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Cross-sectional and prospective associations of early childhood circulating metals with early and mid-childhood cognition in the Project Viva cohort

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Abstract

Background: Relatively little is known about the immediate and prospective neurodevelopmental impacts of joint exposure to multiple metals (i.e., metal mixtures) in early childhood.

Objectives: To estimate associations of early childhood (~3 years of age) blood metal concentrations with cognitive test scores at early and mid-childhood (~8 years of age).

Methods: We studied children from the Project Viva cohort. We measured erythrocyte concentrations of seven essential (Co, Cu, Mg, Mn, Mo, Se, and Zn) and eight non-essential metals (As, Ba, Cd, Cs, Hg, Pb, Sn, and Sr) in early childhood blood samples. Trained research assistants administered cognitive tests assessing vocabulary, visual-motor ability, memory, and general intelligence (standard deviations: ~10 points), in early and mid-childhood. We employed multivariable linear regression to examine associations of individual metals with test scores adjusting for confounders, other concurrently measured metals, and first-trimester maternal blood

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None declared.

Declaration of interests
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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metals. We also estimated joint associations and explored interaction between metals in mixture analyses.

Results: We analyzed 349 children (median whole blood Pb ~1 μg/dL). In cross-sectional analyses, each doubling of Pb was associated with lower visual-motor function (mean difference: −2.43 points, 95% confidence interval (CI): −4.01, −0.86) and receptive vocabulary, i.e., words understood (−1.45 points, 95% CI: −3.26, 0.36). Associations of Pb with mid-childhood cognition were weaker and less precise by comparison. Mg was positively associated with cognition in cross-sectional but not prospective analyses, and cross-sectional associations were attenuated in a sensitivity analysis removing adjustment for concurrent metals. We did not observe joint associations nor interactions.

Discussion: In this cohort with low blood Pb levels, increased blood Pb was robustly associated with lower cognitive ability in cross-sectional analyses, even after adjustment for prenatal Pb exposure, and regardless of adjustment for metal co-exposures. However, associations with mid-childhood cognition were attenuated and imprecise, suggesting some buffering of Pb neurotoxicity in early life.

Introduction

A range of cognitive abilities are critical to social and educational success in childhood, and subsequent health and well-being in adulthood.[1,2] Cognition depends on successful neurodevelopment, which in turn hinges on healthful prenatal and postnatal environments free of neurotoxicants and adequate in essential nutrients. Multiple non-essential metals, such as lead (Pb), are known neurotoxicants, and are pervasive in children's environments. These metals have been evaluated in relation to neurocognitive outcomes,[3] whereas the potential effects of many others, such as strontium (Sr), have only rarely been evaluated. Essential metals, such as zinc (Zn), are obtained through the diet and are co-factors in many cellular processes relevant to neurodevelopment, including neuronal proliferation, migration, and differentiation.[4] Because metals are often correlated due to shared sources in the environment and diet, and therefore public health actions are likely to affect multiple metals simultaneously, there has been significant interest in studying multiple metals jointly as a mixture.[5] Toxicologic evidence has shown that metals can interact to jointly affect brain development and function by well-established mechanisms, such as increased oxidative stress and altered neurotransmitter release.[6,7] Additionally, some epidemiologic studies have borne this out by demonstrating joint effects of particular sets of toxic metals on neurodevelopmental outcomes in children.[8–10] However, relatively few studies have examined the cognitive effects of both non-essential and essential metals in children, as well as persistence of effects later in childhood.

We aimed to address these gaps by leveraging data and samples from Project Viva, a pre-birth cohort in the US. We estimated associations of a panel of 15 non-essential and essential metals measured in early childhood (~3 years of age) erythrocytes (i.e., red blood cells) with child cognitive test scores obtained in early and mid-childhood (~8 years of age) assessing a variety of specific and composite mental faculties including visual-motor ability, language, memory, and general intelligence. We included adjustment for co-occurring metals ("co-metals") and maternal first-trimester erythrocyte metal concentrations to isolate

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the effects of postnatal metal exposure, and evaluated joint effects and interaction using recently developed statistical methods for chemical mixtures.

Methods

Study Population: Project Viva

We conducted analyses within a prospective cohort study, using data from mother-child pairs in Project Viva, a general population pre-birth cohort recruited in Boston, Massachusetts, USA. Initial recruitment and follow-up of the cohort is described in detail elsewhere.[11] Briefly, pregnant persons were approached at one of eight obstetric practices within Atrius Harvard Vanguard Medical Associates, a multi-site group practice in Eastern Massachusetts, during their first prenatal medical visit, between April 1999 and November 2002. Persons were excluded due to multiple gestation, inability to answer questions in English, planning to relocate away from the area prior to delivery, or if gestational age exceeded 21 weeks at recruitment. Blood samples were provided by children at the early childhood study visit (~3 y), and cognitive testing was performed at the early and mid-childhood (~8 y) visits. Written informed consent was obtained from mothers at recruitment and follow-up visits, and verbal assent was obtained from children starting at the mid-childhood visit. The Institutional Review Board of Harvard Pilgrim Health Care approved Project Viva study protocols.

Exposures: Metal Concentrations in Child Erythrocytes

We measured metal concentrations in stored blood samples collected from children at the early childhood visit [mean age (range): 3.3 years (2.9–5.6)]. The metals that were measured included 9 essential metals [cobalt (Co), chromium (Cr), copper (Cu), magnesium (Mg), Manganese (Mn), Molybdenum (Mo), nickel (Ni), selenium (Se), and zinc (Zn)], and 16 non-essential metals or metalloids [aluminum (Al), antimony (Sb), arsenic (As), barium (Ba), beryllium (Be), bismuth (Bi), cadmium (Cd), cesium (Cs), mercury (Hg), lead (Pb), tin (Sn), titanium (Ti), strontium (Sr), uranium (U), vanadium (V), and tungsten (W)]. We limited measurements to blood samples belonging to children who subsequently attended cognitive testing at the early childhood visit or mid-childhood visit [mean age (range): 7.8 years (6.6–10.7)]. Erythrocytes were isolated by centrifuging samples at 2,000 revolutions per minute for 10 minutes at 4°C. Subsequently, we weighed and digested 0.5 g of erythrocytes at room temperature for 48 hours using 1.5 mL of double distilled concentrated nitric acid, followed by another 24 hours after addition of 0.5 mL of 30% hydrogen peroxide. All metals were measured using triple quadrupole inductively coupled mass spectrometry (Agilent 8900; Agilent Technologies, Santa Clara, CA). Quality control measures included initial and continuing calibration verification, National Institute of Standards and Technology traceable mixed-element standard solution, procedural blanks, and duplicate analysis of 4% of samples (N=16 samples). To account for instrument measurement error in each sample, we used the average of five replicate measurements. We imputed values for non-detects as the limit of detection divided by the square root of 2. To limit the impact of measurement error on estimated associations with cognition, we limited our analysis to metals that were well-detected (detected in at least 60% of samples) for associations with cognition, including seven essential metals (Co, Cu, Mg, Mn, Mo, Se, and Zn), and eight non-essential metals (As, Ba, Cd, Cs, Hg, Pb, Sn, and Sr).

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We estimated whole blood Pb concentrations to compare blood Pb levels in this cohort with those in other cohorts, and with the Centers for Disease Control and Prevention whole blood Pb reference value of 3.5 μg/dL.[12] We multiplied child erythrocyte Pb concentrations by child hematocrit (measured in the same blood sample) and divided by 10 to estimate whole blood Pb concentrations (μg/dL).[13] This approach assumes the vast majority of the analyte is bound to erythrocytes, which is the case for blood Pb.[14] We used erythrocyte concentrations for estimating associations with cognition in statistical models.

Outcomes: Child Cognitive Test Scores

Cognitive tests were administered to children at the early and mid-childhood visits to quantify cognitive ability across multiple functional domains, including language, visual-motor function, memory, and general intelligence. Tests were administered by trained research staff, and scores were determined using published guidelines. Staff were blinded to child erythrocyte metal concentrations. At the early childhood visit, the Wide Range Assessment of Visual Motor Abilities (WRAVMA)[15] and the Peabody Picture Vocabulary Test, 3rd edition (PPVT-III)[16] were administered, assessing visual-motor function and receptive vocabulary (i.e., the ability to understand words), respectively. The WRAVMA summary score is the sum of scores on three subtests assessing Visual-Motor, Visual-Spatial, and Fine-Motor abilities. At the mid-childhood visit, we administered the WRAVMA Visual-Motor subtest, as well as the visual memory component of the Wide Range Assessment of Memory and Learning, 2nd edition (WRAML2),[17] and the Kaufman Brief Intelligence Test, 2nd edition (KBIT-II).[18] The visual memory component of the WRAML2 assesses short-term visuospatial memory and is comprised of the Design Memory and Picture Memory subtests. KBIT-II has verbal and non-verbal sub-scores which respectively quantify crystallized intelligence (the ability to solve problems using accumulated knowledge) and fluid intelligence (the ability to learn and reason to solve novel problems). If tests were incomplete for any reason or no questions were answered correctly, the test was not scored. According to the tests' manuals, scores were standardized to age-specific reference samples of the U.S. pediatric population.[15–18] All cognitive tests we utilized have demonstrated high split-half and test-retest reliabilities in construct validation studies, and moderate or strong correlations with full-scale intelligence tests such as the Weschler Intelligence Scale for Children.[15–18] For all tests, higher test scores correspond to higher estimated cognitive function.

Covariates

We identified covariates *a priori* for inclusion in statistical models. Confounders were identified as factors likely to affect both child blood metal concentrations and cognition, and are presented in a causal diagram [Supplementary Material (SM) Figure S1]. Maternal confounders included education (some college/associate's degree or less, four-year college graduate, or graduate school), age at enrollment (years), annual household income ($70,000 or less, $70,001–$100,000, or greater than $100,000), marital status (not married or married), and maternal-reported child confounders included sex (male or female), child race and ethnicity as a proxy for structural racism[19] (non-Hispanic White, non-Hispanic Black, non-Hispanic Asian, Hispanic, or more than one race), breastfeeding duration (months), fish consumption (never, less than once per week, or once or more per week), and attendance

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to daycare (did not attend daycare or did attend daycare). For each well-detected metal, we included the corresponding maternal first-trimester erythrocyte concentration of the metal as a confounder. For a given early childhood metal, we included all other well-detected early childhood metals to account for co-exposure confounding. An additional covariate, maternal intelligence, was assessed with PPVT-III at the early childhood visit, and composite score from the KBIT-II, a combination of the verbal and non-verbal scores, at the mid-childhood visit. Maternal intelligence was included in statistical models to improve precision on estimates of association, as maternal intelligence is a strong predictor of child cognitive test scores that is unlikely to lie on a causal path between child blood metals and child cognition. Child age was not included as a covariate as cognitive test scores were age-standardized.

Preliminary Analyses

As metal concentrations were strongly right-skewed, we considered log-transformation of these variables prior to modeling. Following recommendations from Choi and colleagues,[20] we found $\log_2$-transformed metal concentrations had similar fit to the data as untransformed concentrations, according to Akaike Information Criterion (AIC) in preliminary single-metal linear regression models that included the covariates (data not shown). We proceeded with using $\log_2$-transformed concentrations in analyses to mitigate the impact of outliers on the estimated associations and to retain an interpretable exposure contrast across all metals, where a single-unit increase in $\log_2$-transformed concentration corresponds to a doubling on the original scale. Adding a quadratic term did not lower the AIC for any metal, and therefore we modeled metals as linear terms. In the subsample of children with at least one exposure and outcome, at least one covariate was missing in 12% of subjects in early childhood analyses and 8.9% of subjects in mid-childhood analyses. We conducted complete case analysis, excluding subjects with missing covariate data. We also excluded additional pregnancies beyond the first that occurred during the recruitment period to retain independence of observations for statistical modeling. Spearman correlation coefficients were estimated among pairs of the untransformed metals and Pearson correlation coefficients among pairs of the cognitive test scores.

Statistical Analyses

We used multivariable linear regression to estimate the mean difference in cognitive test score per doubling of a given erythrocyte metal concentration, adjusting for confounders and covariates. Because public health interventions and policies concerning metal exposure are likely to impact multiple metals simultaneously, we estimated the effect of jointly increasing all essential metals (“essential metal mixture”) or non-essential metals (“non-essential metal mixture”) by one quartile using the R package *qgcomp*[21] for implementing quantile g-computation. Quantile g-computation converts each mixture component to a user-specified number of quantiles (in this case, quartiles), models them jointly with adjustment for covariates in a linear regression model, and sums regression coefficients across components to estimate the mean difference in outcome per quartile increase in the mixture, along with 95% confidence intervals (CIs). Finally, we also evaluated statistical interaction using Bayesian kernel machine regression (BKMR), a semi-parametric approach that models mixture components together in a flexible kernel function, permitting estimation of non-linear and non-additive associations, and associated 95% credible intervals (CrIs),

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adjusting for covariates. Using package default priors, we obtained 100,000 samples from the posterior distribution for each of three chains. The initial 50% of each chain was discarded as burn-in and subsequent samples were thinned to every 50th iteration to reduce autocorrelation, resulting in 3,000 samples across the chains used to characterize the posterior distribution. To identify interactions, we visually inspected plots of concentration-response curves for each metal at the 10th, 50th, and 90th percentiles of each other metal in pairwise fashion, with all other metals fixed at their medians.

Sensitivity Analyses

We conducted several sensitivity analyses for multivariable linear regressions and quantile g-computation models. In addition to fish consumption, other components of the diet can be both a significant source of some essential and non-essential metals,[22] and affect neurodevelopment.[23] Therefore, we assessed robustness of the results to adjustment for the Youth Healthy Eating Index (YHEI), a measure of the quality of dietary behaviors and consumption of nutrient-dense foods,[24] estimated at the early childhood visit using a food frequency questionnaire. Because household smoking was reported in only 2% of households at the early childhood visit, we did not include household smoking as a confounder in the main analyses. However, we adjusted for it in an additional sensitivity analysis as a dichotomous variable (smoking present or not present in the household, as reported by the mother). Adjustment for co-exposures to reduce confounding can result in bias in the presence of unmeasured confounders of the co-exposures and the outcome of interest.[25] Therefore, for linear regression models we conducted an additional sensitivity analysis removing adjustment for other early childhood co-metals.

We did not conduct null hypothesis significance testing given known limitations of this approach for inference when the aim is to estimate effect size.[26] We therefore report point estimates and 95% CIs (or, in the case of BKMR models, 95% CrIs) to quantify uncertainty, and did not adjust for multiple testing. We conducted all analyses in R version 4.2.1.[27]

Results

Study Population Characteristics

From 2,128 live births, our analytic sample included 349 children, with 336 in the early childhood cognition analyses and 296 in the mid-childhood cognition analyses (Figure S2). Covariate distributions were similar between children included in the early *versus* mid-childhood cognition analyses (Table S1). In the analytic sample, 44.1% had household income >$100,000, and most mothers were college-educated (44.4% four-year college graduates; 30.7% graduate school), married (88.5%), and enrolled in the study in their early 30's (mean age 33.0 years) (Table 1). Maternal-reported child race and ethnicity was mostly non-Hispanic White (71.9%), roughly half of children reported consuming fish (52.2%), and the majority attended daycare (73.6%). Maternal cognitive test scores were typically higher than the national reference population used to norm the tests, indicated by mean scores for receptive vocabulary (PPVT-III) and general intelligence (KBIT-II composite) (108 and 109, respectively) being greater than the reference population mean score of 100. Mean child test scores for receptive vocabulary (PPVT-III), visual-spatial ability (WRAVMA), and

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crystallized (KBIT-II verbal) and fluid intelligence (KBIT-II non-verbal) (106, 109, 108, and 115, respectively) were also above the reference population mean score of 100.

Exposure and Outcome Distributions

Detection rates and distributions of early childhood erythrocyte metals are provided in Table S2. For all well-detected metals (detected in >60% of subjects), consistency in concentrations between instrument replicates and between duplicate samples was high, with ICC>0.9 for instrument replicates and relative percent difference between duplicates <25% in more than half of duplicate samples (Table S3). Among well-detected metals, medians were within reference ranges except for Zn (median: 6,690 ng/g), which was below the lower bound of the reference range (7,200–13,000 ng/g). Median child metal concentrations were lower than median maternal first-trimester metal concentrations for one essential metal, Zn (6,690 ng/g vs. 10,400 ng/g), and most non-essential metals, particularly Ba (0.826 ng/g vs. 3.18 ng/g). Notably, median child Pb concentration was ~50% higher than median maternal Pb concentration (27.3 ng/g vs. 17.7 ng/g). Early childhood whole blood Pb, estimated by multiplying erythrocyte Pb by hematocrit measured in the same blood sample used to measure the metals (hematocrit range: 22.8%–43.9%), exceeded the US blood lead reference value of 3.5 μg/dL in 2% of children, and the median (range) of estimated whole blood concentrations was 1.0 μg/dL (0.14–6.9). Essential metals were weakly to moderately correlated with each other (highest Spearman ρ=0.42 between Se and Zn) (Figure S3). Non-essential metals were generally weakly correlated with each other. The highest correlation among any metal pair across essential and non-essential metals was observed between As and Hg (ρ=0.61), which was expected as these metals share a common dietary source in fish. Among cognitive test scores, the strongest correlations were, as expected, observed between summary scores (e.g., WRAVMA summary and WRAML2 total) and their constituent subtests. Otherwise, the strongest correlation was between early childhood PPVT-III and mid-childhood KBIT-II verbal (Pearson r=0.6), which was also expected as both tests assess receptive vocabulary with very similar tasks, with KBIT-II additionally assessing expressive vocabulary. WRAVMA visual-motor subtest, the only test conducted at both early and mid-childhood visits, was weakly correlated between visits (r=0.09).

Cross-sectional Associations with Early Childhood Cognition

Results from multivariable linear regression models for associations between early childhood erythrocyte concentrations of individual metals and early childhood cognitive test scores are provided in Figure 1, with numeric values provided in Table S4. Among the non-essential metals, Pb was consistently negatively associated with all tests administered at this visit. Each doubling of Pb was associated with a mean difference of −2.43 points (95% CI: −4.01, −0.86) on WRAVMA summary, −1.76 points (95% CI: −3.44, −0.08) on WRAVMA visual-motor subtest, −2.10 points (95% CI: −3.69, −0.51) on WRAVMA fine-motor subtest, −2.07 points (95% CI: −4.08, −0.06) on WRAVMA visual-spatial subtest, and −1.45 points (95% CI: −3.26, 0.36) on PPVT-III. We found that Cd was positively associated with WRAVMA visual-spatial subtest score, but not other tests: a doubling in erythrocyte Cd concentration was associated with a mean difference of 2.05 points (95% CI: 0.28, 3.82) on the WRAVMA visual-spatial subtest. Increased Ba was associated with lower WRAVMA

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visual-motor subtest score, with a mean difference of −0.87 points (95% CI: −1.52, −0.22) per doubling of Ba; conversely, Ba was also associated with a small increase in PPVT-III score, with a mean difference of 0.77 points (95% CI: 0.05, 1.49). Each doubling of Sn was associated with a small increase in WRAVMA fine-motor subtest score, with a mean difference of 1.15 points (95% CI: −0.02, 2.32).

Among essential metals, Mg was positively associated with all tests. Each doubling of Mg was associated with a mean difference of 8.11 points (95% CI: 1.53, 14.68) on WRAVMA summary, 6.40 points (95% CI: −0.77, 13.56) on WRAVMA visual-motor subtest, 2.69 points (95% CI: −3.98, 9.35) on WRAVMA fine-motor subtest, 10.28 points (95% CI: 1.80, 18.77) on WRAVMA visual-spatial subtest, and 6.69 points (95% CI: −1.05, 14.43) on PPVT-III. In contrast, a doubling of Zn was associated with lower scores on WRAVMA subtests, with the strongest association for visual-spatial function (mean difference: −7.25 points, 95% CI: −13.66, −0.84). A doubling of Se was generally associated with lower scores on early childhood cognitive tests, particularly the WRAVMA visual-motor subtest (mean difference: −7.14 points, 95% CI: −13.35, −0.94).

Prospective Associations with Mid-Childhood Cognition

Results from prospective multivariable linear regression models of associations between early childhood (~3 years) erythrocyte metal concentrations and mid-childhood (~8 years) cognitive test scores are provided in Figure 2, with numeric values provided in Table S5. Pb was negatively associated with scores on some cognitive tests in mid-childhood, however estimates were smaller in magnitude, more imprecise, and less consistent than those observed in cross-sectional analyses. We observed a positive association between Hg and fluid intelligence (mean difference: 1.63 points, 95% CI: 0.08, 3.17), though associations of Hg with other mid-childhood cognitive test scores were much weaker. Each doubling in Ba was associated with higher WRAML2 picture memory score (mean difference: 0.21 points, 95% CI: 0.01, 0.40). Each doubling in Sn was associated with higher WRAML2 design memory score (mean difference: 0.34 points, 95% CI: 0.01, 0.68).

In contrast to cross-sectional analyses, associations of Mg with mid-childhood cognition were inconsistent in direction and imprecise. Zn was negatively associated with some aspects of cognition, though not as consistently across tests as in cross-sectional analyses. A doubling in erythrocyte Zn concentration was associated with a mean difference of −10.01 points (95% CI: −18.83, −1.18) in fluid intelligence, measured by the KBIT-II non-verbal test. Each doubling of Mn was associated with higher scores on tests of fluid intelligence (mean difference: 2.74 points, 95% CI: −2.28, 7.76) and crystallized intelligence (mean difference: 1.07 points, 95% CI: −2.49, 4.64), though estimates were fairly imprecise.

Joint Associations of Metal Mixtures

Associations of the joint non-essential or essential metal mixtures with early childhood cognition were null (Figure S5), as were associations of the non-essential metal mixture with most mid-childhood cognitive tests (Figure 3). Notably, the essential metal mixture was associated with somewhat higher scores in mid-childhood WRAML2 total and KBIT-II

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verbal, with Mn having the highest positive weight in both models, though the estimates were imprecise.

Evaluation of Statistical Interaction

Concentration-response functions for each metal appeared consistent in shape across the 10th, 50th, and 90th percentiles of each other metal in BKMR models, suggesting no interaction between metals (Figures S6–S11).

Sensitivity Analyses

Associations for non-essential metals were largely unaffected in sensitivity analyses, including the negative associations we observed between Pb and early childhood cognition (Figure S12). Except for Mg, associations of essential metals with cognition were also unaffected in sensitivity analyses (Figure S13). For Mg, positive associations with early childhood cognition were attenuated after eliminating adjustment for co-metals. Numeric values of association estimates from multivariable linear regression models without co-metals (the third sensitivity analysis in Figures S12–13) are provided in Tables S6–S7. Quantile g-computation estimates were largely unaffected in sensitivity analyses (Figure S14).

Discussion

We examined associations of early childhood erythrocyte concentrations of nine non-essential metals and six essential metals with cognitive test scores in early childhood and mid-childhood, adjusting for co-occurring metals and prenatal metal concentrations. In cross-sectional analyses, Pb was consistently associated with lower scores on all tests, spanning visual-motor function and receptive vocabulary, whereas Mg was associated with higher scores. In prospective analyses, Pb was also associated with lower test scores, but estimates were weaker and more imprecise, whereas associations were null for Mg. Additionally, after removing adjustment for co-metals, associations of Mg with early childhood cognition were attenuated, whereas associations of Pb with early childhood cognition were robust to this and other sensitivity analyses adjusting for child diet and household smoking. Mixture analyses using quantile g-computation were largely null. BKMR models did not suggest interaction between any pair of metals.

While we estimated associations of Pb with cognition using erythrocyte concentrations, we approximated whole blood Pb concentrations for comparison of exposure levels in this cohort to those in the literature and current reference levels. We found that the median concentration was ~1 μg/dL and only 2% of children exceeded the current blood lead reference level (BLRV) of 3.5 μg/dL. The BLRV is based on the 97.5th percentile of blood Pb in children from the 2015–2016 and 2017–2018 cycles of the nationally-representative National Health and Nutrition Examination Survey (NHANES). NHANES results suggest blood Pb levels in Project Viva are comparable to those of the broader U.S. pediatric population in recent years.[3] Similar to prior studies of low-level Pb exposure, we did not identify a threshold of effect, as a linear relationship was the best fit to the data. Multiple prior cross-sectional studies of children with generally higher blood Pb levels than in Project

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Viva have also reported inverse associations of child blood Pb with visual-motor function,[28] including when measured using the WRAVMA,[29,30] with less consistent associations for verbal ability tests such as the PPVT-III,[28,31] though these other studies typically did not adjust for co-metals and prenatal metals. The magnitude of the associations we observed, while difficult to compare to other studies of metals due to different biological media, exposure transformations, and targeted contrasts, were comparable to the beneficial impact of breastfeeding on cognition observed in a prior Project Viva study.[32] We observed an average 1.45 points decrease in receptive vocabulary score (PPVT-III) per doubling of erythrocyte Pb, roughly equivalent to the average increase in score on this test per three months of exclusive breastfeeding (~1.50 points).[32]

We observed relatively weak and imprecise associations of Pb with mid-childhood cognition (~8 years), in contrast with previous large prospective studies of Pb exposure in early childhood and prospective measures of neurodevelopment.[33,34] Notably, average whole blood Pb concentrations in these cohorts were around 3-fold that of the concentrations estimated in our study by hematocrit-based conversion of erythrocyte concentrations. Additionally, their sample sizes were more than an order of magnitude greater than ours. Together, these factors may explain these studies' ability to detect small adverse effects of early life Pb exposure on cognition later in childhood. Multiple studies have reported that the effect of Pb on neurodevelopment is attenuated in children belonging to households of higher socioeconomic status (e.g., more education and higher income),[35,36] potentially due to reduced concurrent exposure to other developmental neurotoxicants and greater presence of counteracting nutritive factors.[37] Project Viva families have relatively more education and higher income than the general US population, which may have mitigated neurodevelopmental deficits in later childhood, though we could not test this hypothesis with our data given the lack of variability in socioeconomic indicators within Project Viva.[11] Additionally, it is plausible that repeated longitudinal assessments of the same cognitive domains are needed to observe persistent effects if effects are domain-specific, as has been observed on some occasions for the preferential effect of Pb on visual-motor function over abilities in other areas such as language.[29] In our study, one cognitive test, the WRAVMA visual-motor subtest, was conducted at both early and mid-childhood visits, but associations with the metals were not consistent between visits, including for Pb. Strikingly, scores on this test were only weakly correlated between visits, suggesting potential variability in the underlying construct measured by the test at different ages, measurement error, or genuine change in relative visual-motor ability over childhood, potentially driven by other modifiable factors.

While significant strides in public health policy have been made in recent decades to prevent Pb exposure, Pb remains detectable in most people, with no safe detectable level established. This suggests some form of secondary prevention to mitigate Pb-induced cognitive damage may, if identified, yield significant public health benefits if widely adopted.[38] Recent studies in rats suggest environmental cognitive enrichment (e.g., increased number and variety of toys) after Pb exposure may recover the pre-exposure transcriptome, and subsequently mitigate some Pb-induced deficits in memory.[39] While direct cellular damage drives the immediate consequences of Pb exposure, these effects may persist due to Pb-induced impaired structural and functional plasticity in the brain.[38] Recent studies have suggested

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impaired plasticity is remediable. Increased caregiver support and improved quality of childcare have appeared to mitigate poverty-induced structural and functional deficits in neuroplasticity.[40] For example, a randomized study of a six-week-long, family-based brain training program led to significant improvements in selective attention, cognitive function, and behavior, in comparison to active control groups.[41] However, it remains to be seen if these results are translatable to Pb-induced deficits.[42] Our finding that associations between Pb and early childhood cognition appear to have attenuated by mid-childhood may be interpreted as providing some indirect support for this theory, as Project Viva mothers, in contrast to other cohorts that have observed longitudinal effects of Pb exposure on cognition,[33] had relatively high levels of education and household income, factors strongly linked to features of the home environment supportive of cognitive development.[43] In fact, previous epidemiologic studies have demonstrated that higher SES may reduce the magnitude and persistence of Pb neurotoxicity in children,[44] suggesting that modifying SES can ameliorate Pb neurotoxicity. However, SES is a complex construct that is measured by, or correlates strongly with, diverse sets of factors, such as income, education, diet, and psychosocial well-being. SES is therefore a sensitive but crude indicator for many aspects of the child's home environment and lifestyle. The development of effective post-exposure interventions to make Pb-induced cognitive deficits less persistent and devastating will depend on the conduct of larger studies with socioeconomically diverse populations that can systematically identify which factors drive the modulation of Pb neurotoxicity by SES.

Positive associations of erythrocyte Mg with cognitive test scores at the early childhood visit attenuated when adjustment for the 14 other co-metals was removed. We reported results both with and without adjustment for co-metals as adjustment for co-exposures can amplify bias in the presence of unmeasured sources.[25] It is possible adjustment for co-metals caused an upward bias in our estimates for associations between Mg and early childhood cognition, though the magnitude of the bias is in part determined by the magnitude of correlations among co-exposures, which were relatively weak in this study. There is some mechanistic and clinical basis for a cognitive benefit of Mg in early life. Antenatal administration of magnesium sulfate in preterm births has been shown to reduce the risk of cerebral palsy, with Mg functioning as a neuroprotectant by several potential mechanisms, including reducing oxidative stress and pro-inflammatory cytokines, and preventing excitotoxicity in the developing brain by reducing extracellular glutamate.[45]

We observed negative associations between early childhood erythrocyte Zn concentrations and some domains of cognition in early childhood and mid-childhood. Associations were stronger in cross-sectional analyses, and weaker and less consistent in prospective analyses. Laboratory studies have revealed that Zn plays a critical role in many developmental processes, including synthesis of DNA, RNA, and proteins, and, specifically in the developing brain, establishment of axonal and synaptic transmission between neurons.[46] However, the accumulation of Zn also causes oxidative stress, and has been associated with neuronal death in the context of several diseases including Alzheimer's disease.[47] Randomized controlled trials of the effect of early life Zn supplementation on cognitive development have not shown evidence of cognitive benefit.[48] In general, excess intake of nutrients can be harmful. For example, Amorós and colleagues observed an inverted U-shaped relationship between serum Se during pregnancy and cognitive function in Spanish

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preschool children, suggesting the existence of an optimal range of Se.[49] In contrast, we did not observe non-linear relationships between essential metals and cognition, potentially due to limited sample size, the range of metal concentrations available in Project Viva children, and modifying factors.

We observed relatively imprecise associations between some metals, such as Ba, Sn, Se, Hg, and Mn, and some domains of cognitive function. As with Pb,[29] exposure to some metals may affect only particular brain functions strongly enough to be detected in epidemiologic studies. We found that Ba displayed associations with early childhood cognition that were in different directions for different tests. There is some observational epidemiologic evidence that Ba exposure in prenatal and later life periods is associated with worse cognitive function,[50–52] however additional studies similar to ours examining early postnatal exposure are needed, as well as animal studies to understand potential mechanisms of action at low doses.[53] Positive associations between Mn and fluid and crystallized intelligence scores, while individually fairly weak, contributed substantially to positive associations of the essential metal mixture with mid-childhood cognition. The literature concerning associations of Mn biomarkers with child neurodevelopment is inconsistent, particularly for blood biomarkers, however increased hair concentrations of Mn have been consistently associated with adverse effects on neurodevelopment, as demonstrated in a recent meta-analysis.[54] While Mn is an essential metal that plays important roles in neurotransmitter release, an excess of Mn in the brain has been shown to be toxic, suggesting there may be an optimal range of this metal.[55] This hypothesis has been supported by several epidemiologic studies that found inverted U-shaped concentration-response relationships between Mn biomarkers and neurodevelopment.[56,57]

Associations between metal mixtures and cognition in our study were largely null, and we did not observe interaction between metals. Mechanistic studies have established that some heavy metals such as As, Hg, and Pb, exert their toxicity on shared pathways, such as oxidative stress, causing synergy and increased toxicity beyond the sum of their individual effects.[58] While the literature is still in its infancy, some epidemiologic studies of associations between metal biomarkers and cognitive function in children have observed statistical interactions. For example, Bauer and colleagues observed an adverse effect of a mixture of hair concentrations of Mn, Pb, and Cr on verbal IQ in Italian adolescents, but only when the concentration of Cu was at its 10th percentile, suggesting Cu may buffer metal neurotoxicity.[59] The presence of, and ability to identify, statistical interaction between metal exposures is dependent on many factors, including exposure timing, sufficient internal doses of the candidate metals, appropriate biomarker selection, and sufficient statistical power,[60] as well as other biological and social factors, including, for example, sex,[61] socioeconomic status,[37] and psychological status.[62] Interestingly, while we did not observe a strong joint effect of the metal mixtures on cognition using quantile g-computation, we did find that in traditional linear regression, there was strong and consistent signal for an adverse cross-sectional effect of Pb, and a beneficial cross-sectional effect of Mg. The mixture effect estimated by quantile g-computation is the sum of coefficients of the mixture components, permitting cancelation of positive and negative effects of the components, meaning Mg and Pb were insufficient on their own to drive an overall effect of the essential or non-essential metal mixtures, respectively.

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Our study has several strengths. We adjusted for a range of relevant confounders identified *a priori*, including co-exposure to other metals and prenatal metals. Also, we leveraged a battery of neurodevelopmental tests assessing both specific and composite measures of cognitive function over two time points, enabling us to evaluate the consistency and specificity of associations across cognitive domains and over time. Further, we leveraged multiple statistical approaches spanning traditional and modern methods for evaluating the effects of metal mixtures.

We note several limitations in this study. Total metal concentration in erythrocytes may not be a desirable biomarker for some metals. For example, total As in blood or urine is not a sensitive proxy for inorganic As (the toxic form) in the presence of seafood consumption, which was frequently reported in Project Viva children.[63] However, erythrocytes are a sensitive marker for recent and ongoing Pb exposure, particularly in children, with the vast majority of blood Pb bound to erythrocytes.[64] It is also sensitive for Mg exposure.[65] Exposure biomarkers aim to approximate the internal dose from all sources, and the use of inappropriate biomarkers will likely cause bias towards the null due to measurement error that is non-differential by the outcome. This may at least in part explain our null findings for classically neurotoxic metals such as As. Additionally, as most mothers in Project Viva were college-educated and non-Hispanic White, and all had health insurance and health care at study enrollment, the results of this study may not generalize to more disadvantaged and marginalized segments of the US population, where exposure ranges and the prevalence of modifying factors are likely to vary. Notably, though, we observed blood Pb concentrations in this cohort that were comparable to recent nationally representative estimates. We conducted complete case analysis in which we excluded some children for whom at least one covariate was missing. This may lead to some selection bias if missingness is not completely random after conditioning on the other covariates in the models. Notably, however, we did not find evidence of differential selection in cohort follow-up, suggesting this larger potential source of selection bias is unlikely to have strongly impacted our results. Our use of complete cases also resulted in a smaller analytic sample, another limitation of this study, particularly because the effects we aimed to estimate are likely small in magnitude.

In this prospective study of children from the Project Viva cohort, we observed robust cross-sectional associations of low-level erythrocyte Pb with visual-motor ability and receptive vocabulary in early childhood (~3 years) while adjusting for co-metals and prenatal Pb concentrations. Associations of Pb with mid-childhood (~8 years) cognitive test scores were weaker by comparison. A safe, detectable level of Pb in child blood remains elusive,[66] highlighting the importance of epidemiologic studies of low-level Pb exposure for identifying such a threshold, if it exists. Further research is needed to understand which particular modifiable factors might buffer the neurodevelopmental toxicity of Pb to avoid life-long impacts. If developmental Pb neurotoxicity can indeed be buffered by modifiable factors in the home or school environment, this can lead to the development of scalable interventions, including changes in clinical practice, recommendations for parents and caregivers, and school policies, that can reduce the massive existing public health burden of Pb exposure.[67] Further methodological research is also needed to understand and mitigate threats to causal inference due to potential bias amplification from co-exposure adjustment.

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Supplementary Material

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What this study adds:

Relatively few studies have comprehensively separated the effects of neurotoxic metals such as lead (Pb) from pre- and postnatal co-occurring metals, nor examined persistence of associations across childhood. In a cohort of middle-class children, we found higher early childhood (~3y) blood Pb was associated with lower scores on cognitive tests, independent of other metals and prenatal blood Pb. However, early childhood Pb was only weakly associated with cognition in mid-childhood (~8y). Our results suggest the effects of low-level Pb exposure may attenuate over time in some populations, implying the presence of factors that may buffer Pb neurotoxicity in early life.

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Highlights

- There is no known safe concentration of lead (Pb) in the blood.
- Child blood Pb was associated with lower cognitive ability cross-sectionally.
- These associations were weakened in later childhood.
- Adverse cognitive effects of Pb may be modifiable.

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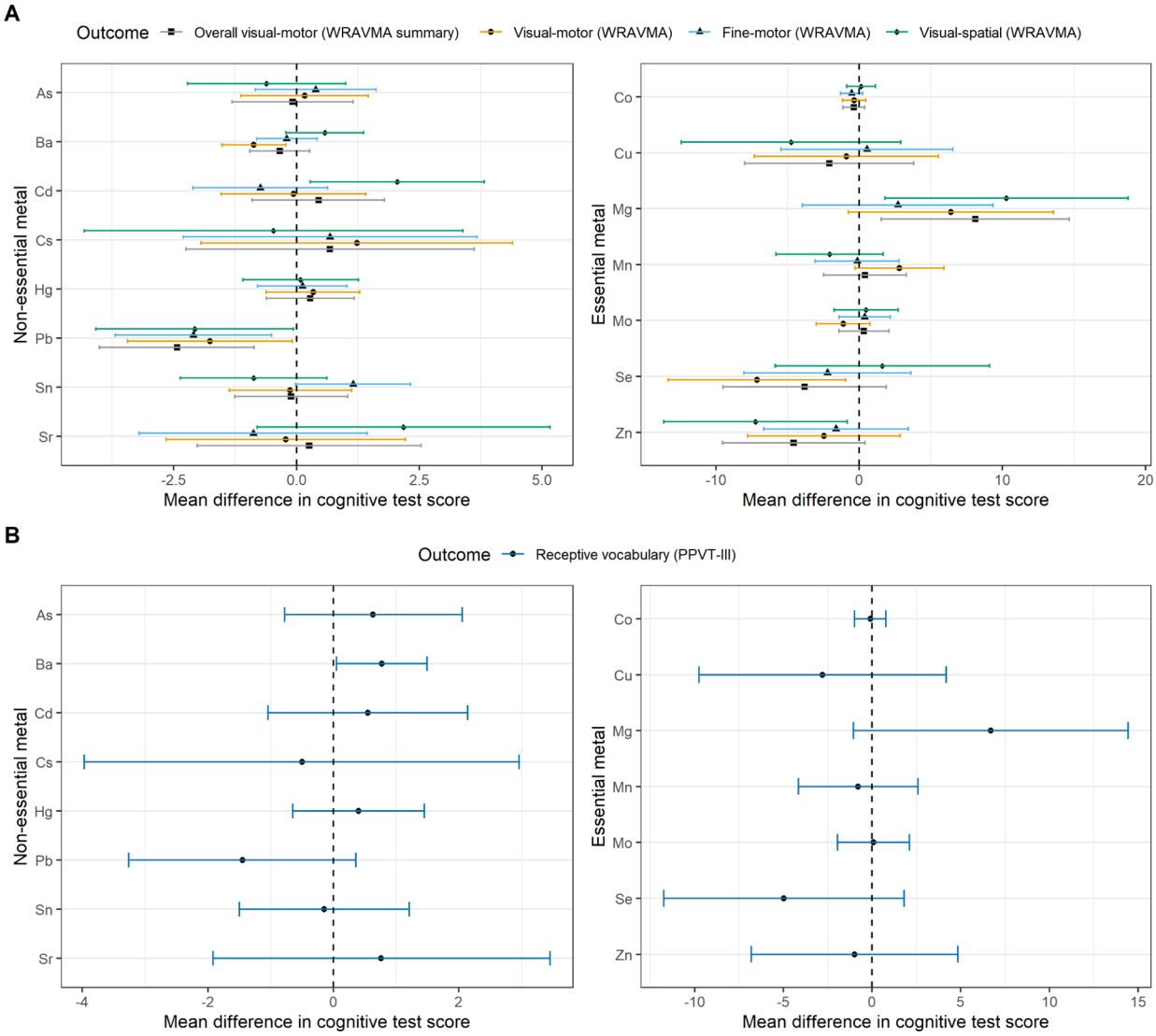

Figure 1.

Multivariable linear regression estimates and 95% confidence intervals of adjusted mean difference in early childhood (mean age: 3.2 years; range: 2.9–4.0 years) cognitive test scores assessing visual-motor ability (WRAVMA) (**A**) or receptive vocabulary (PPVT-III) (**B**), associated with a doubling in early childhood erythrocyte concentration of a non-essential (left) or essential (right) metal. N=336 mother-child pairs from the Project Viva pre-birth cohort in Boston, Massachusetts, USA, were included. Models adjusted for child factors, including race and ethnicity, fish consumption, daycare attendance, breastfeeding duration, annual household income, and maternal factors, including PPVT-III score at early childhood visit, education, marital status, and age at enrollment. For each metal, we also adjusted for the maternal first trimester erythrocyte concentration of the metal, and early childhood erythrocyte concentrations of the 14 other concurrently measured metals. PPVT-III: Peabody Picture Vocabulary Test, 3rd edition; WRAVMA: Wide Range Assessment of Visual Motor Abilities.

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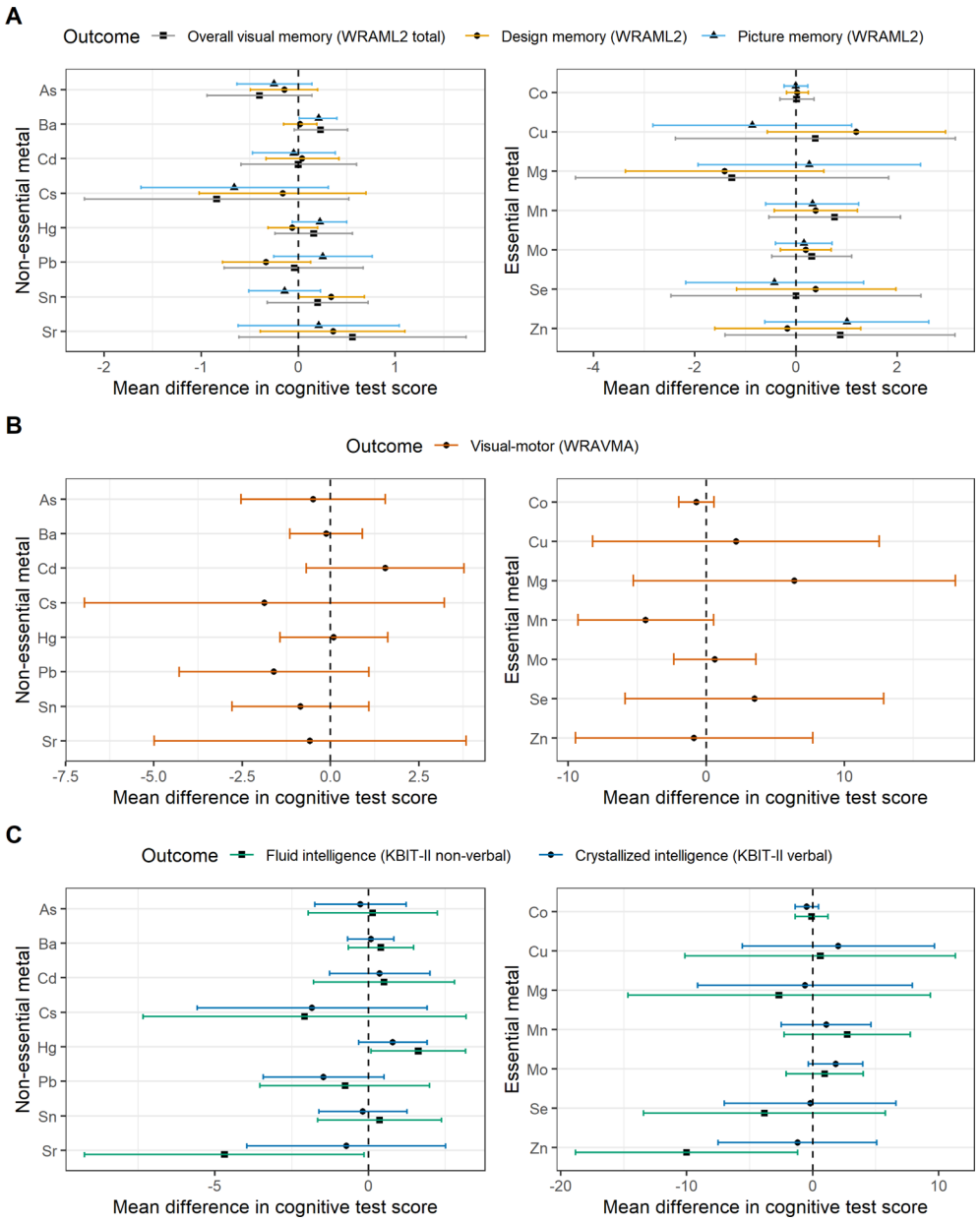

Figure 2.

Multivariable linear regression estimates and 95% confidence intervals of adjusted mean difference in mid-childhood (mean age: 7.8 years; range: 6.6–10.7 years) cognitive test scores assessing visual memory (WRAML2) (**A**), visual-motor ability (WRAVMA) (**B**), crystallized intelligence (KBIT-II verbal), or fluid intelligence (KBIT-II non-verbal) (**C**), associated with a doubling in early childhood (mean age: 3.2 years; range: 2.9–4.0 years) erythrocyte concentration of a non-essential (left) or essential (right) metal. N=296 mother-child pairs from the Project Viva pre-birth cohort in Boston, Massachusetts, USA, were included. Models adjusted for child factors, including race and ethnicity, fish consumption, daycare attendance, breastfeeding duration, annual household income, and maternal factors, including KBIT-II composite score at mid-childhood visit, education, marital status, and age

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at enrollment. For each metal, we also adjusted for the maternal first trimester erythrocyte concentration of the metal, and early childhood erythrocyte concentrations of the 14 other concurrently measured metals. KBIT-II: Kaufman Brief Intelligence Test, Second Edition; WRAML2: Wide Range Assessment of Memory and Learning, Second Edition; WRAVMA: Wide Range Assessment of Visual Motor Abilities.

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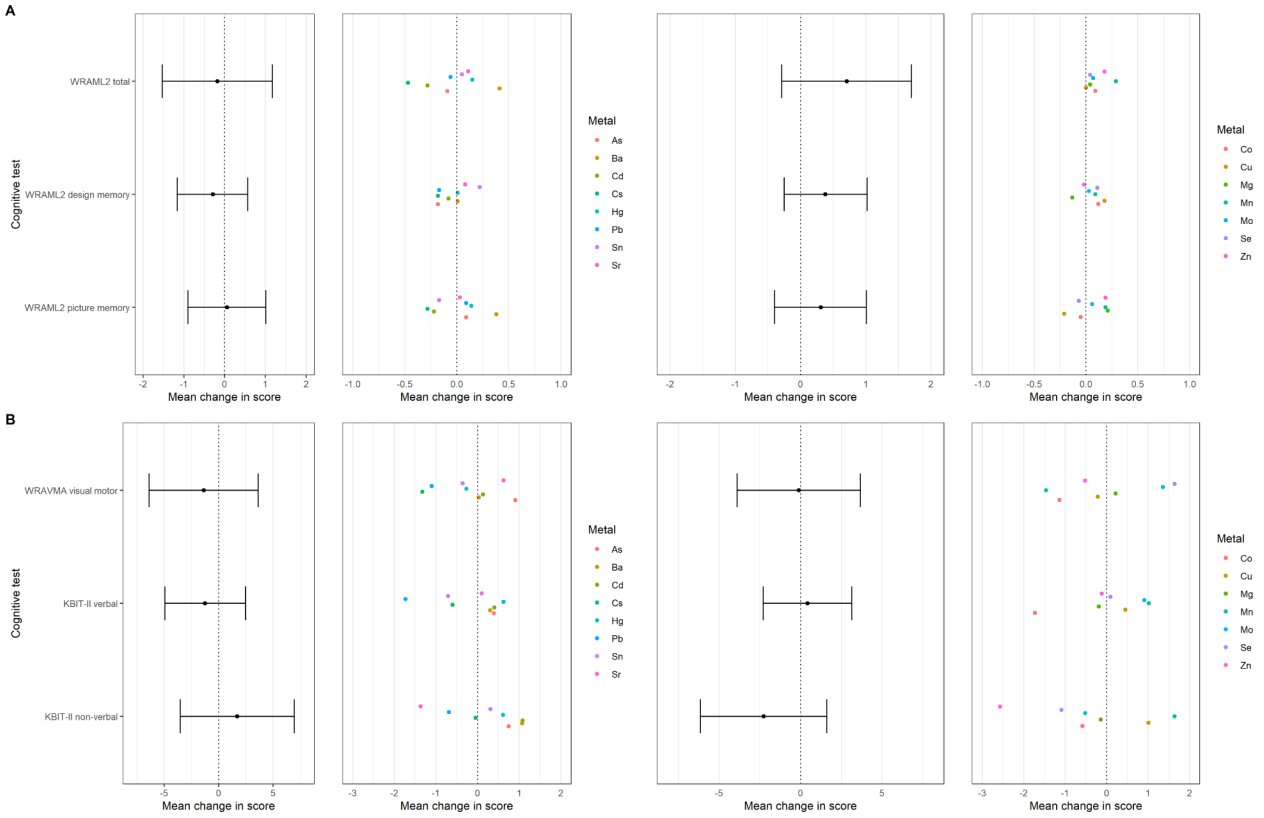

Figure 3.

Quantile g-computation estimates and 95% confidence intervals of adjusted mean difference in mid-childhood cognitive test scores for each quartile increase in early childhood erythrocyte concentration of a mixture of non-essential metals (arsenic (As), barium (Ba), cadmium (Cd), cesium (Cs), mercury (Hg), lead (Pb), tin (Sn), and strontium (Sr)) (**A**) or essential metals (cobalt (Co), copper (Cu), magnesium (Mg), manganese (Mn), molybdenum (Mo), selenium (Se), and zinc (Zn)) (**B**). Cognitive tests assessed visual memory (WRAML2), visual-motor ability (WRAVMA), crystallized intelligence (KBIT-II verbal) or fluid intelligence (KBIT-II non-verbal). Component point estimates are provided to the right of the mixture point estimates and intervals. Models adjusted for child factors, including race and ethnicity, fish consumption, daycare attendance, breastfeeding duration, annual household income, and maternal factors, including KBIT-II composite score at mid-childhood visit, education, marital status, age at enrollment, maternal first trimester erythrocyte concentrations of the mixture components, and early childhood erythrocyte concentrations of metals in the other mixture (e.g. non-essential metal mixture estimate is adjusted for essential metal mixture). KBIT-II: Kaufman Brief Intelligence Test, Second Edition; WRAML2: Wide Range Assessment of Memory and Learning, Second Edition; WRAVMA: Wide Range Assessment of Visual Motor Abilities.

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Table 1.

Mother-child pair characteristics in the analytic sample from the Project Viva pre-birth cohort, Boston, Massachusetts, USA.

Mother-child pair characteristic (N=349)	N (%) or mean ± SD
Covariates	
Annual household income	
$70,000 or less	109 (31.2%)
$70,001-$100,000	86 (24.6%)
Greater than $100,000	154 (44.1%)
Maternal education	
Graduate school	107 (30.7%)
Four-year college graduate	155 (44.4%)
Some college/Associate's, or less	87 (24.9%)
Maternal marital status	
Not married	40 (11.5%)
Married	309 (88.5%)
Child biological sex	
Male	187 (53.6%)
Female	162 (46.4%)
Child race and ethnicity	
NH White	251 (71.9%)
NH Black	42 (12.0%)
Hispanic	9 (2.6%)
NH Asian	6 (1.7%)
More than one race	41 (11.7%)
Child fish consumption, times per week	
Never	167 (47.9%)
Less than once per week	84 (24.1%)
Once per week or more	98 (28.1%)
Childcare	
Did not attend a daycare	92 (26.4%)
Attended a daycare	257 (73.6%)
Maternal age at enrollment, years	33.0 ± 4.59
Early childhood visit cognitive testing (n=336)	
Maternal PPVT-III score	108 ± 13.5
Child age, years	3.20 ± 0.181
Overall visual-motor (WRAVMA summary) (n=318)[a]	103 ± 10.7
Visual-motor (WRAVMA) (n=330)[a]	99.3 ± 11.1
Fine-motor (WRAVMA) (n=331)[a]	97.8 ± 10.5
Visual-spatial (WRAVMA) (n=325)[a]	109 ± 14.0
Receptive vocabulary (PPVT-III) (n=329)[a]	106 ± 13.3

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Mother-child pair characteristic (N=349)	N (%) or mean ± SD
Mid-childhood visit cognitive testing (n=296)	
Maternal KBIT-II composite score	109 ± 14.2
Child age, years	7.84 ± 0.773
Overall visual memory (WRAML2 total) (n=295)[a]	17.3 ± 4.26
Design memory (WRAML2) (n=296)[a]	8.26 ± 2.76
Picture memory (WRAML2) (n=295)[a]	9.03 ± 2.96
Visual-motor (WRAVMA) (n=293)[a]	93.4 ± 16.1
Crystallized intelligence (KBIT-II non-verbal) (n=296)[a]	108 ± 17.3
Fluid intelligence (KBIT-II verbal) (n=296)[a]	115 ± 13.5

KBIT-II: Kaufman Brief Intelligence Test, 2nd edition; NH: Non-Hispanic; PPVT-III: Peabody Picture Vocabulary Test, 3rd edition; SD: standard deviation; WRAML2: Wide Range Assessment of Memory and Learning, 2nd edition; WRAVMA: Wide Range Assessment of Visual Motor Abilities

[a]n denotes number of mother-child pairs included in analyses of the outcome, i.e., with data on at least one exposure, the indicated outcome, and all covariates.