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INVERSE ASSOCIATION OF INTELLECTUAL FUNCTION WITH VERY LOW BLOOD LEAD BUT NOT WITH MANGANESE EXPOSURE IN ITALIAN ADOLESCENTS

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Abstract

Background—Pediatric lead (Pb) exposure impacts cognitive function and behavior and co-exposure to manganese (Mn) may enhance neurotoxicity.

Objectives—To assess cognitive and behavioral function in adolescents with environmental exposure to Pb and Mn.

Methods—In this cross sectional study, cognitive function and behavior were examined in healthy adolescents with environmental exposure to metals. The Wechsler Intelligence Scale for Children (WISC) and the Conners-Wells' Adolescent Self-Report Scale Long Form (CASS:L) were used to assess cognitive and behavioral function respectively. *ALAD* polymorphisms rs1800435 and rs1139488 were measured as potential modifiers.

Results—We examined 299 adolescents (49.2% females) aged 11–14 years. Blood lead (BPb) averaged 1.71 µg/dL (median 1.5, range 0.44 – 10.2), mean Blood Manganese (BMn) was 11.1 µg/dL (median 10.9, range 4.00 – 24.1). Average total IQ was 106.3 (verbal IQ = 102, performance IQ = 109.3). According to a multiple regression model considering the effect of other covariates, a reduction of about 2.4 IQ points resulted from a two-fold increase of BPb. The

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Benchmark Level of BPb associated with a loss of 1 IQ-point (BML01) was 0.19 µg/dL, with a lower 95% confidence limit (BMLL01) of 0.11 µg/dL. A very weak correlation resulted between BPb and the ADHD-like behavior (Kendall's tau rank correlation = 0.074, $p = 0.07$). No influence of *ALAD* genotype was observed on any outcome. Manganese was not associated with cognitive and behavioral outcomes, nor was there any interaction with lead.

Conclusions—These findings demonstrate that very low level of lead exposure has a significant negative impact on cognitive function in adolescent children. Being an essential micro-nutrient, manganese may not cause cognitive effects at these low exposure levels.

INTRODUCTION

Lead effects on the central nervous system (CNS) are prominent in the developing brain, and cognitive dysfunction may persist into adulthood (Mazumdar et al., 2012; Needleman et al., 1990). Lead-induced deficits in children have been reported in most functional domains, including total Intelligence Quotient (IQ) and academic skills such as reading and mathematics (Bellinger, 2008). Lead exposure has been also associated with hyperactivity (Nigg et al., 2008) with a large effect-size (Nicolescu et al., 2010). Attentional deficits may affect the performance on the Intelligence Scales (Jepsen et al., 2009); therefore the cognitive effects of lead may be mediated by a basic impairment of attention level. Manganese can affect cognitive function in children (Bouchard et al., 2011; Menezes-Filho et al., 2011; Riojas-Rodriguez et al., 2010; Wasserman et al., 2006; Wright et al., 2006), and co-exposure of lead and manganese may further affect neuro-development beyond exposure to either one alone (Henn et al., 2012). There is large variation in the individual susceptibility to the effects of lead exposure that may be explained, at least in part, by genetic factors. Polymorphisms in the δ -aminolevulinic acid dehydratase (*ALAD*) gene modify the effects of lead on heme synthesis, kidney function, central and peripheral nervous system (Pawlas et al., 2012; Scinicariello et al., 2010; Zheng et al., 2011). Lead inhibits the *ALAD* enzyme, which catalyzes a step in the heme biosynthetic pathway. Heterozygote and homozygote carriers of the variant allele (often called *ALAD2*) of *ALAD* rs1800435 appear protected against lead toxicity compared to the *ALAD1* homozygote carriers. Pawlas et al. recently reported that *ALAD* rs1139488 TT and CT carriers were more susceptible to lead impairment of IQ compared to CC carriers (Pawlas et al., 2012).

Intense scientific discussion has focused on the identification of adverse neurocognitive effects at very low BPb levels. Blood lead levels above 10 µg/dL are clearly associated with adverse outcomes on the IQ (Pocock et al., 1994). Moreover, a notable body of evidence has emerged showing cognitive declines in children at BPb levels below 10 µg/dL (Canfield et al., 2003; Lanphear et al., 2005). Several cohort and cross sectional studies and meta-analysis support that there is no identified BPb without deleterious effects (Wigle and Lanphear, 2005), consistent with estimates that BPb levels in contemporary humans are >50-fold higher than natural levels (Flegal and Smith, 1992; Smith and Flegal, 1992). Recently, U.S. CDC accepted the recommendation by the Advisory Committee on Childhood Lead Poisoning Prevention that a blood lead level reference value of 5 µg/dL, based on the 97.5th percentile of the NHANES blood lead level distribution in children 1–5 years old, be used to identify children with elevated blood lead levels (ACCLPP, 2012). The European Food and Safety Authority (EFSA) did not establish a guidance level for lead, in absence of a clear threshold below which adverse effects would not occur for fetuses, infants and children (EFSA, 2010).

Here, we examined cognitive and behavioral functions in 299 adolescents aged 11–14 years with environmental exposure to various metals including lead and manganese in the Valcamonica and Garda Lake regions of Northern Italy. Our prior epidemiological studies in this region had shown high prevalence of Parkinsonism in aged adults in the vicinities of

ferroalloy emission points, with significant associations between regional prevalence of Parkinsonism and manganese levels in deposited dust (Lucchini et al., 2007). Increased tremor, impaired motor coordination and odor identification have been also observed in adolescents (Lucchini et al., 2012) and elderly (Rentschler et al., 2012) from the same area as associated to manganese exposure.

METHODS

Study area and subjects

In this cross sectional study, cognitive function and behavior were examined in healthy adolescents with environmental exposure to metals, including lead and manganese, from anthropogenic emissions in the Province of Brescia, Italy.

The study sites were Valcamonica, a valley in the pre-Alps where ferroalloy plants had been operating for about a century until 2001, and the Garda Lake, a tourist area with limited industrial activity. Analysis of metals in deposited dust (Zacco et al., 2009), airborne particles (Borgese et al., 2011) and surface soil (Lucchini et al., 2012) showed higher levels of manganese, lead, iron, zinc, in the area of Valcamonica compared to Garda Lake. Subjects were recruited from junior high schools (total 20 schools) of the local public school district. Children, parents and teachers were invited to group discussions with the research team at the schools, where the study aims and methodology were explained in details and informed consent was obtained from the primary caregiver of each child. Inclusion criteria were: i) to be born in the respective areas within a family residing in the area for at least two generations, ii) have been a resident in the study areas since birth, and iii) being between 11 and 14 years old. Exclusion criteria included: i) presence of a diagnosed neurological, hepatic, metabolic, endocrine or psychiatric clinical condition, ii) previous total parental nutrition as it may contain high manganese levels (Chalela et al., 2011), iii) family history of neurodegenerative disease, iv) consumption of medications with known neuro-psychological side-effects, v) deficits in hand and/or finger function, and vi) visual deficits not adequately corrected.

Exposure assessment

Venous whole blood (4 mL) was collected from the cubital vein using a 19 gauge butterfly catheter into a lead-free Li-heparin Sarstedt Monovette Vacutainer. A spot urine sample (50–200 mL) was collected into a clean, sterile polyethylene container. All samples were stored at 4°C until analyzed at the laboratory facility of the University of Brescia. Lead and manganese were measured in blood and urine with Zeeman graphite furnace atomic absorption spectrometry (GFAAS, Varian SpectrAA) in the Industrial Hygiene laboratory at the University of Brescia, Italy, using methods previously reported (Apostoli et al., 2000). Quality control was assured within the Inter-comparison Program 44, 2009, by the Institute and Outpatient Clinic for Occupational, Social and Environmental Medicine of the University of Erlangen-Nuremberg, Germany. The detection limit for BPb measurements by GFAAS was 0.40 µg/dL. Blood lead values that fell near or below the detection limit by GFAAS (i.e., 0.6 µg/dL, n=32 samples) were reanalyzed by high resolution inductively coupled plasma mass spectrometry (Finnigan XR ICP-MS) at the trace metal clean laboratory of the University of California, Santa Cruz. The detection limit for these analyses was 0.010 µg/dL blood, with an accuracy of 95% or better based on repeated analyses of U.S. National Institutes of Standards and Technology Standard Reference Material 955c, and analytical reproducibility of 5% relative standard deviation based on triplicate processing and analyses of blood samples.

Because of the lack of predictivity of manganese biomarkers (Apostoli et al., 2000; Smith et al., 2007), manganese was measured also in environmental media including PM10 airborne particles and soil, in addition to blood, urine, hair. Further details on exposure assessment methodology are available in a separate publication (Lucchini et al., 2012).

Genetic analysis

DNA was extracted from peripheral whole blood using the QIAamp DNA Blood Mini kit (QIAGEN, Hilden). *ALAD* rs1139488 (also referred to as *RsaI*, T/C exchange) and rs1800435 (also referred to as *MspI* with *ALAD1* and 2 as alleles, C/G exchange) were analyzed. The genotyping was determined by Taqman assay on an ABI7900 instrument (Applied Biosystems, CA, USA) according to the manufacturer's protocol. Five percent of the samples were re-assayed for each SNP and there was a perfect concordance between the runs. The distribution of genotypes for *ALAD* showed no deviation from the Hardy–Weinberg equilibrium calculated by Fisher's exact test.

Assessment of cognitive behavioral functions

The adolescents were examined in the morning, according to standardized conditions in well illuminated and quiet rooms within the local schools. The Wechsler Intelligence Scale for Children-Third Edition was administered. This scale is a test of general intelligence, developed for the use with children aged 6–16 years. In addition to providing a rating of children's overall intelligence, the test is composed by a verbal IQ, which provides a rating for verbal comprehension and output, and a performance IQ, which provides a rating for perceptual organization. Two trained neuro-psychologists administered the 10 subtests for the determination of the total IQ. The mean standard score is 100 ± 15 . All IQ scores are normalized by age.

The Conners-Wells' Adolescent Self-Report Scale-Long Form (CASS.L) (Conners et al., 1997) was administered to all children. It contains 87 items which yield scores for eight subscales concerning adolescent's behavior and is recommended for adolescents aged from 12 to 17 years. We included the 11 years old children for this test, as the percentage of this age class was lower and equally distributed in the study areas. Adolescents rate their feelings and behaviors over the past month on a 4 point Likert scale. The following 10 subscales are assessed: family problems, emotional problems, conduct problems, cognitive problems/inattention, anger control problems, hyperactivity, attention deficit hyperactivity disorder (ADHD) index, DSM-IV (disattention), DSM IV (hyperactivity/impulsivity), and DSM IV (Total).

Subjects' examination took place at school, which did not allow for blind assessment from the target area. Therefore we adopted a repeated blinded test scoring procedure that was performed by different examiners from the one who administered the test on site. Repeated scoring did not show significant differences between blind and not blind examiners.

Potential confounders

The demographic factors included: adolescent's gender and age at testing, the educational level of both parents and a measure of socioeconomic status (SES), family size, parity order and the body mass index (BMI). The SES was assessed according to a methodology developed in Italy that combines parental education and occupations (Cesana et al., 1995). Education was divided into three levels: low (elementary and junior high school), medium (senior high school) and high (university degree and post-degree). Occupations were grouped in three categories according to an ordinal scale according to WHO criteria (WHO, 1988) and based on information provided by the Italian National Institute for Statistics. The low SES category included housewives, low-unskilled workers, hospital ancillaries, social

workers; the middle SES employees, teachers, educators, nurses, craftsmen, carpenters; the high SES lawyers, engineers, entrepreneurs, physicians. We considered also the effect of the area of residence (Lower, Median, Upper Valcamonica and Garda Lake), to account for possible unmeasured socio-cultural, genetic differences. Alcohol consumption was ascertained by questionnaire as both a dichotomic variable (yes/no) and a quantitative estimate of the weekly intake in grams, based on the number of declared alcoholic beverages. Smoking habits was also assessed as a dichotomic variable (yes/no) and number of cigarettes per week.

Hematological parameters were hemoglobin and ferritin levels, based on the observation of negative effect on cognition in anemic conditions (Lanphear et al., 2005). However, as hemoglobin and ferritin were collinear, only hemoglobin was retained in the statistical analysis.

Statistical analysis

Study participants were enrolled from various classes within different sections from twenty different schools, therefore we used a hierarchical mixed-effects regression (ME) model (Pinheiro, 2000), to account for the possible non independence of the observations within the same classes, sections and schools. The analysis of the variance component showed that the structure of hierarchical error (i.e., class within section within school) accounted for only 2% of the residual variance and therefore it was considered as negligible. Consequently, we reduced the ME regression model to a simpler Ordinary Least Squares regression model (OLS). To obtain a parsimonious model and test the conjoint effects of all variables on the IQ scores, a full regression model was used and the unrelated variables were eliminated in a backward fashion, using an AIC-based (Akaike's Information Criterion) stopping rule (Venables, 2002). The BPb variable was log transformed in its natural logarithm in order to meet the following regression requirements: i) approximate normality of residuals, and ii) homogeneity of residual variance. Log-transformation of BPb is a common procedure to obtain a linear relationship between BPb and IQ (Budtz-Jørgensen, 2001), and in fact the transformation yielded a better fit of the data. Deviations from linearity was evaluated by comparing the OLS model with a semi-parametric model (GAM), where the degree of smoothness of the non-parametric part (that represents here the effect of Pb and/or Mn) was determined by generalized cross validation (Wood, 2006). The possible interaction between blood Pb and the Mn-related exposure variables (Blood Mn, hair Mn, soil Mn and air Mn) was tested in the final model. Main genetic effects of *ALAD* polymorphisms as well as the effect of an interaction between *ALAD* (considered here as a categorical variable with 3 levels) and Pb (by including an interaction term *ALAD**ln(BPb)) considering the genotypes as categorical variables in the OLS model).

The methodology described by Budtz-Jørgensen et al. (Budtz-Jørgensen, 2001) was used to derive the Benchmark Dose (BMD) of BPb for a loss of 1 IQ-point (BMD₀₁) and its 95 %-confidence lower bound (BMDL) from the regression model. The BMD is defined as the dose which induces a pre-specified loss in the outcome. This loss is known as the Benchmark response (BMR). We used a BMR of 1 IQpoint (BMR₀₁). Analyses and graphics were made with R (R Core Team, 2011).

RESULTS

Descriptive statistics

We successfully tested 299 subjects, or 72% of the 414 consented adolescents. A total of 115 subjects were excluded for the following reasons: not meeting the residency requirement (14.7%); presence of neurological or psychiatric disease (2.9%); refusal to

participate after initial consent (5.3%); not signing the informed consent (1.2%); endocrine disease (0.25%), metabolic disease (0.25%), history of total parental nutrition (0.25%). Enrolled subjects (49.2% female) were from different families and households, 151 residing in Valcamonica and 148 in the Garda Lake area. Categorical socio-demographic variables and *ALAD* allele frequencies are reported in table 1. Participant subjects had mostly one sibling (61%) and they were mostly the first born (52%). No siblings were included in the cohort. Participants' age was on average 154 months (median 155), and the average BMI was 21.3 (median 20.0, range 13–40). Nine subjects declared a history of alcohol consumption (mean: 1.3 g/week), and three subjects declared a history of smoking cigarettes (mean: 0.1/week). The frequency for the variant allele of rs1800435 (*ALAD2*) was 8.2% and for the variant allele of rs1139488 (C) 34.3%.

Table 2 shows the descriptive statistics of the exposure biomarkers to lead and manganese. The average BPb was 1.71 $\mu\text{g/dL}$ (median 1.5). Only one case exceeded the BPb level of 10 $\mu\text{g/dL}$. Manganese in blood (BMn) averaged 11.1 $\mu\text{g/L}$ (median 10.9) whereas hair manganese averaged 0.166 $\mu\text{g/g}$ (median 0.096). The analysis of PM10 particles and surface soil substrates showed air manganese averaging 42.0 $\mu\text{g/m}^3$ (median 29.4), air Pb 17.8 $\mu\text{g/m}^3$ (median 14.1), soil manganese 711 $\mu\text{g/g}$ (median 579), and soil Pb 43.1 $\mu\text{g/g}$ (median 40.4). The scores of WISC and CASS-L subscales are reported in table 3. The total IQ averaged 106.3 points (median 106), while the verbal IQ averaged 102.0 points (median 103) and the performance IQ averaged 109.2 (median 109).

Regression analysis and BMD calculation

The OLS regression analysis considered firstly the IQ scores as the dependent variable. All biological and environmental exposure parameters were included, as well as area of residency, *ALAD* polymorphisms, and the potential confounders. Unrelated variables were eliminated according to the AIC-based stopping rule. They included all internal and external parameters of manganese exposure, *ALAD* genetic variants and most potential confounders like age, gender, BMI, family size, SES, self-reported alcohol consumption, area of residence, hemoglobin, ferritin, and parity order. The regression on the IQ total score yielded a negative association with $\ln(\text{BPb})$ (beta coefficient: -3.5 ; Figure 2), alcohol consumption (beta coefficient: -12.7 as dichotomous variable yes Vs no), and a positive association with SES-medium Vs low (beta coefficient: 4.8), SES-high Vs low (beta coefficient: 8.8), hemoglobin (beta coefficient: 1.5 for a 1 g/dl increase) (see table 4). The overall effect of the area of residence was significant ($p=0.027$ - the comparisons in table 4 are done with the reference area, i.e. the Garda Lake Area), Table 5 shows also the results for verbal and performance IQ, with the 95% C.I. of the estimates. The relation with alcohol consumption and SES was stronger for the verbal compared to the performance IQ score. The estimated BMD_{01} value from the regression model was 0.19 $\mu\text{g/dL}$, and the BMDL value 0.11 $\mu\text{g/dL}$. The regression analysis was also conducted using the 10 subscales of CASS:L as dependent variable and showed a weak border-line association only between BPb and the ADHD subscale (Kendall's rank correlation $z = 1.82$, $\tau = 0.0742$, $p\text{-value} = 0.069$) (Figure 3). To ascertain a possible role of manganese exposure on both cognitive and behavioral scores, all biological and environmental indicators of manganese exposure were considered in the final regression model as interaction variables between the $\ln(\text{BPb})$ and the log-transformed Mn levels and both the main effect of Mn and its interaction with lead was found to be not significant from a statistical or biological point of view. Also, no interaction between $\ln(\text{BPb})$ and either of the two *ALAD* polymorphisms was detected.

DISCUSSION

Our results are the first, which we are aware of, to demonstrate significant adverse cognitive effects of lead at such very low BPb levels. This finding further supports the growing

awareness of adverse effects of lead at increasing lower levels of exposure over the past 15 years and our BPb is the lowest showing cognitive effects (table 6). Lead exposure levels reported here actually overlap those currently observed among children in most European Countries (Hruba et al., 2012).

In our study, the regression coefficient associated with the $\ln(\text{BPb})$ indicates that after controlling for the other variables considered we expected a reduction of about 2.4 ($-3.483 \cdot \ln(2)$) IQ points in the IQ score for a two-fold increase of the BPb (figure 1 & 2). The log-transformation of the BPb values not only linearized the BPb-IQ relationship but conceptually implies that the effect is not constant over this relatively narrow range of BPb levels (i.e., 0.44 – 10.2 $\mu\text{g/dL}$), and depends on the ratio (not on the difference) between two BPb levels. Therefore, the expected absolute IQ reduction depends on the BPb level itself and is greater at the lower BPb levels (where a minor increase causes a greater percent variation). This is consistent with observations showing that the relative partitioning of Pb in plasma, expressed as the percentage of whole blood Pb in plasma, increases with decreasing BPb levels of 10 $\mu\text{g/dL}$ or lower, suggesting that the relative proportion of whole blood Pb in the more readily exchangeable, and likely more toxicologically active plasma fraction is actually higher at blood lead levels <10 than at moderately elevated blood lead levels (Smith et al., 2002).

A Benchmark for effective prevention of lead neurotoxicity was suggested at BPb level of 2 $\mu\text{g/dL}$ (Gilbert and Weiss, 2006). In 2007 the “Declaration of Brescia on Prevention of the Neurotoxicity of Metals” proposed a reduction of the BPb action level to 5 $\mu\text{g/dL}$ worldwide *“as a temporary level that may need to be revised further downward in future years as new evidence accumulates on toxicity at still lower blood lead levels”* (Landrigan et al., 2007). The same concentration of 5 $\mu\text{g/dL}$ resulted as a BMDL estimated by Murata (Murata et al., 2009). The Benchmark Dose modeling performed by Budtz Jørgensen (Budtz-Jørgensen, 2010) for the European Food and Safety Authority (EFSA) on the cohorts summarized by Lanphear (Lanphear et al., 2005) yielded a BMDL value of 0.21 $\mu\text{g/dL}$. The BMDL calculated from our dose-response curve (BMDL = 0.11 $\mu\text{g/dL}$) is slightly lower than the BMDL calculated by Budtz-Jørgensen (Budtz-Jørgensen, 2010) (BMDL = 0.21 $\mu\text{g/dL}$). It is important to note that since the blood lead – IQ relationship is non-linear, the BMDL would be specific to the range of blood lead level, being higher at the lower range of blood lead. Our estimate further supports the lack of a safe threshold for pediatric lead exposure, supporting the EFSA’s indication on this matter.

The BPb association was stronger with the performance IQ compared to verbal IQ score, differently from other studies that found a stronger association between BPb and verbal IQ (Surkan et al 2007; Kim et al. 2009). Some studies observed a consistent performance decrements associated with higher lead exposure throughout the preschool/elementary school ages (Bellinger et al, 1991; Wasserman et al. 1997) while verbal decrements were not apparent until 10–11 years of age. Min et al (2009) observed a significant relation, at 4 years, between blood lead level and Performance IQ, but not Verbal IQ; while verbal decrements became apparent only at age 11. Mazumdar et al. (2011) found that Performance IQ was significantly related to blood-lead concentration but in the earlier childhood: at age of 6 months and 4 years and average blood-lead concentration, while Verbal IQ was significantly related to blood-lead concentration at 10 years. The model also confirmed the positive influence on IQ of socio-economic status (SES) and parental education levels. No influence on the IQ was noted by family size, nor by birth order, confirming a previous observation (Kristensen and Bjerkedal, 2007). A limitation of our study is that we collected some information about the environment (caregivers’ level of study and type of job, number of siblings, birth order) and parents’ education through questionnaires; there was no direct evaluation of the home environment and a measurement of parents’ IQ.

Genetic modifications by *ALAD* on the lead toxicity was not observed, which may be due to limited statistical power for *ALAD* rs1800435, for which the variant genotype was quite rare in our population. That may explain why we could not prove or disregard our main hypothesis, i.e., that carriers of *ALAD2* are more protected against neurotoxic effects of lead. For *ALAD* rs1139488, the gene frequencies were more favorable from a statistical power point of view, but no significant effects were observed. Pawlas (Pawlas et al., 2012) reported an influence of *ALAD* rs1139488 T on lead. However, the average BPb (4.66 µg/dL) was about three times higher compared to our study.

Alcohol can influence the child's IQ, as shown for mother's prenatal alcohol consumption (Mazumdar et al., 2011) or caregivers' home alcohol consumption (Chiodo et al., 2007). We observed a strong and negative association of alcohol consumption by the adolescents with their total, verbal and performance IQ. However it should be noted that very few subjects reported alcohol consumption and that our data are self-reported. It is possible those children with lower IQ have a greater tendency towards drinking, and that our self-reporting questionnaire did not capture this.

A weak association was also observed between BPb and ADHD index of the Conners Scales. Nigg (Nigg, 2008) found that lead was related to weakened cognitive control, a mechanism often associated with risk for ADHD via breakdowns in frontal-striatal neural circuits. In our study the association of lead exposure and the ADHD score was weak and this may be due to several factors: i) the very low BPb levels observed in our cohort; ii) the exclusion of clinical ADHD cases from enrollment; and/or iii) the cut-off minimum value of the ADHD score, which precludes our assessment of this relationship at lower scores.

No association was found between manganese exposure and cognitive/behavioral functions. The co-exposure between lead and manganese in previous pediatric studies (Menezes-Filho et al., 2011; Riojas-Rodriguez et al., 2010) has been approached using BPb as a covariate to which manganese exposure was adjusted for. In those studies, there was an effect of blood manganese on IQ but not BPb as reported by the authors. The studies on lead-manganese co-exposure in maternal-infant pairs by Kim (Kim et al., 2009) and Henn (Henn et al., 2012) considered instead both manganese and lead in blood as independent variables in the regression models. These studies found evidence of a positive interaction between lead and manganese on IQ and Mental Development respectively. The lack of a manganese influence on the IQ in our study should be interpreted in light of the fact that manganese is a micronutrient and the effect on neurodevelopmental functions are based on an inverse U-shaped curve. Manganese exposure in this study is historical and currently quite low, therefore it may not be sufficient to cause cognitive impairment. The ferroalloy emission ceased in 2001, therefore exposure intensity was likely greatest when our subjects were 0 – 2 yrs of age. This implies that cognitive effects from manganese may have occurred at a younger age but became reversible after exposure cessation. Reversibility did not occur with the effects of manganese on tremor, motor and odor function, that are still persistent today, as shown in a different publication (Lucchini et al., 2012). Exposure to lead is, instead, more ubiquitous and depending from different sources other than ferroalloy emission. These hypotheses may explain the current effect of lead, and not of manganese, on the IQ.

In conclusion, the present study shows a significant negative association between BPb level on IQ and a borderline positive correlation between BPb and hyperactivity in adolescents. The fact that these effects were observed over a range of BPbs considered near 'background' in many countries underscores the importance of primary prevention and of further reducing the levels of lead in the environment.

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The work described has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). The research protocol was approved by the Ethical Committees of the local Public Health agencies of Valcamonica and Brescia, Italy.

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Highlights

- We examined 299 children exposed to Pb and Mn from industrial ferroalloy emission
- BPb averaged 1.71 $\mu\text{g/dL}$ (median 1.5) and was inversely related to the IQ scores
- The Benchmark of BPb was 0.19 $\mu\text{g/dL}$, and the lower 95% confidence limit 0.11 $\mu\text{g/dL}$
- No Mn x Pb interaction and no separate effect of Mn on the IQ scores was observed
- This study shows cognitive effects of lead at the lowest exposure level

Figure 1.
Relationship between blood lead and IQ: natural scale (Restricted Cubic Spline fit).

Figure 2.
BPb-IQ relationship: logarithmic transformation of the BPb axis (Tick marks are labeled in the original (untransformed) scale (Ordinary Least Square fit).

Figure 3. BPb-ADHD relationship: logarithmic transformation of the BPb axis (Tick marks are labeled in the original (untransformed) scale (OLS fit).

Table 1

Socio-demographic and genetic variables (total n.: 299 subjects)

Variable	Levels	n	%
Geographic area	GL	148	49.5
	LVC	46	15.4
	MVC	83	27.8
	UVC	22	7.4
Gender	F	147	49.2
	M	152	50.8
Age (years)	11	53	17.7
	12	102	34.1
	13	122	40.8
	14	22	7.4
Socioeconomic	low	73	24.5
	medium	124	41.6
	high	109	40.0
Mother's education	low	112	37.5
	medium	157	52.5
	high	30	10.0
Siblings	none	47	15.7
	one	183	61.2
	two	58	19.4
	three	11	3.7
Birth order	first	155	51.8
	second	114	38.1
	third-fourth	30	10.0
ALADrs1800435	2-2 (CC)	3	1.0
	1-2 (CG)	43	14.5
	1-1 (GG)	251	84.5
ALADrs1139488	CC	32	10.8
	CT	140	47.1
	TT	125	42.1

VC Lower ValCamonica, MVC – Mid ValCamonica, UVC – Upper ValCamonica

Table 2

Exposure indicators and biomarkers to lead and manganese

Variable	n	Min	1 st q.le	Mean	Median	3 rd q.le	Max	IQR
Blood lead (BPb) (µg/dL)	299	0.44	1.10	1.71	1.50	2.10	10.2	1.00
Blood manganese (µg/L)	299	4.00	8.80	11.1	10.9	12.9	24.1	4.10
Hair manganese (µg/g)	186	0.024	0.062	0.166	0.096	0.182	3.45	0.120
Air Manganese (ng/m ³)	189	1.24	15.38	41.99	29.37	47.20	516.70	31.82
Soil Manganese (ppm)	299	159.76	424.92	722.07	529.12	1001.10	1729.10	483.14

IQR: InterQuartile Range

Table 3

WISC and CASS:L scores

Variable	Min	1 st Q.le	Mean	Median	3 rd Q.le	Max	IQR
WISC							
Verbal IQ	69	93	102.0	103	111	135	18
Performance IQ	66	100	109.2	109	118	136	18
Total IQ	71	98	106.3	106	115	138	17
CASS:L							
Family problems	35	38	45.0	42	50	92	12
Emotional problems	35	40	47.6	45	54	88	14
Conduct problems	37	42	45.9	45	49	79	7
Cognitive problems/Inattention	35	41	47.5	46	52	95	11
Anger control problems	35	41	47.3	45	51	76	10
Hyperactivity	35	41	50.5	48	58	88	17
ADHD Index	36	40	46.7	45	51	89	11
DSM-IV (disattention)	35	44	49.7	48	55	77	11
DSM IV (hyperactivity/impulsivity)	35	41	47.0	45	52	87	11
DSM IV (total)	35	42	48.3	47	53	83	11

IQR: InterQuartile Range, total n. 299 subjects

Table 4

Multiple regression model with total IQ as dependent variable

	Estimate	Std. Error	T value	Pr(> t)
Intercept	82.362	10.538	7.816	<0.0001
ln(B-Pb)	-3.483	1.481	-2.351	0.0194
SES (medium Vs low)	4.790	1.698	2.821	0.0051
SES (high Vs low)	8.826	1.781	4.955	<0.0001
Area* (lower VC Vs Garda)	-2.735	1.938	-1.411	0.1592
Area (mid VC Vs Garda)	2.512	1.602	1.568	0.1180
Area (upper VC Vs Garda)	-4.328	2.663	-1.625	0.1053
Hemoglobin	1.501	0.760	1.975	0.0493
Alcohol (yes Vs no)	-12.698	3.905	-3.252	0.0013

VC Lower ValCamonica, MVC – Mid ValCamonica, UVC – Upper ValCamonica, n.s. – not significant.

Table 5

Regression models with total, verbal and performance IQ as dependent variables

	Total IQ			Verbal IQ			Performance IQ		
	Estimate	95% C.I.		Estimate	95% C.I.		Estimate	95% C.I.	
Intercept	82.4	(61.7, 103.0)		86.5	(64.7, 108.2)		82.7	(61.4, 104.0)	
Ln(BPb)	-3.5	(-6.4, -0.6)		-2.9	(-5.9, 0.1)		-3.1	(-6.1, -0.1)	
SES (medium Vs low)	4.8	(1.5, 8.1)		5.1	(1.6, 8.6)		3.2	(-0.2, 6.7)	
SES (high Vs low)	8.8	(5.3, 12.3)		8.7	(5.1, 12.4)		6.5	(2.9, 10.1)	
Area (LVC Vs GL)	-2.7	(-6.5, 1.1)		-2.7	(-6.7, 1.3)		-2.6	(-6.5, 1.3)	
Area (MVC Vs GL)	2.5	(-0.6, 5.7)		1.9	(-1.4, 5.2)		2.7	(-0.6, 5.9)	
Area (UVC Vs GL)	-4.3	(-9.5, 0.9)		-4.3	(-9.8, 1.2)		-3.5	(-8.8, 1.9)	
Alcohol	-12.7	(-20.4, -5.0)		-12.6	(-20.7, -4.6)		-9.3	(-17.2, -1.4)	
Hemoglobin	1.5	(0.0, 3.0)		0.9	(-0.7, 2.5)		1.8	(0.2, 3.3)	

VC Lower ValCamonica, MVC – Mid ValCamonica, UVC – Upper ValCamonica

* the overall effect of the residence area was considered significant ($p=0.027$).

Table 6B-Pb levels (in $\mu\text{g/dL}$) in studies on cognitive effects of lead in children

Reference	Subjects #	Study site	Study design	Age (years)	BPb Mean (SD)
Chen et al., 2005	780	Baltimore, MD, USA,	longitudinal	2	26.2 (5.1)
	731	Cincinnati, OH, USA,	longitudinal	5	12.0 (5.2)
	622	Newark, NJ, USA,	longitudinal	7	8.0 (4.0)
		Philadelphia, PA, USA	longitudinal		
Canfield et al., 2003	154	Rochester, NY, USA	longitudinal	0.5	3.4
				2	9.7
				5	6.0
Roy et al., 2011	651	Chennai, India	cross-sectional	3–7	11.42 (5.43)
Bellinger et al., 2005	74	Chennai, India	cross-sectional	4–14	11.10 (5.6)
Schnaas et al., 2006	150	Mexico City, Mexico	longitudinal	1–5	9.8
				6–10	6.2
Ahamed et al., 2005	62	Lucknow, India	cross-sectional	4–12	7.47 (3.06)
Despres et al., 2005	110	Nunavik, Canada	cross-sectional	4–6	5.0 (3.7)
Chiodo et al., 2007	506	Detroit, MI, USA	cross-sectional	7	5.0 (3.0)
Pawlas et al., 2012	175	Upper Silesia, Poland	cross-sectional	6–10	4.66 (1.23)
Nicolescu et al., 2010	83	Bucharest and Pantelimon, Romania	cross-sectional	8–12	4.15
This study	299	Brescia, Italy	cross-sectional	11–14	1.71 (0.99)