IV – Vocabulary because the child was not able to complete the test. We choose not to replace this value.

Linear regression models (SAS PROC GLM), both simple and multivariate, were used to test the association between blood Mn and psychomotor outcomes. WISC-IV subtests, NEPSY II, TEA-Ch and DCDQ scores - treated as continuous variables - were entered as dependent variables, while blood Mn was used as independent variable. Child age, sex, family income, caregiver intelligence, blood Pb, alcohol consumption (yes/no) and cigarette consumption of the mother during pregnancy (yes/no) were tested as potential confounders. Blood Mn was correlated with sex using a T-Test. Dependent variables were correlated with children sex, family income, age, caregiver intelligence and current use of tobacco in pregnancy. Only sex was correlated with both blood Mn and most outcome measures. Based on previous literature, we also considered blood Pb as a potential confounder in our models. Finally, all multivariate models included children sex, family income, age, caregiver intelligence, current use of tobacco in pregnancy and blood Pb as covariates. A statistical interaction with sex was tested for each outcome separately. In addition, a quadratic Mn term was included in the model to test for a potential nonlinear, U-shape, dose-response relationship.

We conducted three sensitivity analyses to evaluate the robustness of our results. First, we re-ran models excluding children with any reported neurodevelopmental disorder (i.e.: Autism spectrum disorder, pervasive development disorder and language delay) (n = 30). Second, we conducted an analysis to evaluate if there were any differences in the psychomotor outcomes between children non medicated for ADHD (*i.e.* suspected for ADHD) (n = 10) and medicated for ADHD (*i.e.* diagnosed for ADHD) (n = 8). Third, we investigated potential differences between children with medicated ADHD (n = 8) and those without any reported neurodevelopmental disorder (n = 169).

To examine the risk of diagnosis of ADHD and other neurodevelopmental disorders, we used logistic regression (SAS PROC LOGISTIC) to estimate the odds ratio (OR) and 95% confidence intervals (CI) of blood Mn using an unadjusted and adjusted model. To examine the risk of being suspected of Developmental Coordination Disorder (DCD), we used a logistic regression (SAS PROC LOGISTIC). The dependent variable was the DCDQ-FC > 45 (n = 13). Potential confounders were children age, sex, blood Pb, alcohol and cigarette consumption of the mother during pregnancy. Dependent variables were not correlated with any of the potential confounders. We kept children sex, blood Pb, alcohol and tobacco use during pregnancy in our models to be consistent with literature data (28, 29).

Results

Cohort data

Table 2 shows participants demographics and distribution of model covariates and outcomes data. There were no differences between participants included in present analyses and those who declined to give a blood sample (n = 148) for any of the characteristics. A total of 13 included participants were using medication for other disease (like asthma) and 5 in the excluded group.

Table 2
Distribution of outcomes variables. [a] Median (range); [b] Number (percent)

Characteristic	Median (range) or Number (percent)	
	Included children (n = 210)	Excluded children (n = 148)
Child age (years)	6.3 (6.00–7.97) [a]	6.53 (6.00–7.98) [a]
Sex: Boy	120 (57) [b]	75 (51) [b]
Girl	90 (43) [b]	73 (49) [b]
Family annual Income (CA$)	80,000 (10,000–600,000) [a]	87,500 (13,000–500,000) [a]
Maternal smoking during pregnancy	7 (3) [b]	14 (9) [b]
Maternal alcohol consumption during pregnancy	18 (9) [b]	7 (5) [b]
Reconstituted family	39 (18) [b]	30 (20) [b]
Maternal education, university level	90 (42) [b]	66 (45) [b]
ADHD, no medication	10 (5) [b]	4 (3) [b]
under medication	8 (4) [b]	9 (6) [b]
Other neurodevelopmental disorders	30 (15) [b]	17 (12) [b]
Mother		
Age at the 6-7y/o follow-up (years)	36.1 (25.2–46.8) [a]	35.7 (26.8–47.9) [a]
Raven raw score	54 (33–60) [a]	54 (39–60) [a]
Mn concentration (µg/L)	9.9 (4.67–21.4) [a]	
Pb concentration (ng/L)	6.94 (2.07–24.0) [a]	

There was no difference between results obtained with the whole study group and those from the analysis restricted to neurotypical children only (for more details see additional file 1). For this reason, we show results from the whole study group. The second sensitivity analysis found no difference in psychomotor outcomes between ADHD children with or without medication. The third sensitivity analysis found no difference between children with medicated ADHD and those without any reported neurodevelopmental disorder.

Insert Table 2

Blood Mn and psychomotor scores

Table 3 shows simple and multivariate model parameters for psychomotor scores in relation blood Mn. Globally, only NEPSY II- Design Copying fine motor score was slightly positively correlated with blood Mn in both adjusted and non-adjusted models.

Table 3
Generalized linear model slope estimation for cognitive and motor outcomes [β (95% CIs)]. Adjustment variables: Children sex, family income, age, caregiver intelligence, cigarette consumption and blood lead as covariates

	Blood Mn model (µg/L)	**Blood Mn (µg/L)**
	β (95% CI)	β (95% CI) **adjusted**
WISC IV		
Digit span		
Forward	0.09 (-0.06 to 0.23)	0.10 (-0.04 to 0.25)
Reverse	0.05 (-0.07 to 0.18)	0.08 (-0.05 to 0.20)
Combined	0.09 (-0.03 to 0.22)	0.11 (-0.02 to 0.23)
Coding	0.04 (-0.10 to 0.18)	0.05 (-0.09 to 0.19)
Block design	0.00 (-0.14 to 0.14)	0.00 (-0.14 to 0.14)
Vocabulary	0.10 (-0.03 to 0.24)	0.10 (-0.03 to 0.24)
Information	0.08 (-0.04 to 0.19)	0.09 (-0.03 to 0.20)
NEPSY		
Design copying		
Fine motor score	0.17 (0.03 to 0.32) **	0.16 (0.01 to 0.30) **
Visuomotor Precision		
Total error	-0.80 (-2.08 to 0.46)	-0.73 (-1.98 to 0.51)
Visuomotor precision score	0.10 (-0.07 to 0.27)	0.09 (-0.08 to 0.26)
TEACH		
Sky search		
Score B	0.17 (-0.005 to 0.35)	0.17 (-0.01 to 0.35)
Score C	-0.12 (-0.28 to 0.04)	-0.105 (-0.27 to 0.06)
Score F	-0.01 (-0.31 to 0.29)	-0.05 (-0.35 to 0.25)
Score G	-0.06 (-0.21 to 0.09)	0.05 (-0.20 to 0.11)
Score !	0.12 (-0.01 to 0.25)	0.11 (-0.02 to 0.24)
Score DT	0.11 (-0.04 to 0.27)	0.11 (-0.04 to 0.26)

CI: confidence interval; **p < 0.05

	Blood Mn model (µg/L)	Blood Mn (µg/L)
DCDQ – FQ total	0.30 (-0.12 to 0.72)	0.27 (-0.15 to 0.69)
Global	0.09 (-0.08 to 0.27)	0.07 (-0.11 to 0.25)
Writing	0.16 (0.01 to 0.32) **	0.13 (-0.02 to 0.28)
Control	0.05 (-0.17 to 0.22)	0.07 (-0.10 to 0.24)
CI: confidence interval; **p < 0.05		

A model including a quadratic Mn term found no evidence of nonlinearity (data not shown).

The sex-stratified analyses yielded one statistically significant association. Boys performed better on the Score DT test (β: 0.29, CI: 0.098 to 0.49) when exposed to higher Mn concentration, while no association was found among girls (β: -0.09, 95% CI: -0.33 to 0.14).

The writing score of the DCD-FQ questionnaire was significantly and positively correlated with blood Mn in the non-adjusted models, but not in the adjusted model.

Insert Table 3

Blood Mn and at risk of ADHD/DCD

We observed no association between blood Mn and ADHD diagnosis (OR unadjusted: 0.87; 95% CI: 0.68–1.12; adjusted: 1.06; 0.90–1.24) or risk for DCD (OR unadjusted: 0.89; 95% CI: 0.69–1.15; adjusted: 1.06; 0.90–1.25).

Discussion

Our study shows no correlation between blood Mn and psychomotor skills in school-age children from the general population, contrary to our hypothesis. This finding aligns with previous reports by Lucchini (28) and Wasserman (18). In addition, we observe no association with the diagnosis or suspicion of ADHD, as well as non-significant associations found in the attention scores from TEA-Ch. We also found no association between high blood Mn and the risk of DCD, confirmed by our results from the motor scores in NEPSY II.

One potential explanation for these non-significant findings are the low absolute levels of Mn in the blood that may not reach a toxic level in our population, thus not affecting children psychomotor skills. Also, at 6–7 years of age the physiological regulation of Mn in the body -reduction of absorption in the intestines and increase excretion by the liver- may be sufficient to reduce blood Mn levels and its potential toxic effect (30). Another potential explanation is that our population is not exposed to any Mn-releasing industry as seen in other studies (31, 32). The only Mn-releasing industry in the Eastern Townships is a small paper mill plant, that was closed approximately 2 years before our study. Thus, we may speculate

that for the majority of children in our study group the major source of Mn would be water and/or food. However, we do not have any individual measurement of Mn concentrations in drinking water or air to confirm this hypothesis. Blood Mn levels in our study group are similar to those reported in U.S. samples (33). Moreover, our findings regarding blood Mn concentration (mean : 9.9 µg/L, range : 4.67–21.4) are similar to those for children aged 6 to 14 published by Lucchini *et al* (mean: 11.24 µg/L, range: 4.25–24.10) (34), Bhang *et al* (mean: 14.10 µg/L, range: 4.00–24.10) (35) and Haynes *et al* (36) (Geometric mean: 9.67 µg/L, range: 6.1–18.8) in Italy, Korea and Bangladesh, respectively. A similar range of studies highlight the effects of blood Mn on psychomotor scores (see Table 1).

A recent publication discusses the presence and the role of Type III error in environmental health science. Type III error is defined as correctly rejecting the null hypothesis (in our case Mn in drinking water is not toxic for school age children) for the wrong reasons (37, 38). This error occurs most often when a causal factor, such as socio-economic status or other environmental factors, etc., is homogeneously distributed in the population. Type III error mostly comes from a dichotomic reasoning. We may inconsistently conclude that only one factor is the cause rather than the interaction between factors (e.g. genetic and environment, socio-economic status and environment, etc.) (38).

It is possible that some studies did not consider the interactions that may lead to a toxic effect of Mn. Studies based on socio-economically disadvantaged populations often show a toxic effect of Mn. These are predominantly people living in poor rural areas in Mexico, Brazil (31, 32), and Bangladesh (39). Low socio-economic status can lead to an increasing of malnutrition in the population. The exposure to Mn is higher among malnourished children, they absorb Mn more efficiently and eliminate it less efficiently (40, 41). Socio-economically disadvantaged communities also face higher concentrations of pollutants in their environment (42), thus increasing the risk of toxic interaction with Mn.

Studies focused mainly on communities exposed to Mn through food and water remain inconsistent. Bouchard *et al* (2011) (19) and Oulhote *et al* (2014) (43), found a negative association between water and hair Mn and IQ. In contrast, Wasserman *et al* (2006) (18), Khan *et al* (2011) (39) and Bouchard *et al* (2018) (7) found no association between Mn levels in blood, hair, or water and decreased psychomotor performances.

In adults, the route of exposure plays a crucial role in total intake and Mn accumulation. When exposed to Mn through air, it will bypass physiological control and leads to a rate of absorption of almost 70%. This inhaled Mn will be directly transported to the brain through the olfactive nerve (14). The ingestion of significant quantities of Mn is tightly controlled by the liver, which allows only about 2% of ingested Mn to reach the general circulation, the rest being eliminated through bile into the GI tract (14). Elimination of Mn by hepatobiliary mechanisms is decreased in children (44), however only evidence from neonates are cited to support this claim. There is no evidence that the hepatic elimination of Mn in school aged children might be different compared to adults. The route of exposure may also have an important role in Mn toxicity. This was proposed by Winder et al. (2010) (45), when they estimated that at the same exposure level a child's Mn inhalation is at a greater proportion of the maximum recommended levels

compared to adults. Inhalation provides more rapid uptake of Mn into the blood from lungs, as shown in welders and mice (45). In a review of the inhalation dosimetry methods applied to children's risk assessment, the authors (46) recommended to use a higher uncertainty factor due to increased toxicity of most inhaled chemicals in children.

Mn is suspected to have gender-dependent effect (6). In our study, only one attentional score is positively affected in boys, and tends to be negatively affected in girls. To date, mechanisms underlying this relationship remain unclear and there is no published data on the gender-dependent toxic effect. As only one score was affected, we presume that this result may be due to chance.

Our study has several strengths. Children included in the analyses participated in a prospective population-based birth cohort decreasing the risk of selection bias. We also adjusted for lead levels and most of confounders and effect modifiers we had access to. Our statistical analyses included non-linear modelling to account for a potential U shape association. Our study population is relatively homogenous socially, economically and racially, given that our root population is stable, composed of historical descendants from French and Irish families. This homogeneity is advantageous when we are looking for small effects of environmental pollutants because it reduces a background noise from potential confounders (i.e. "quasi-experimental design"). Blood Mn, our biomarker of exposure, has been shown to better approximate pallidal index, an indicator of Mn accumulation in the brain (relevant in the context of studying its neurodevelopmental effects), than other potential markers including hair or water Mn.

This study has some limitations. The homogeneity of our population can decrease the external validity of our results, which should be carefully extrapolated to a community with different genetic or socioeconomic characteristics. In the same way, we noticed that more educated families tend to stay within the follow up (data not shown), as it was recently observed for other prospective cohorts (47).

Another potential limitation is that we used blood Mn as the only biomarker of exposure, and blood Mn is weakly corelated with ingested Mn (18, 48). However, blood Mn has been reported to have longitudinal stability (33). Furthermore, data on iron deficiency in the cohort was not collected, but there is low prevalence – 3.5% of the children population - of iron deficiency in Canada (49). Children with iron deficiency tend to have high blood Mn (50). Yet increased blood Mn in patients with iron deficiency do not typically increase Mn accumulation in the brain (51, 52). However, even if iron deficiency increases blood Mn, we observed no effect of blood Mn on psychomotor scores thus we expect the direction of results to be the same. In addition, we have no estimation of Mn intake. Most of the Mn in our region comes from water (19) and there is no association between dietary Mn intake and cognitive scores in children (19). Moreover it has been reported that blood Mn is poorly correlated with intake in children and is not affected by subtle intake variation (18). Thus, the lack of estimation of Mn intake should not alter our results.

For this study we used five subscales of the WISC-IV - which did not allow us to estimate the children's full IQ -, two of the NEPSY II and three of the TEACh. These subscales were chosen in order to cover a variety of non-verbal skills within a limit of about 1.5 hour of evaluation, which is feasible in children aged 6–7.

ADHD group selection was based on whether the child took prescription ADHD medication and if the caregiver reported a diagnosis. We may have misclassified ADHD cases as controls if their caregiver did not report physician's diagnosis. Children with ADHD were not asked to stop taking their medicines during the visit, meaning they may appear more neurotypical on continuous assessments of their behaviors, which could weaken any true association. TEA-Ch is a test commonly used to diagnose ADHD and is known to differentiate children medicated for ADHD and those non-medicated (24). However, because children diagnosed with ADHD who were medicated had similar scores on the psychomotor evaluations compared to those who were unmedicated (for more details see additional file 2), any effect of medication is likely minimal. Although we could not confirm DCD diagnosis in medical records, our finding of no association between Mn and DCD as assessed by the parentally-administered DCDQ is supported by our null findings with the NEPSY-II, which also measures dexterity but is more objective because it is administered by study staff.

In conclusion, our study in school-aged children from the general population we did not show evidence of Mn toxicity related to psychomotor and attention skills. Our study does not rule out prenatal Mn toxicity given that fetuses/neonates have immature hepatobiliary mechanisms of Mn elimination (44). Pre and postnatal exposure to Mn has toxic effects in toddlers (48). Those effects may be persistent during the childhood and alter the developmental trajectories of the child (53). There is a need for further comprehensive studies using different matrices and MRI to confirm these results. New biomarker that can better reflect ingested Mn (like meconium or stools) should also be considered in consort with other established biomarkers. Additionally, MRIs can be used to confirm Mn accumulation in children's brains and provide more information on the related anatomical or functional modifications.

Declarations

Ethics approval and consent to participate

The study protocol was approved by the ethical review board of the Centre Hospitalier Universitaire de Sherbrooke. All participants provided written informed consent before their inclusion.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

LS analyzed and interpreted the data regarding blood Mn and psychomotor outcomes. NA enrolled families. ASD conducted psychomotor testing. VG was in charge of the biobank and sample analysis. AB was the coordinator of the project. EW, CB, KB and HEL read and critically reviewed this article. MF assisted in enrolment of participants. AAB and LT are the principal investigator of the GESTE study and LT created the GESTE cohort. All authors read and approved the final manuscript.

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Tables

Table 1: Summary of studies on manganese toxicity. * Arithmetic mean (standard deviation). ** Geometric mean (geometric standard deviation). Blood-Mn: BMn, Hair-Mn: HMn, Water-Mn: WMn

Study	Country	Study population	Mn measurement	Association with Neurodevelopment
Bhang *et al*, 2013(35)	South-Korea	Children 8-11 y	Blood: 4.25 – 31.50 µg/L	Þ blood-Mn (BMn) à à scores in thinking, reading, calculation, and learning scores and Þ cognitive inhibition test scores.
Bouchard *et al*, 2006(54)	Quebec, Canada	Children 6-15 y	Hair: 0.28 – 20.0 µg/g	Þ hair-Mn (HMn) à Þ hyperactive and oppositional behavior was associated.
Carvalho *et al*, 2018(31)	Bahia, Brazil	Children 7-12	Hair: 0.52 – 55.74 µg/g	Þ HMn à Þ hyperactivity and a à of verbal performances.
Chung *et al*, 2015 (55)	South-Korea	Mother-infant pairs recruited prenatally	Maternal blood: 22.5 ± 6.5 µg/L *	à and Þ levels of BMn à à psychomotor development scores in infants at 6 months.
Haynes *et al*, 2015 (36)	Ohio, U. S	Children 7-9 y	Hair: 416.51 (2.44) ng/g ** Blood: 9.67 (1.27) µg/L **	BMn and HMn à à full scale IQ, perceptual reasoning, à processing speed.
Henn *et al*, 2010 (56)	Mexico City, Mexico	Children enrolled before birth and followed until 3 y	Blood: 24.3 (4.5) µg/L* at 12 months, 21.1 (6.2) * µg/L at 24 months	BMn à Ø with the Bayley Scales of Infant Development-II.
Hernández-Bonilla *et al*, 2011 (57)	Hidalgo, Mexico	Children 7-11 y	Exposed: Hair: 4.2 – 48 µg/g Blood: 5.5 – 18 µg/L Non-exposed: Hair: 0.06 – 3.6 µg/g Blood: 5.0 – 14 µg/g	HMn à Ø neuromotor outcomes (grooved pegboard, finger tapping and Santa Anna test in children. Þ BMn à à finger tapping. BMn à Ø Other motor function measures.
Khan *et al*,	Bangladesh	Children 8-	Water: 40.0 –	BMn à Ø externalizing (attention

2011 (39)		11 y	3442.5 µg/L Blood: 6.3 – 33.9 µg/L	problems and aggression) and internalizing (anxiety) behaviors and a total behavioral score. Þ Water Mn (WMn) was associated with Þ externalizing and internalizing behaviors.
Kim *et al*, 2009 (58)	South-Korea	Children 8-11 y	Blood: 5.30–29.02 µg/g	Þ BMn à à overall and verbal IQ.
Lucchini *et al*, 2012a (34)	Valamonica et Garda lake, Italia	Children 11-14 y	Hair: 0.024 – 3.45 µg/g Blood: 4 – 24.1 µg/L	Þ WMn and BMn à Þ Tremor intensity in dominant hand.
Lucchini *et al*, 2012b (28)	Valamonica et Garda lake, Italia	Children 11-14 y	Hair: 0.024 – 3.45 µg/g Blood: 4 – 24.1 µg/L	Mn à Ø IQ (full scale, verbal and performance) or behavioral (hyperactivity, attention deficit) scores in adjusted analyses.
Menezes-Filho *et al*, 2011 (32)	Salvador, Brazil	Children 7-12 y	Hair: 0.10 – 86.68 µg/g Blood: 2.7 – 23.4µg/L	Þ HMn à Þ Externalizing behaviors and attention problems on the Child Behavior Checklist for girls, but not boys. BMn à Ø IQ scores.
Oulhote *et al*, 2014 (43)	Quebec, Canada	Children 6-13 y	Hair: 0.1 – 20.7 µg/g Water: 1 – 2,701 µg/L	Mn à significant à in memory (hair and water), attention (hair), and motor function (water).
Riojas-Rodríguez *et al*, 2010 (59)	Hidalgo, Mexico	Children 7-11 y	Exposed: Hair: 4.20 – 48 µg/g Blood: 5.5 – 18 µg/L Non-exposed: Hair: 0.06 – 3.64 µg/g Blood: 5.0 – 13 µg/g	Þ HMn à à full scale, verbal and performance IQ scores in children. Ø associations à BMn with à full scale, verbal and performance IQ scores.
Takser *et al*, 2003 (60)	Paris, France	Mother-infant pairs followed until 6y	Hair: 0.05 – 13.33 µg/g	Þ cord BMn at birth à à attention and non-verbal memory in three-year olds. Placental, mother's BMn and

			Cord blood: 14.9 – 92.9 µg/L Mother's blood: 6.3 – 151.2 µg/L Placenta: 0.01 – 0.49 µg/g	Child's HMn à Ø general psychomotor developmental indices.
Wasserman *et al*, 2005 (18)	Bangladesh	Children 9.5-10.5 y	Blood: 12.8 (3.2) µg/L* Water: 4 – 3,908 µg/L.	WMn and BMn concentrations à Ø overall, verbal and performance IQ scores.

Table 2: Distribution of outcomes variables. [a] Median (range); [b] Number (percent)

Characteristic	Median (range) or Number (percent)	
	Included children (n= 210)	**Excluded children** (n=148)
Child age (years)	6.3 (6.00 – 7.97) [a]	6.53 (6.00 – 7.98) [a]
Sex: Boy	120 (57) [b]	75 (51) [b]
Girl	90 (43) [b]	73 (49) [b]
Family annual Income (CA$)	80,000 (10,000 – 600,000) [a]	87,500 (13,000 – 500,000) [a]
Maternal smoking during pregnancy	7 (3) [b]	14 (9) [b]
Maternal alcohol consumption during pregnancy	18 (9) [b]	7 (5) [b]
Reconstituted family	39 (18) [b]	30 (20) [b]
Maternal education, university level	90 (42) [b]	66 (45) [b]
ADHD, no medication	10 (5) [b]	4 (3) [b]
under medication	8 (4) [b]	9 (6) [b]
Other neurodevelopmental disorders	30 (15) [b]	17 (12) [b]
Mother		
Age at the 6-7y/o follow-up (years)	36.1 (25.2 – 46.8) [a]	35.7 (26.8 – 47.9) [a]
Raven raw score	54 (33 – 60) [a]	54 (39 – 60) [a]
Mn concentration (µg/L)	9.9 (4.67 – 21.4) [a]	
Pb concentration (ng/L)	6.94 (2.07 – 24.0) [a]	

Table 3: Generalized linear model slope estimation for cognitive and motor outcomes [β (95% CIs)]. Adjustment variables: Children sex, family income, age, caregiver intelligence, cigarette consumption and blood lead as covariates

	Blood Mn model (µg/L)	**Blood Mn** (µg/L)
	β (95% CI)	β (95% CI) **adjusted**
WISC IV		
Digit span		
Forward	0.09 (-0.06 to 0.23)	0.10 (-0.04 to 0.25)
Reverse	0.05 (-0.07 to 0.18)	0.08 (-0.05 to 0.20)
Combined	0.09 (-0.03 to 0.22)	0.11 (-0.02 to 0.23)
Coding	0.04 (-0.10 to 0.18)	0.05 (-0.09 to 0.19)
Block design	0.00 (-0.14 to 0.14)	0.00 (-0.14 to 0.14)
Vocabulary	0.10 (-0.03 to 0.24)	0.10 (-0.03 to 0.24)
Information	0.08 (-0.04 to 0.19)	0.09 (-0.03 to 0.20)
NEPSY		
Design copying		
Fine motor score	0.17 (0.03 to 0.32) **	0.16 (0.01 to 0.30) **
Visuomotor Precision		
Total error	-0.80 (-2.08 to 0.46)	-0.73 (-1.98 to 0.51)
Visuomotor precision score	0.10 (-0.07 to 0.27)	0.09 (-0.08 to 0.26)
TEACH		
Sky search		
Score B	0.17 (-0.005 to 0.35)	0.17 (-0.01 to 0.35)
Score C	-0.12 (-0.28 to 0.04)	-0.105 (-0.27 to 0.06)
Score F	-0.01 (-0.31 to 0.29)	-0.05 (-0.35 to 0.25)
Score G	-0.06 (-0.21 to 0.09)	0.05 (-0.20 to 0.11)
Score !	0.12 (-0.01 to 0.25)	0.11 (-0.02 to 0.24)
Score DT	0.11 (-0.04 to 0.27)	0.11 (-0.04 to 0.26)
DCDQ – FQ total	0.30 (-0.12 to 0.72)	0.27 (-0.15 to 0.69)
Global	0.09 (-0.08 to 0.27)	0.07 (-0.11 to 0.25)
Writing	0.16 (0.01 to 0.32) **	0.13 (-0.02 to 0.28)
Control	0.05 (-0.17 to 0.22)	0.07 (-0.10 to 0.24)

CI: confidence interval; **p<0.05

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfile1.docx
- Additionalfile2.docx