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Assessing windows of susceptibility to lead-induced cognitive deficits in Mexican children

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Abstract

Background—The identification of susceptible periods to Pb-induced decrements in childhood cognitive abilities remains elusive.

Objective—To draw inferences about windows of susceptibility using the pattern of associations between serial childhood blood lead (BPb) concentrations and children's cognitive abilities at 4 years of age among 1035 mother-child pairs enrolled in 4 prospective birth cohorts from Mexico City.

Methods—Multiple longitudinally collected BPb measurements were obtained from children (1, 2, 3, and 4 years) between 1994 and 2007. Child cognitive abilities were assessed at 4 years using the general cognitive index (GCI) of the McCarthy Scales of Children's Abilities. We used multivariable linear regression to estimate the change in cognitive abilities at 4 years of age with a 10 µg/dL increase in childhood BPb concentrations adjusting for maternal IQ, education, marital status, child sex, breastfeeding duration, and cohort.

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Conflict of interest

None.

Results—In separate models for each BPb measurement, 2 year BPb concentrations were most strongly associated with reduced GCI scores at 4 years after adjusting for confounders (β : -3.8 ; 95% confidence interval CI: -6.3 , -1.4). Mutual adjustment for other BPb concentrations in a single model resulted in larger, but less precise estimate between 2 year BPb concentrations and GCI scores at 4 years of age (β : -7.1 ; 95% CI: -12 , -2.0). The association between 2 year BPb and GCI was not heterogeneous ($p = 0.89$), but some BPb and GCI associations varied in magnitude and direction across the cohorts. Additional adjustment for child hemoglobin, birth weight, gestational age, gestational BPb concentrations, or test examiner did not change the pattern of associations.

Conclusions—Higher BPb concentrations at 2 years of age were most predictive of decreased cognitive abilities among these Mexico City children; however, the observed pattern may be due to exposure, outcome, or cohort related factors. These results may help developing countries more efficiently implement childhood Pb prevention strategies.

Keywords

Lead; Children; Epidemiology; Cognitive abilities; Windows of development

1. Introduction

The theory of critical windows of development posits that environmental exposures at specific life stages are more toxic than others (Selevan et al., 2000). Examples include the relationship between in utero diethylstilbestrol exposure and vaginal clear cell adenocarcinoma, as well as gestational methyl mercury exposure and brain development (Axelrad et al., 2007; Swan, 2000). Such windows may be especially relevant for brain development since neural structures and synaptic connections develop and remodel in response to environmental events at discrete life stages (Rice and Barone, 2000b). Chemical exposures at specific developmental windows may interfere with time-dependent processes that lead to toxicity, whereas exposures outside those windows may be less toxic.

Despite the consistent inverse associations between BPb concentrations and cognitive abilities, the results of epidemiological studies do not provide a clear picture regarding the time of greatest susceptibility to Pb-induced neurotoxicity during childhood (Braun and Lanphear, 2007). Results from some epidemiological studies suggest that early childhood Pb exposures may be more important than late childhood Pb exposures (Bellinger et al., 1992; Tong et al., 1996; Wasserman et al., 1994), while other studies suggest that gestational (Schnaas et al., 2006; Tellez-Rojo et al., 2006) or late childhood (Chen et al., 2005; Hornung et al., 2009) BPb exposure is associated with decrements in cognitive development.

Identifying unique windows of susceptibility to Pb exposure is complicated by the intensity, timing, duration, of Pb exposures, as well as the timing of the outcome assessment. Typically, Pb exposures exhibit high within individual correlation over time, making it difficult for any study to identify unique windows of susceptibility (Hornung et al., 2009). Furthermore, the developing brain may adapt to prior environmental insults and associations between Pb exposures and early life cognitive abilities may disappear if cognitive abilities are tested later in life.

Using four prospective birth cohorts from Mexico City, we examined the pattern of association between serial BPb concentrations and children's cognitive abilities at 4 years of age to assess whether BPb concentrations at different times during early childhood were more strongly associated with deficits in cognitive abilities.

2. Methods

Data for this study come from four prospective birth cohorts conducted between 1993 and 2006 in Mexico City. All four cohorts enrolled low to moderate income mothers either during pregnancy or at delivery from the National Institute of Perinatology, Hospital General Dr. Manuel Gea Gonzalez, or clinics affiliated with the Mexican Social Security Institute. Low or moderate socioeconomic position was not a requirement for enrollment into any of the cohorts and was a feature of the women served at these facilities. Women were excluded if they planned to leave the area within 5 years; had a history of infertility, diabetes, or psychosis; consumed alcoholic beverages daily during pregnancy; were addicted to illegal drugs; were diagnosed as a high-risk pregnancy; were pregnant with multiples; or did not intend to breastfeed (Cohort 1 only). Exposure, outcome, and covariate data from these cohorts was collected in a standardized fashion by the same group of investigators and field staff, allowing us to pool the data and generate summary effect estimates.

Each of the four cohorts was designed to either study the impact of Pb on child development or identify interventions to reduce gestational and infancy Pb exposures. Cohort 1 enrolled women at delivery between 1994 and 1995 and tested the impact of postnatal calcium supplementation (randomized trial) on maternal BPb levels during lactation (Hernandez-Avila et al., 2003; Tellez-Rojo et al., 2002). Cohort 2 had two observational studies that examined the relationships between periconceptional and gestational Pb biomarkers in two different groups of women. We designate these cohorts as 2A and 2B. Cohort 2A recruited women between 1997 and 2001 before pregnancy or during the 1st trimester, while 2B recruited women between 1999 and 2003 at delivery (Hu et al., 2006; Tellez-Rojo et al., 2004). Cohort 3 recruited women between 2001 and 2004 for a randomized trial designed to assess the impact of gestational calcium supplementation on Pb mobilization during pregnancy (Ettinger et al., 2009). Women were enrolled either before becoming pregnant or during the 1st trimester of their pregnancy.

2.1. Blood lead measurements

Venous blood was collected in trace metal-free tubes from children at 1 and 2 years of age in our study clinic and immediately refrigerated until analyzed for Pb. BPb concentrations from these samples were determined using graphite furnace atomic absorption spectrophotometry (GFAAS) (Model 3000; Perkin Elmer, Wellesley, MA, USA) at the American British Cowdray Hospital Trace Metal Laboratory in Mexico City (Miller et al., 1987). With the exception of cohort 1, which continued using venous BPb sampling and GFAAS, capillary blood samples were collected with trace metal free materials at 3 and 4 years of age using a finger stick and BPb concentrations were measured with the LeadCare System (Magellan Biosciences, Chelmsford, MA, USA). Women in cohorts 2A and 3 provided 1 venous blood in each trimester of pregnancy, which was analyzed for Pb using GFAAS.

External blinded quality control samples (concentrations ranging from 2 to 88 $\mu\text{g}/\text{dL}$) were analyzed and provided by the Maternal and Child Health Bureau and the Wisconsin State Laboratory of Hygiene Cooperative Blood Lead Proficiency Testing Program. These analyses demonstrated good precision and accuracy with quality control specimens (Pearson $r > 0.98$; mean difference $< 1 \mu\text{g}/\text{dL}$).

2.2. Childhood cognitive abilities

We assessed children's cognitive abilities at 4 years of age using a translated version of the McCarthy Scales of Children's Abilities (MSCA) (McCarthy, 1972). The MSCA is a valid and reliable assessment of verbal, perceptual-performance, quantitative, memory, and motor abilities of preschool-aged children. This version of the MSCA was translated (from English

to Spanish) and normalized for use among Spanish children 2.5–8.5 years of age. We used the US normalizations since the Spanish version was missing normalization data for some age groups. Trained examiners administered the test to children in our study clinic at the National Institute of Perinatology. We used the General Cognitive Index (GCI) from the MSCA in our analyses. GCI scores were normalized to a mean of 100 and standard deviation (SD) of 15 among children in the United States by test developers. Previous studies, including ones conducted in these cohorts, have successfully used translated versions of the MSCA (Julvez et al., 2007; Kordas et al., 2011; Puertas et al., 2010).

2.3. Confounding variables

We used directed acyclic graphs (DAGs) to select confounding variables associated with childhood Pb exposure and child cognitive abilities, but not causal intermediates or direct descendants of both the exposure and outcome (Greenland, 2008). We started by using a list of variables that were available across all 4 cohorts so that we could maximize our sample size. We then used the DAG to identify variables associated with both the exposure and outcome. Our final set of variables included maternal education, IQ, marital status, duration of breastfeeding (months), child sex, and study cohort (Ettinger et al., 2004; Wasserman and Factor-Litvak, 2001). These variables were collected using standardized instruments at delivery, 1-month partum, and during subsequent study visits. Maternal IQ was estimated using selected subtests of the Wechsler Adult Intelligence Scale-Spanish (Information, Comprehension, Similarities, and Block Design) (Wechsler, 1981). We controlled for study cohort since childhood BPb concentrations and cognitive test examiners varied across cohort (Schnaas et al., 2004).

2.4. Statistical analyses

First, we examined the univariate (mean, SD, and proportions) characteristics of demographic, maternal and child factors, and BPb concentrations among women–infant pairs with and without follow-up. We also calculated mean and SD 4 year GCI scores according to categorizations of our covariate data. Next, we described the temporal pattern of BPb concentrations over the first 4 years of life by study cohort. We calculated the Intraclass Correlation Coefficient (ICC) for $\log_{10}$ -transformed BPb concentrations among children with all four BPb concentrations to gauge the degree of within-child correlation between BPb measurements. We used $\log_{10}$ -transformation for this analysis because of the right-skewed distribution of BPb concentrations. The ICC is a measure of reproducibility, with values ranging from 0 (no reproducibility) to 1 (perfect reproducibility) (Rosner, 2000). We repeated the ICC analysis separately for each cohort to gauge the variability of childhood BPb concentrations over time within each cohort.

Locally weighted scatterplot smoothing analyses revealed an approximately linear relationship between BPb concentrations (in $\mu\text{g}/\text{dL}$) and GCI. Thus, we did not use mathematical transformations (e.g., logarithms) of BPb concentrations in our analyses and estimated the adjusted change in GCI scores for a 10 $\mu\text{g}/\text{dL}$ increase in BPb concentrations. We constructed four multivariable linear regression models to estimate the change in GCI scores for a 10 $\mu\text{g}/\text{dL}$ increase in each childhood BPb biomarker (Model set 1). Next, we ran the same four models among children with all four BPb concentrations to assess the impact of missing data on our results (Model set 2). Finally, we constructed a model that included all four childhood BPb variables as predictors and GCI score as the outcome (Single model). We assessed potential multicollinearity between predictor variables in this final regression model using variance inflation factors and eigenvalues (Rosner, 2000).

We used the magnitude of the BPb–GCI estimate to determine which BPb measurement was most strongly associated with cognitive abilities. This method is more biologically

meaningful and has greater public health relevance than *p*-values or correlation, path, or standardized regression coefficients (Greenland et al., 1986; Poole, 2001).

We examined the heterogeneity of our results by individual study cohorts two ways. First, we created four separate models (one for each BPb biomarker) with a product interaction term between the cohort and BPb concentration variables. We used the *p*-value from this interaction term to test the null hypothesis that BPb estimates were homogenous across cohorts. Second, we examined the magnitude and direction of the different BPb effect estimates for each cohort.

2.5. Secondary analyses

First, we examined a subset of 448 mother–child dyads from Cohorts 2A and 3 to determine if gestational Pb exposures were associated with GCI scores and if they confounded the association between childhood BPb concentrations and cognitive abilities at 4 years. After calculating the arithmetic mean gestational or childhood BPb concentration for these mother–child pairs, we constructed three statistical models using average gestational, average childhood, or both average gestational and childhood BPb concentrations as predictors of 4 year GCI scores. These models were adjusted for the same confounders used in our primary models.

Second, we examined whether the pattern of our results changed after adjustment for variables that might be causal intermediates in our DAG or only associated with GCI scores. These included birthweight, gestational age at birth, children's 2 year hemoglobin (Hgb) levels, and psychological test administrator (Kordas et al., 2007; Wasserman and Factor-Litvak, 2001). Third, we conducted residual regression as an additional sensitivity analysis for collinearity between serial BPb measurements. We regressed the 3 year or 4 year BPb concentrations against the 2 year BPb concentration and included the residuals from these models in the models with 2 year BPb predicting 4 year GCI scores. This model allowed us to estimate the relationship between 2 year BPb concentrations and GCI scores, independent of the subsequent variation in BPb concentrations at 3 and 4 years of age. Finally, we examined whether excluding women who participated in lactational (Cohort 1) or gestational (Cohort 3) calcium supplementation changed the pattern of our results.

2.6. Ethical considerations

Participants were informed about the nature of the study and written consent was obtained before enrollment. The Institutional Review Boards at the National Institute of Public Health of Mexico, Harvard School of Public Health, and participating hospitals approved all study materials and procedures.

3. Results

Of the 2098 women who enrolled in one of the four cohorts and delivered a live-born singleton infant, 1643 (78.3%) returned with their child for at least one follow-up visit. Women who returned for a visit had similar demographic characteristics as women without any follow-up. Due to financial and logistical limitations, children in cohort 3 were less likely to have completed any follow-up (59%) than children in the other three cohorts (>77%). Average childhood BPb concentrations were slightly higher among children with complete follow-up at 4 years of age (geometric mean [GM]: 5.3 vs. 4.9 µg/dL; % difference: 7%, 95% confidence interval CI: 0, 13%).

Among dyads with complete covariate data and at least one childhood BPb concentration, 1035 (49.3%) returned at 4 years. Most women were <25 years of age, had <12 years of education, and breastfed their infants <6 months (Table 1). On average, children were 48.4

months of age at follow-up (standard deviation [SD]: 0.7, range: 45.8–55.6 months). The mean age of follow-up was similar across cohorts (48.2–48.7 months). Children's GCI scores were lower among mothers with lower IQ, younger age at delivery, and less education (Table 1). GCI scores were also lower in cohort 1 than the other cohorts. Compared to infants who were breastfed <6 months, those breastfed –12 months had decreased GCI scores at 4 years of age (β : -1.2; 95% CI: -3.2, 0.8). Confounding due to elevated average childhood BPb concentrations among infants breast fed for –12 months (GM: 6.0 vs. 5.0 $\mu\text{g/dL}$) was likely responsible for this finding and the association between >12 months of breastfeeding and GCI scores was attenuated toward the null after adjustment for BPb concentrations at 2 years of age (β : -0.6; 95% CI: -2.8, 1.5).

Child BPb concentrations rose across the first 4 years of life (Fig. 1 and Table 2). We observed a temporal trend in BPb concentrations, where children born more recently had lower BPb concentrations than infants born earlier (Fig. 1 and Supplemental Table 1). The highest concentrations were observed among children from cohort 1 (year of birth: 1994–1995) and lowest among children in cohorts 2A, 2B, and 3 (years of birth: 1998–2004). Median 1 and 2 year BPb concentrations were lower than BPb concentrations at 3 and 4 years of age in cohorts 2A, 2B, and 3. Median concentrations declined slightly between age 3 and 4 in cohorts 2A and 2B. There was a moderate degree of correlation between $\log_{10}$ -transformed childhood BPb measurements (ICC = 0.35, Pearson's r ranged from 0.37 to 0.62). Spearman rank correlation coefficients between childhood BPb concentrations were very similar to the Pearson's correlation coefficients. The ICCs ranged from 0.19 in cohort 2B to 0.50 in cohort 1. Consistent with the ICCs, Pearson correlations between BPb concentrations were highest in cohort 1 (r = 0.41–0.64) and lower in the other three cohorts (r = 0.03–0.59).

Among individual BPb measures, the 2 year BPb concentration showed the largest inverse association with GCI scores at 4 years of age (Table 3, Model Set 1). Each 10 $\mu\text{g/dL}$ increase in 2 year BPb concentrations was associated with a 3.8 point (95% CI: -6.3, -1.4) decrease in GCI scores at 4 years of age. Concurrent and 1 year BPb concentrations were also inversely associated with GCI scores at 4 years of age. However, mutual adjustment for other BPb concentrations attenuated the 1 and 4 year BPb point estimate (Table 3, Single model). The 2 year BPb concentration remained inversely associated with GCI scores while the other BPb biomarkers were not (Table 3, Single model, β : -6.5; 95% CI: -11, -7). Exclusion of children with incomplete BPb data strengthened the 2 year BPb association (Model set 1 vs. Model set 2 in Table 3). None of the BPb variables in our regression model containing all four BPb concentrations had variance inflation factors >2.0, indicating an absence of multicollinearity. Examination of the eigenvalues revealed that the most collinear variables were maternal education and IQ (Pearson r = 0.52).

Statistical tests of heterogeneity did not suggest that the BPb–GCI relationships varied across cohorts (Table 4, p -values > 0.10). All four cohorts showed inverse associations between 2 year BPb concentrations and GCI scores and there was no evidence of heterogeneity (Interaction term p -value = 0.89). While the direction and magnitude of the 1, 3, and 4 year BPb–GCI associations varied across cohorts, their 95% CIs overlapped and in some cases the 95% CI of one cohort's estimate included the point estimate of another cohort. For example, higher 4 year BPb concentrations predicted higher GCI scores in cohort 2B (β : 2.4; 95% CI: -3.4, 8.2) and lower GCI scores in cohorts 1 (β : -4.6; 95% CI: -9.0, 0.2), 2A (β : -0.4; 95% CI: -5.8, 5.8), and 3 (β : -5.8; 95% CI: -11, -0.2).

We repeated the analyses presented in Table 3 excluding children from cohort 1 since their BPb concentrations were much higher than the children from the other cohorts. These

analyses showed a similar pattern of results and do not suggest that cohort 1 children had a disproportionate influence on the regression estimates.

3.1. Secondary analyses

Among the 448 mother–child dyads from cohorts 2A ($n = 188$, 74.6%) and 3 ($n = 260$, 66.2%), average gestational (GM: 4.8; geometric standard deviation GSD: 1.8) and childhood (GM: 4.7; GSD: 1.6) BPb concentrations were modestly correlated (Pearson $r = 0.41$). After adjustment for confounders, average gestational BPb concentrations were not associated with 4 year GCI scores (β : -0.8 ; 95% CI: -4.3 , 2.8), while average childhood BPb concentrations were inversely associated with GCI scores (β : -4.6 ; 95% CI: -9.2 , -0.2). When we included both average gestational and average childhood BPb concentrations in the same model the gestational (β : 0.7 ; 95% CI: -3.1 , 4.6) and childhood (β : -5.0 95% CI: -9.9 , -0.2) estimates only changed marginally compared to estimates from the two separate models for each average BPb concentration.

Additional adjustment for whole blood Hgb concentrations at 2 years of age, gestational age at birth, birth weight, or psychological tester did not appreciably change the associations between BPb concentrations and 4 year GCI scores (Covariate sets 2, 3, and 4 vs. Covariate set 1, Table 5). The estimated association between 2 year BPb concentrations and 4 year GCI scores was unchanged when we used residual regression to account for variations in BPb concentrations between 2 and 3/4 years of age.

Compared to our Model set 1 results (Table 3), the exclusion of women participating in the calcium supplementation trials in cohorts 1 and 3 did not substantially change our point estimates from the 3 year (β : -0.7 ; 95% CI: -3.1 , 1.6) BPb models. The point estimates from the 1 year (β : -0.6 ; 95% CI: -4.5 , 3.2), 2 year (β : -3.1 ; 95% CI: -6.1 , -0.1), and 4 year (β : -1.5 ; 95% CI: -4.6 , 1.6) BPb models moved slightly toward the null.

4. Discussion

Compared to other childhood BPb biomarkers, 2 year BPb concentrations had the strongest inverse association with childhood cognitive abilities at 4 years of age in this study. The magnitude of this association was similar to prior studies of Pb exposure and childhood IQ and had a high degree of precision because of our large sample size (Bellinger et al., 1992; Chen et al., 2005; Lanphear et al., 2005; Tong et al., 1996). The association was not confounded by gestational Pb exposures and became stronger after adjustment for other childhood BPb measurements.

Our results are consistent with some prior studies (Bellinger et al., 1992; Tong et al., 1996; Wasserman et al., 1994), but not others (Chen et al., 2005; Hornung et al., 2009). Two other studies have reported that early childhood BPb concentrations were more strongly associated with cognitive abilities than later BPb concentrations (Bellinger et al., 1992; Tong et al., 1996). Consistent with our results, Bellinger et al. (1992) reported that BPb concentrations at 2 years of age were more strongly associated with IQ at 10 years of age than were other BPb measurements taken between birth and 10 years of age among children from Boston. Tong et al. (1996) reported that BPb concentrations at 3 and 5 years of age were most strongly associated with IQ at 11–13 years of age among Australian children. Using the MSCA at 4 years of age in Yugoslavian children, Wasserman and colleagues reported that BPb concentrations between 2 and 4 years of age were most strongly associated with GCI scores. However, they did not report whether mutual adjustment for multiple BPb concentrations changed the pattern of their results.

The inverse association between concurrent BPb concentrations and GCI scores was attenuated by adjustment for prior BPb concentrations, suggesting the later childhood Pb exposures may not impact child cognition after accounting for prior Pb exposures in these children. This is inconsistent with prior studies that observed larger declines in IQ associated with concurrent BPb levels (Chen et al., 2005; Hornung et al., 2009). Chen and colleagues found that concurrent BPb concentrations were most strongly associated with decrements in IQ at 5 and 7 years of age among children participating in the Treatment of Lead Exposed Children Trial. Furthermore, their results suggest that associations between concurrent BPb concentrations and IQ were not confounded because of elevated 2 year BPb levels. However, their results are not generalizable to most children since all the children in this trial were Pb-poisoned (BPb > 25 µg/dL) and half received chelation therapy. A recent re-analysis of the Cincinnati and Rochester lead studies used ratios of BPb concentrations to demonstrate that increasing BPb concentrations between 2 and 6 years of age were associated with decreased IQ (Hornung et al., 2009).

Several factors limit the comparability of prior studies to the present one and generalizability of these results to other populations. First, prior studies were conducted on children with different sources, patterns, and intensities of Pb exposure that resulted in higher BPb concentrations than those observed in this cohort (Hornung et al., 2009; Lanphear et al., 2005). Second, the high intra-individual correlation of BPb concentrations in prior studies (i.e., tracking) may limit the ability to identify windows of susceptibility. We observed lower correlations between serial BPb concentrations than prior studies and our statistical models were not unduly influenced by multicollinearity between BPb concentrations. Second, cohort-specific features including genetics, co-morbidities, and nutritional factors may produce unique developmental windows of susceptibility in different source populations. Finally, these results are based on measures of cognitive abilities at 4 years of age. While the GCI shows good correlation with other concurrent measures of IQ, correlations between later GCI measures and IQ tend to be stronger at older ages (Arinoldo, 1982).

Animal and epidemiological literature suggests that specific neurobehavioral domains may be sensitive to environmental exposures during distinct windows of vulnerability throughout gestation and childhood (Braun et al., 2011; Rice and Barone, 2000a). It is unlikely that Pb-induced damage to any single brain region or function is responsible for decrements in cognitive abilities since Pb has multiple targets in the developing brain and cognitive abilities are dependent on multiple brain processes (Lidsky and Schneider, 2003). Pb-induced interference with synaptic network formation is one potential mechanism for the inverse association between 2 year BPb concentrations and cognitive abilities. Pb can inhibit neuronal transmission by blocking calcium channels, but also stimulates spontaneous neurotransmitter release in the absence of environmental stimuli, thus interfering with the formation of efficient synaptic networks by disrupting synaptic pruning, which peaks around two years of age in children (Bressler et al., 1999; Bressler and Goldstein, 1991).

Trajectories of BPb concentrations varied according to calendar time and child age. BPb concentrations were lower among children born after 1997 compared to children born between 1994 and 1995. This is due, in part, to the phase-out of leaded gasoline in Mexico in 1998, resulting in decreases in both ambient air Pb and childhood BPb concentrations. (Schnaas et al., 2004) The pattern of BPb concentrations over early childhood in these cohorts is different than that observed in Australian, European, and US cohorts, which exhibit a parabolic shape that peaks between 2 and 3 years of age (Hornung et al., 2009; Tong et al., 1996; Wasserman et al., 2003). Investigators observed this same parabolic shape in a different cohort of children from Mexico City born between 1987 and 1992 (Schnaas et al., 2004). Differences in childhood BPb trajectories could be due to the relative contribution

of different Pb sources (e.g., air, leaded ceramics, paint, and water) to childhood BPb concentrations across source populations. Alternatively, exogenous contamination may have artificially raised the BPb concentration in capillary measurements from Cohorts 2A, 2B, and 3; thus, obscuring the expected parabolic relationship between age and BPb concentrations.

The lack of statistically significant heterogeneity of the BPb and GCI effect across cohorts does not safeguard against cohort specific factors from impacting the pattern of results. As noted, several of the point estimates between BPb concentrations and GCI scores varied in direction and magnitude across cohorts. This heterogeneity could be due to differences in the field methods used in the separate cohorts. While great care was taken to ensure that exposure, outcome, and covariate information were collected in a consistent and systematic fashion, we had to adapt and change our protocols over the 15 year period of study. For example, we began measuring 3 and 4 year BPb concentrations using capillary finger stick collection the LeadCare instrument in cohorts 2A, 2B, and 3. This was done to provide parents with timely feedback about their children's BPb concentrations. While BPb concentrations from the LeadCare System are highly correlated with GFAAS measures, capillary measurements could have been contaminated with exogenous Pb, resulting in the higher observed values at 3 and 4 years of age in these cohorts (Fig. 1). However, the pattern of regression estimates from Table 4 does not suggest that this difference was responsible for variations in the pattern of our results across cohorts. Another potential systematic bias could be changes in our cognitive test administration procedures or examiners over time. While we controlled for both cohort and examiner in our analyses, it is possible that other time-dependent factors biased the pattern of our results.

While we controlled for many factors typically included in studies of Pb neurotoxicity and selected confounding factors based on causal knowledge, adjustment for additional factors that are associated with both childhood Pb exposure and cognitive abilities may attenuate our observed 2 year BPb estimate (Hernan et al., 2002; Mink et al., 2004). Maternal care giving environment and alcohol or drug use are factors that may be associated with higher BPb concentrations and lower cognitive abilities scores. Adjustment for such factors might attenuate our observed estimates towards the null.

5. Conclusions

Compared to other infant and early childhood BPb concentrations, BPb concentrations at 2 years of age were most strongly associated with decreased cognitive abilities at 4 years of age. The relationship between BPb concentrations at other ages and cognitive abilities varied in magnitude and direction across cohorts, suggesting that within-cohort factors could have impacted the pattern of our results. While the persistence of the observed relationship between BPb concentrations at 2 years of age and cognitive ability deserves further study, the neurotoxic effects of Pb are well documented and efforts should be made to reduce or eliminate childhood Pb exposures. Childhood BPb concentrations have declined in the United States (US), but childhood Pb exposure remains a persistent problem in developing countries (Ahamed et al., 2010; He et al., 2009; Roy et al., 2009; Shi et al., 2010; Zhang et al., 2009). If specific periods of development are found to be sensitive to Pb, then public health interventions could be better targeted to reduce or eliminate exposures prior to susceptible developmental windows. This may be of particular importance in developing and newly industrialized countries where childhood Pb exposure is endemic and public health resources are scarce.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

BPb	blood lead
CI	confidence interval
GCI	general cognitive index
GFAAS	graphite furnace atomic absorption spectrophotometry
GM	geometric mean
ICC	intraclass correlation coefficient
MSCA	McCarthy Scales of Children's Abilities
Pb	lead
SD	standard deviation

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.neuro.2012.04.022>.

Fig. 1.

Box plot of blood lead concentrations at 1, 2, 3, and 4 years by study cohort.* Bars represent the minimum and maximum, boxes are the 25th and 75th percentiles, lines represent the mean, and diamonds are the median.

Table 1Descriptive characteristics of 1035 women–child pairs with complete follow-up.^b

	Mean covariate value (SD)	N (%)	Mean 4 year GCI ^a (SD)
Maternal age at delivery (years)	25.5 (5.3)		
<25		484 (47)	95.1 (13.1)
25 to <35		326 (32)	95.4 (14.4)
35		225 (22)	98.4 (13.4)
Maternal education (years)	10.4 (2.9)		
<9		182 (18)	90.6 (11.8)
9 to <12		432 (42)	94.1 (12.6)
12		295 (29)	98.3 (14.2)
>12		126 (12)	104.1 (13.4)
Maternal IQ	88.2 (21.9)		
1st quartile (23–76)		268 (26)	89.9 (12.6)
2nd quartile (77–87)		258 (25)	95.4 (13.0)
3rd quartile (88–98)		267 (26)	97.3 (12.9)
4th quartile (>98)		242 (23)	101.5 (13.6)
Marital status	N/A		
Non-married		96 (9)	96.1 (11.7)
Common law marriage		211 (20)	93.1 (14.1)
Married		728 (70)	96.7 (13.6)
Duration of breastfeeding (months)	8.9 (7.8)		
0 to <6		403 (39)	95.7 (13.3)
6 to <12		308 (30)	97.6 (14.3)
12		324 (31)	94.5 (13.3)
Child birthweight (g)	3125 (430)		
2500 g		977 (95)	96.1 (13.5)
<2500 g		56 (5)	92.4 (15.0)
Child gestational length (weeks)	38.9 (1.6)		
Term (≥ 37 weeks)		977 (94)	96.2 (13.6)
Preterm (<37 weeks)		57 (6)	90.9 (14.2)
Child sex	N/A		
Female		505 (49)	97.8 (13.5)
Male		530 (51)	94.1 (13.6)
Cohort (years of child birth)	N/A		
1 (1994–1995)		287 (28)	93.5 (13.1)
2A (1997–2002)		189 (18)	97.9 (14.3)
2B (1998–2001)		298 (29)	97.0 (13.5)
3 (2001–2004)		261 (25)	95.8 (13.6)

^aGCI: general cognitive index.^bWomen–child dyads with GCI scores, complete confounder data, and at least one childhood BPb measurement.

Table 2

Descriptive statistics of child blood Pb concentrations in Mexico City children at 1, 2, 3, and 4 years of age.^a

Child BPb	N	Min	5th percentile	25th percentile	Median	75th percentile	95th percentile	Max
1 year	758	0.3	1.3	2.7	4.2	6.4	10.6	27.4
2 year	900	0.6	1.5	3.0	4.6	7.0	13.4	36.8
3 year	806	0.6	2.3	4.0	5.5	7.8	13.8	50.1
4 year	874	0.3	2.5	4.1	5.9	8.0	12.8	30.3

All units are µg/dL.

^a Among children with complete covariate data and follow-up at 4 years of age.

Table 3

Adjusted change in 4 year GCI with a 10 µg/dL increase in child blood lead concentrations.^a

Child BPb	Model set 1 ^b		Model set 2 ^c		Single model ^d	
	N	β (95% CI)	N	β (95% CI)	N	β (95% CI)
1 year	758	-2.5 (-5.6, 0.5)	479	-2.3 (-6.7, 2.0)	479	1.5 (-3.7, 6.7)
2 year	900	-3.8 (-6.3, -1.4)	479	-5.4 (-9.1, -1.6)	479	-6.5 (-11, -1.7)
3 year	806	-0.7 (-3.1, 1.6)	479	-1.0 (-4.6, 2.6)	479	1.8 (-2.3, 5.8)
4 year	874	-2.5 (-5.1, 0.1)	479	-2.5 (-6.4, 1.4)	479	-1.3 (-5.5, 3.0)

^a Adjusted for maternal education (continuous in years), maternal IQ (continuous), marital status (common law marriage or non-married vs. married), child sex, breast feeding duration (continuous in months), and study cohort (1, 2A, and 2B vs. 3).

^b Model set 1: separate covariate-adjusted regression models for each blood lead measurement (i.e., four separate linear regression models).

^c Model set 2: separate covariate-adjusted regression models for each blood lead measurement among mother–infant pairs with non-missing BPb concentrations, covariates, and GCI score (i.e., four separate linear regression models).

^d Single model: single regression model mutually adjusted for covariates and all 4 BPb concentrations (i.e., one linear regression model).

Table 4

Adjusted change in 4 year GCI with a 10 µg/dL increase in child blood lead concentrations by study cohort.^{a,b}

BPb	Cohort 1		Cohort 2A		Cohort 2B		Cohort 3		Heterogeneity <i>p</i> -value ^b
	N	<i>β</i> (95% CI)	N	<i>β</i> (95% CI)	N	<i>β</i> (95% CI)	N	<i>β</i> (95% CI)	
1 year	135	-5.4 (-12, 0.8)	156	-1.4 (-7.3, 4.5)	283	2.1 (-4.2, 8.4)	184	-4.9 (-11, 0.9)	0.29
2 year	222	-4.8 (-9.0, -0.6)	171	-3.5 (-8.5, 1.5)	279	-2.1 (-7.6, 3.4)	228	-4.1 (-38.9, 0.7)	0.89
3 year	188	-1.2 (34.8, 2.3)	159	33.3 (38.5, 1.8)	265	1.4 (33.2, 6.0)	194	2.0 (36.8, 11)	0.52
4 year	249	34.6 (39.0, 30.2)	185	30.4 (35.8, 5.0)	280	2.4 (33.4, 8.2)	160	35.8 (311, 30.2)	0.14

^a Adjusted for maternal education (continuous in years), maternal IQ (continuous), marital status (common law marriage or non-married vs. married), child sex, and breast feeding duration (continuous in months).

^b Each model included a cohort by blood lead interaction term.

Table 5

Adjusted change in 4 year GCI with a 10 μ g/dL increase in child blood lead concentrations with additional adjustment for perinatal factors, child hemoglobin, and psychological tester.^{a, b, c, d}

BPb	Unadjusted		Covariate set 1 ^a		Covariate set 2 ^b		Covariate set 3 ^c		Covariate set 4 ^d	
	N	β (95% CI)	N	β (95% CI)	N	β (95% CI)	N	β (95% CI)	N	β (95% CI)
1 year	758	-4.8 (-7.8, -1.9)	758	-2.4 (-5.5, 0.6)	756	-2.3 (-5.4, 0.8)	630	-2.1 (-5.5, 1.3)	758	-2.4 (-5.5, 0.6)
2 year	900	-6.4 (-8.7, -4.0)	900	-3.8 (-6.2, -1.3)	897	-3.6 (-6.0, -1.1)	813	-3.7 (-6.3, -1.1)	900	-3.6 (-6.0, -1.1)
3 year	806	-2.5 (-4.8, -0.1)	806	-0.8 (-3.2, 1.5)	804	-0.7 (-3.1, 1.7)	664	-0.6 (-3.3, 2.1)	806	-1.2 (-3.6, 1.2)
4 year	874	-4.0 (-6.6, -1.3)	874	-2.3 (-4.9, 0.3)	871	-2.1 (-4.7, 0.5)	722	-2.2 (-5.1, 0.7)	874	-2.8 (-5.5, -0.2)

^a Adjusted for maternal education (continuous in years), maternal IQ (continuous), marital status (common law marriage or non-married vs. married), child sex, and breast feeding duration (continuous in months), and study cohort (1, 2A, 2B, vs. 3).

^b Adjusted for Covariate set 1 and gestational age at birth (continuous in weeks) and birth weight (continuous in grams).

^c Adjusted for Covariate set 1 and child's 2 year hemoglobin concentration (continuous in mg/dL).

^d Adjusted for Covariate set 1 and psychological tester.