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Mixtures of Metals and Micronutrients in Early Pregnancy and Cognition in Early and Mid-Childhood: Findings from the Project Viva Cohort

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Background: The developing fetal brain is sensitive to many environmental exposures. However, the independent and joint effects of prenatal exposure to metals and micronutrients on child cognition are not well understood.

Objectives: Our aim was to evaluate associations of first-trimester (∼10 wk) maternal erythrocyte concentrations of mixtures of nonessential and essential metals and micronutrients with early (∼3 y) and mid-childhood (∼8 y) cognitive test scores in Project Viva, a prebirth cohort in Boston, Massachusetts, USA.

Methods: We measured concentrations of five essential metals (Cu, Mg, Mn, Se, Zn) and two micronutrients (vitamin B12 and folate), together termed the "nutrient mixture," as well as six nonessential metals (As, Ba, Cd, Cs, Hg, Pb), together termed the "neurotoxic mixture," in first-trimester (∼10 wk) maternal erythrocytes (metals) or plasma (micronutrients). We assessed visual-motor function and receptive vocabulary in early childhood (∼3 y), and visual-motor function, visual memory, and fluid and crystallized intelligence in mid-childhood (∼8 y). We employed adjusted quantile g-computation and linear regression to estimate mixture and individual component associations, respectively.

Results: Analyses included 900 mother–child pairs (74% college graduates; 52% male children). In mixture analyses, a quartile increase in the nutrient mixture was associated with a mean difference in early childhood receptive vocabulary score of 1.58 points [95% confidence interval (CI): 0.06, 3.10], driven by Zn and Se. A quartile increase in the neurotoxic mixture was associated with a mean difference in mid-childhood visual-motor score of −3.01 points (95% CI: −5.55, −0.47), driven by Ba and Cs. Linear regressions supported quantile g-computation findings for mixture component contributions.

Discussion: Maternal circulating concentrations of several essential (Zn and Se) and nonessential (Ba and Cs) metals were associated with some domains of child cognition. In this folate-replete cohort, first-trimester circulating concentrations of known neurotoxic metals, such as Pb, were not associated with child cognition. https://doi.org/10.1289/EHP12016

Introduction

Subclinical deficits in cognitive ability are common and contribute to reduced academic achievement and greater risk of physical and mental health problems across the life span.[1] Brain development occurs rapidly *in utero* and is highly susceptible to exogenous factors.[2] For example, folic acid supplementation during pregnancy helps prevent neural tube defects and has been shown to improve cognitive development.[3]

Metals are a class of elements that have been studied for decades as modifiable early-life determinants of neurodevelopment. Multiple metals, such as mercury (Hg) and lead (Pb), are known to cross the placenta and accumulate in the fetus, where they may play essential or detrimental roles in neurodevelopment through multiple pathways such as DNA synthesis, oxidative stress, and neurotransmitter production, potentially differentially by sex.[4] Human exposure to heavy metals, such as Pb and Hg, is ubiquitous and largely a consequence of modifiable anthropogenic industrial activity, whereas essential metals, such as zinc (Zn) and magnesium (Mg), are often obtained through diet and sometimes through supplements.[5]

Policies removing Pb from paint and gasoline have conferred large public health benefits in the United States.[6] However, there is an urgent need to evaluate the effects of low-level exposure to Pb and other metals and metalloids on child cognition, given widespread exposure among U.S. pregnant persons[7] and disparities in exposure by race/ethnicity and socioeconomic status (SES).[8,9] In nationally representative surveys, cadmium (Cd), Pb, and Hg were detected in 66%, 96%, and 86% of U.S. pregnant women, respectively,[7] and Mexican-American pregnant women had higher blood Pb concentrations than non-Hispanic White individuals.[9] Although most previous studies of prenatal metal exposure and cognition have examined metals individually, joint modeling of multiple environmental exposures is a national research priority[10] because this approach can reduce bias and permits the estimation of joint effects of the mixture, more closely reflecting the real-world impact of modulating shared exposure sources in diet and the environment. Among the limited number of studies of prenatal metal mixtures and cognition,[11–13] none consider concurrent micronutrient status, such as vitamin B12 or folate, despite the critical need for these nutrients in neurodevelopment via one-carbon metabolism and epigenetic programming.[14]

In the present study, we analyzed longitudinal data from the Project Viva prebirth cohort in Boston, Massachusetts, USA, with the objective of estimating adjusted associations of concentrations of 13 essential and nonessential metals and micronutrients in maternal first-trimester erythrocytes and plasma with child performance on 11 tests spanning multiple domain-specific

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and general measures of cognition, administered across early childhood (~ 3 y) and mid-childhood (~ 8 y). In addition to estimating single-exposure associations, we implemented a recently developed approach, quantile g-computation,[15] to estimate the associations of cognitive test scores with increases in multiple nonessential metals (neurotoxic mixture) or essential metals and micronutrients (nutrient mixture) simultaneously. This approach better reflects the complexity of human exposure and may more closely approximate the impacts of interventions that would modulate multiple exposures. We hypothesized that the nutrient mixture would be associated with higher cognitive test scores, and the neurotoxic mixture would be associated with lower cognitive test scores.

Methods

Study Population: Project Viva

We conducted a prospective cohort study of mother–child pairs in Project Viva. Project Viva is a prebirth cohort with ongoing follow-up designed to study the effects of prenatal diet and other factors on maternal and child health. Details about recruitment and follow-up in Project Viva are provided elsewhere.[16] Briefly, pregnant persons were approached for enrollment at their first prenatal medical visit to one of eight obstetric practices belonging to Atrius Harvard Vanguard Medical Associates, a multisite group practice in eastern Massachusetts, between April 1999 and November 2002. Exclusion criteria included multiple gestation, inability to answer questions in English, planning to move out of the area before delivery, and gestational age >21 wk at recruitment. To avoid violating the assumption of independent observations in statistical models, in the analyses we retained only the first birth that occurred to a mother during the recruitment period. Mothers of all participating children gave written informed consent at enrollment and follow-up visits, and children provided verbal assent starting at the mid-childhood visit. The institutional review board of Harvard Pilgrim Health Care approved Project Viva study protocols.

Metals in First-Trimester Erythrocytes

Blood samples were collected at recruitment from consenting pregnant persons (median gestation 9.9 wk). Circulating metal concentrations (ng/g) were measured in the erythrocyte fraction of the sample. To isolate erythrocytes, samples were centrifuged at 2,000 rpm for 10 min at 4°C. Subsequently, concentrations of all metals, except Hg, were quantified using triple quadrupole inductively coupled plasma–mass spectrometry [Agilent 8800 triple quadrupole inductively coupled plasma mass spectrometry (ICP-QQQ); Agilent Technologies, Inc.], with all aliquots stored at −70°C prior to laboratory analyses. Briefly, 0.5 mL of stored packed erythrocyte was weighted and digested for 48 h in 2 mL of ultrapure concentrated HNO_3 acid, diluted in 10 mL of deionized water, and then digested further using 1 mL of 30% ultrapure HNO_3 acid. Metal concentrations were then measured on a single run of ICP-QQQ using tandem mass spectrometry. Hg concentrations were measured using the Direct Mercury Analyzer 80 (Milestone, Inc.). Three quality control methods were used to ensure valid concentration estimates: procedural blanks, initial and continuous calibration verification, and blinded technical replicates in 2% of samples. Of the 20 metals initially measured, we selected the subset with <10% of measurements below the limit of detection (LOD) for the metal and an intraclass correlation coefficient (ICC) across blinded technical replicates >0.5 to reduce the impact of measurement error on the results. Metals meeting these criteria included six nonessential metals [arsenic (As), barium (Ba), Cd, cesium (Cs), Hg, and Pb] and five essential metals [copper (Cu), Mg, manganese (Mn), selenium (Se), and Zn].

Vitamin B12 and Folate in First-Trimester Plasma

We measured Vitamin B12 (pg/mL) and folate (ng/mL) concentrations in the plasma of the same first-trimester blood samples used to measure the metals and specifically among pregnant persons whose children participated in cognitive testing at early- or mid-childhood follow-up visits. We sent samples to Boston Children's Hospital's Clinical and Epidemiological Research Laboratory (CERLab) for measurements. We measured vitamin B12 using an electrochemiluminescence immunoassay (Elecsys Vitamin B12 II; Roche Diagnostic) and folate using an electrochemiluminescence binding assay (Elecsys Folate III; Roche Diagnostic). Briefly, vitamin B12 or folate are complexed with biotinylated and ruthenium-labeled antibodies and magnetically entrapped on an electrode. Voltage is applied to the electrode, stimulating a chemiluminescent reaction, with the generated light intensity proportional to vitamin B12 or folate concentration. Both assays were conducted on the Roche Cobas 6000 system (Roche Diagnostics) and are approved by the U.S. Food and Drug Administration for clinical use. The assay for Vitamin B12 has day-to-day imprecision values for concentrations of 203, 481, and 1,499 pg/mL of 7.6%, 4.4%, and 3.2%, respectively. The assay for folate has day-to-day imprecision values for concentrations of 7.6, 14.3, and 19.2 ng/mL of 3.9%, 3.1%, and 2.0%, respectively. The protocols for vitamin B12 and folate assays do not require repeated measurements due to high reliability of the assays. Hemolysis (destruction of erythrocytes) and/or lipemia (accumulation of lipoproteins, causing turbidity) may affect the validity of concentration estimates, and these conditions were present in some plasma samples. Sensitivity analyses were performed adjusting for these observations in regression models using an indicator variable (see "Sensitivity Analyses" section).

Child Cognition in Early and Mid-Childhood

Trained research staff measured cognitive ability in early childhood [mean age (range): 3.3 y (2.9–5.6)] and mid-childhood [mean age (range): 7.9 y (6.6–10.7)] using neuropsychological tests spanning several cognitive domains, including receptive vocabulary (the range of words understood by a person, in contrast to expressive vocabulary, which consists of words a person can express or produce), visual-motor ability, visual memory, crystallized intelligence (the ability to solve problems using relevant accumulated knowledge), and fluid intelligence (the ability to learn and reason to solve new problems). All assessments were double-scored using published scoring guidelines, as well as supplementary guidelines developed by a pediatric neuropsychologist, to improve consistency among scorers. Staff administering and scoring the tests were blinded to maternal first-trimester metal and micronutrient concentrations.

At the early childhood visit, the Wide Range Assessment of Visual Motor Abilities (WRAVMA)[17] and the Peabody Picture Vocabulary Test, 3rd edition (PPVT-III),[18] were administered. The WRAVMA consists of three subtests: Visual-Motor (copying a stimulus shape or structure by drawing it), Visual-Spatial (matching an image to a visual stimulus), and Fine Motor (fitting as many pegs into a pegboard as possible within 90 s). The subtest scores are combined to yield a summary score. The PPVT-III measures receptive vocabulary, the ability to recognize words, by asking participants to identify the image that best matches a word spoken by the test administrator. The PPVT-III produces a single score.

At the mid-childhood visit, the WRAVMA visual-motor subtest was again administered, in addition to the visual memory component of the Wide Range Assessment of Memory and Learning, 2nd edition (WRAML2),[19] and the Kaufman Brief Intelligence Test, second edition (KBIT-II).[20] The WRAML2 consists of two subtests to assess short-term visuospatial memory: Design Memory (draw, from memory, a stimulus geometric figure that is shown briefly) and Picture Memory (identify, from memory, differences between an image that is shown briefly and a near-replicate). We summed these to create a total visual memory score. The KBIT-II yields intelligence quotient (IQ) scores quantifying verbal (crystallized) and nonverbal (fluid) intelligence. The verbal component is composed of the Verbal Knowledge subtest, which assesses receptive vocabulary in fashion similar to that of the PPVT-III, and the Riddles subtest, which assesses expressive vocabulary by asking participants to select an image or provide a word that best answers a spoken riddle. The nonverbal component is composed of the Matrices test, in which participants select an image that best matches the underlying patterns in a matrix of images.

For all tests, higher scores indicate higher cognitive function. All tests have demonstrated strong split-half and test-retest reliabilities, and moderate-to-strong correlations with validated full-scale intelligence tests such as the Weschler Intelligence Scale for Children (WISC).[17–20] For a given test, a score was not calculated for children who did not complete the test in its entirety or who were unable to correctly answer any questions. Raw scores were standardized by norming to age-specific reference samples of the U.S. pediatric population, according to the tests' reference manuals.[17–20] In the reference samples, the WRAML2 design and picture memory subtests each have a standardized mean [standard deviation (SD)] of 10 (3), whereas all other tests have a standardized mean ± SD of 100 (15).

Covariates

We identified confounders as common causes of the exposures and outcomes using subject matter knowledge and the extant literature. Confounders and other covariates are presented in a conceptual diagram (Figure S1). We obtained the following maternal confounders via questionnaires or interviews at the recruitment visit: self-identified race/ethnicity as a proxy for structural racism[21] (non-Hispanic White, non-Hispanic Black, Hispanic, non-Hispanic Asian, or more than one race/ethnicity), annual household income (less than or equal to USD $70,000, or >USD $70,000), education (less than college degree or at least college degree), smoking (never smoked, former smoker, or smoked during pregnancy), age at enrollment (years), prepregnancy body mass index (BMI) (kg/m^2) using self-reported weight and height, and weekly fish consumption in the first trimester (servings of fish per week) estimated via semi-quantitative food frequency questionnaire (FFQ). Child biological sex (male or female) and maternal intelligence (PPVT-III and KBIT-II composite IQ score, for early- and mid-childhood analyses, respectively), strong predictors of child cognition that are not confounders, were included as additional covariates in statistical models to improve precision on estimates of associations. Because cognitive test scores were age-standardized, we did not include child age at cognitive testing as a covariate.

Preliminary Analyses

For all metals and micronutrients, we imputed concentrations below the LOD as the LOD divided by the square root of 2. Two plasma samples with vitamin B12 concentrations above the assay upper limit of 4,000 pg/mL were imputed as 4,000 pg/mL. Given the presence of outliers in the exposure data, we considered log-transformation of exposures prior to analyses. Following recommendations by Choi et al. to base exposure transformation on model fit,[22] we compared the fit of multivariable linear regression models for untransformed vs. $\log_2$-transformed exposures using the Akaike information criterion (AIC). The percent difference in AIC between models was extremely small for all models (<0.3%), indicating either approach resulted in similar fit to the data. Given that $\log_2$-transformation helps mitigate the potential disproportionate impact of outliers while yielding an interpretable and comparable contrast across element biomarkers, where a 1-unit increase in $\log_2$-transformed metal or nutrient concentration corresponds to a doubling of concentration on the original untransformed scale, we $\log_2$-transformed all exposures prior to analyses. For Bayesian kernel machine regression (BKMR) models used to evaluate interaction, exposures were standardized after $\log_2$-transformation to increase comparability of scale and thereby increase model stability.[13]

We calculated Pearson correlations among the $\log_2$-transformed exposures, and Pearson correlations among the standardized cognitive test scores. We compared erythrocyte metal concentrations to laboratory reference ranges for adult women and compared plasma micronutrient concentrations to reference ranges in serum for first-trimester pregnant women.[23] For the micronutrients, we additionally compared concentrations to clinical deficiency thresholds (<200 pg/mL for vitamin B12 and <3 ng/mL for folate[24]) In single-exposure multivariable linear regression models with adjustment for confounders and covariates, including a square term for each exposure did not improve model fit according to the Bayesian information criterion, and therefore we included $\log_2$-transformed exposures as linear terms in statistical models.

Mixture Analyses: Quantile g-Computation

Estimating associations for individual exposure, although relevant for understanding etiology, can have limited real-world interpretability because potential interventions would be very likely to impact exposure to multiple environmental contaminants or nutrients.[10] We used quantile g-computation to estimate the associations of a "neurotoxic" mixture comprising the nonessential metals (As, Ba, Cd, Cs, Hg, Pb), and a "nutrient" mixture comprising the essential metals (Cu, Mg, Mn, Se, Zn), vitamin B12, and folate, with each of the child cognition outcomes, using the R package qgcomp.[15] For normally distributed continuous outcomes, such as the cognition scores, quantile g-computation fits a multivariable linear regression model with the exposures comprising the mixture divided into quantiles, adjusting for covariates. The joint effect of increasing all exposures within the mixture by one quantile is estimated by summing exposure coefficients, and 95% confidence intervals (CIs) are constructed. Assuming a linear association across exposure quartiles because square terms did not improve model fit in preliminary models, we used quantile g-computation to estimate the difference in mean child cognition score and 95% CIs associated with jointly increasing all components in either the neurotoxic or nutrient mixture by one quartile, adjusting for all confounders and covariates identified in the conceptual model (Figure S1). For a given mixture (e.g., neurotoxic), we excluded components of the other mixture (e.g., nutrients) to maximize precision, given the large number of model parameters and correlations among the exposures (see "Results" section), and minimize potential bias amplification from unmeasured sources.[25] For estimates where the 95% CI excluded the null (mean difference of 0), we plotted weights of the mixture components, corresponding to their relative contributions to the total effect in each direction, in the Supplemental Material.

Individual Metal Analyses

We used multivariable linear regression models to estimate single element or nutrient associations. A separate model was fitted for each continuous exposure with no other exposures in the model to maximize comparability with previous single-exposure analyses, with adjustment for covariates. We estimated the mean difference in cognitive test score and 95% CI associated with a 1-unit increase in each $\log_2$-transformed exposure, equivalent to a doubling in each exposure on the original scale.

Effect Measure Modification by Child Sex

We fitted sex-stratified quantile g-computation models to assess additive scale effect measure modification by sex. We compared point estimates and CIs across outcomes to identify trends in the differences between associations by sex.

Statistical Interaction among Mixture Components: BKMR

BKMR with component-wise variable selection was employed to explore potential interaction between standardized exposures in relation to WRAVMA summary score and PPVT-III for early childhood, and WRAML2 total score, WRAVMA visual-motor subtest, KBIT-II verbal, and KBIT-II nonverbal for mid-childhood. BKMR is described in detail elsewhere.[26,27] Briefly, this approach models potentially correlated components of an exposure mixture as a multivariate kernel function, allowing for interactions and nonlinear concentration–response within the mixture, while adjusting for covariates. Using the R package *bkmr*, we fit one BKMR model for each of these outcomes with all metals and micronutrients included in the kernel function, with the same covariate set as in other statistical models. Using package default priors, models were fitted by taking 100,000 samples from the posterior for each of three chains, with 50% of each chain discarded as burn-in and thinning every 50th iteration to reduce autocorrelation, resulting in 1,000 iterations used to characterize the posterior distribution. Interaction was explored pairwise among the exposures by visually assessing differences in the shape of concentration–response functions for each mixture component, holding each of the other components in turn at their 10th, 50th, and 90th percentile values, and holding all other components at their median values.

Sensitivity Analyses

Among mother–child pairs with information on at least one exposure and one outcome, the covariate with greatest missingness was first-trimester fish consumption, absent in 5%–7% of mothers. Given this small percentage, we proceeded with conducting complete case analysis excluding these observations. In sensitivity analyses, we fit quantile g-computation models with adjustment for the following factors that may affect erythrocyte metal or micronutrient concentrations: *a*) maternal gestational age–standardized hematocrit (the fraction of blood volume comprising erythrocytes) and *b*) in nutrient mixture models, an indicator variable for presence of hemolysis and/or lipemia in plasma samples used to measure vitamin B12 and folate. We additionally conducted sensitivity analyses adjusting for maternal first-trimester Prudent diet index score, estimated by FFQ and reflecting similarity to a dietary pattern dominant in green leafy vegetables, cabbages, fruits, tomatoes, poultry, and fish, to assess potential confounding by dietary factors other than fish consumption. Because effect estimation is more informative for public health policy and practice than null hypothesis significance testing,[28] and given the implausibility of a global null hypothesis across all exposures and outcomes given numerous previous studies of prenatal metal exposure and child neurodevelopment that observed significant associations,[29] we focused interpretations on point estimates and width of CIs rather than *p*-values and did not correct for multiple hypothesis testing. All statistical analyses were conducted in R (version 3.6.3; R Development Core Team).

Results

Study Population

Of 2,128 live births, we excluded 28 children who were not the first birth during the recruitment period and 697 mothers with insufficient or no blood sample for metal or micronutrient measurement (Figure S2). We analyzed a total of 900 mother–child pairs on whom there was complete information for at least one exposure, one cognitive test (requiring participation in the test, completion of the test, and at least one correct answer on the test), and all confounders and covariates (Table 1), including 834 mother–child pairs in early childhood cognition analyses and 705 in mid-childhood cognition analyses. Among mothers in the analytic sample, 75.4% were non-Hispanic White, 73.7% had a college degree, 64.3% had an annual household income >USD $70,000, and 68.4% reported never smoking in their lifetime. Mean ± SD of maternal prepregnancy BMI was $24.7 \pm 5.2\ \mathrm{kg/m^2}$, and mean ± SD of maternal age at enrollment was 32.7 ± 4.6 y . At the early-childhood visit, the mean ± SD child age was 3.25 ± 0.29 y, and at the mid-childhood visit it was 7.92 ± 0.81 y. Overall, cognitive test scores among females were higher than those of males at both visits. Maternal intelligence test scores in this population were generally higher than that of the tests' reference populations, indicated by average scores being above the normalized mean of 100. In comparison with those excluded from the analyses, mothers in the analytic sample were more likely to be non-Hispanic White, have higher income and education, and were less likely to smoke during pregnancy (Table S1).

Exposure and Outcome Distributions

For the exposures, the distributions, LOD, percent detection, ICCs, laboratory reference ranges, and, for vitamin B12 and folate, percentage of clinically deficient mothers, are provided in Table 2. The geometric means of metals were within laboratory reference ranges, whereas geometric means of vitamin B12 and folate were above the upper bound of their respective reference ranges. No mothers were deficient in folate. Six mothers ($\sim 1\%$) were deficient in vitamin B12. In blood samples that were used to measure vitamin B12 and folate, hemolysis occurred in 13% of samples, and lipemia or hemolysis and lipemia occurred in $\sim 5\%$ of samples (Table S2). Pearson correlations among the $\log_2$-transformed exposures were weak to moderate (range of $r = -0.12$ to 0.41) (Figure S3). Correlations among nonessential metals were generally weaker (maximum correlation $r = 0.29$ between As and Hg) than correlations among essential metals and micronutrients (maximum correlation $r = 0.41$ between Mg and Se). Vitamin B12 and folate were generally not correlated with the metals or with each other ($r = 0.08$). For child cognition tests composed of subtests (e.g., WRAVMA and WRAML2), subtests were weakly correlated with each other, as would be expected if the subtests captured unique aspects of cognition within the domain of the overall test (Figure S4). The strongest correlation between different tests was observed between PPVT-III score in early childhood and KBIT-II verbal IQ in mid-childhood ($r = 0.57$). Both tests capture knowledge and abilities related to receptive vocabulary. For the WRAVMA visual-motor subtest, the only test that was administered at both the early and mid-childhood visits, correlation was weak between visits ($r = 0.18$).

Table 1. Mother–child pair characteristics of the study sample, overall and stratified by child sex, from the Project Viva cohort in Boston, Massachusetts.

Mother–child pair characteristic	Overall *n* (%) or mean ± SD	Male *n* (%) or mean ± SD	Female *n* (%) or mean ± SD
n	900	464	436
Maternal self-reported race/ethnicity			
NH White	679 (75.4%)	349 (75.2%)	330 (75.7%)
NH Black	88 (9.8%)	41 (8.8%)	47 (10.8%)
Hispanic	57 (6.3%)	34 (7.3%)	23 (5.3%)
NH Asian	39 (4.3%)	22 (4.7%)	17 (3.9%)
More than one race/ethnicity	37 (4.1%)	18 (3.9%)	19 (4.4%)
Maternal education			
College graduate	663 (73.7%)	337 (72.6%)	326 (74.8%)
Less than college graduate	237 (26.3%)	127 (27.4%)	110 (25.2%)
Annual household income			
>USD $70,000	579 (64.3%)	302 (65.1%)	277 (63.5%)
≤USD $70,000	321 (35.7%)	162 (34.9%)	159 (36.5%)
Maternal smoking			
Never smoked	616 (68.4%)	311 (67.0%)	305 (70.0%)
Former smoker	188 (20.9%)	95 (20.5%)	93 (21.3%)
Smoked during pregnancy	96 (10.7%)	58 (12.5%)	38 (8.7%)
Hemolysis or lipemia of blood during folate or vitamin B12 measurement			
Yes	156 (17.3%)	87 (18.8%)	69 (15.8%)
No	744 (82.7%)	377 (81.3%)	367 (84.2%)
Maternal prepregnancy BMI (kg/m^2)	24.7 ± 5.2	24.8 ± 5.3	24.7 ± 5.1
Maternal age at enrollment (y)	32.7 ± 4.6	32.5 ± 4.6	32.8 ± 4.5
Maternal first-trimester fish consumption, servings/week	1.68 ± 1.41	1.64 ± 1.40	1.72 ± 1.42
Maternal Prudent diet index	0.000211 ± 0.926	−0.0213 ± 0.926	0.0231 ± 0.927
Maternal hematocrit (%)	70.8 ± 26.0	70.9 ± 25.7	70.7 ± 26.4
Early childhood visit ($n = 834$)			
Maternal receptive vocabulary (PPVT-III)	107 ± 14.1	107 ± 14.3	107 ± 14.0
Child age (y)	3.25 ± 0.29	3.25 ± 0.27	3.25 ± 0.30
Overall visual-motor (WRAVMA summary) ($n = 793$)[a]	103 ± 11.4	100 ± 11.4	105 ± 10.8
Visual-motor (WRAVMA) ($n = 825$)[a]	99.2 ± 11.3	96.7 ± 10.6	102 ± 11.4
Fine-motor (WRAVMA) ($n = 818$)[a]	98.2 ± 10.8	96.8 ± 11.0	99.7 ± 10.4
Visual-spatial (WRAVMA) ($n = 796$)[a]	109 ± 13.6	107 ± 13.4	111 ± 13.5
Receptive vocabulary (PPVT-III) ($n = 810$)[a]	105 ± 13.8	104 ± 14.2	107 ± 13.3
Mid-childhood visit ($n = 705$)			
Maternal overall intelligence (KBIT-II composite)	109 ± 14.4	109 ± 14.9	108 ± 13.8
Child age (y)	7.92 ± 0.81	7.93 ± 0.80	7.90 ± 0.83
Overall visual memory (WRAML2 total) ($n = 701$)[a]	17.0 ± 4.4	16.8 ± 4.6	17.2 ± 4.3
Design memory (WRAML2) ($n = 703$)[a]	8.09 ± 2.78	8.16 ± 2.94	8.03 ± 2.62
Picture memory (WRAML2) ($n = 702$)[a]	8.89 ± 3.02	8.66 ± 3.00	9.13 ± 3.03
Visual-motor (WRAVMA) ($n = 699$)[a]	92.7 ± 16.7	90.7 ± 16.8	94.6 ± 16.4
Crystallized intelligence (KBIT-II non-verbal) ($n = 705$)[a]	108 ± 16.8	107 ± 16.8	108 ± 16.8
Fluid intelligence (KBIT-II verbal) ($n = 697$)[a]	114 ± 14.0	113 ± 14.4	115 ± 13.6

Note: BMI, body mass index; CI, confidence interval; 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.

Neurotoxic and Nutrient Mixture Associations from Quantile g-Computation

Quantile g-computation estimates of associations of neurotoxic and nutrient mixtures with cognitive test scores at early and mid-childhood are provided in Table 3, with quartile values for each exposure provided in Table 2. The nutrient mixture was associated with higher PPVT-III score in early childhood (mean difference per quartile increase in nutrient mixture: 1.58 points, 95% CI: 0.06, 3.10). The largest contributor to the positive association was Zn, followed closely by Se and Mg (Figure S5). The nutrient mixture was also weakly associated with higher WRAVMA visual-motor score, though the CI included the null (0.55 points, 95% CI: −0.80, 1.90).

Associations between the neurotoxic mixture and early childhood cognitive test scores were relatively small and imprecise (Table 3).

In mid-childhood, the neurotoxic mixture was associated with lower WRAVMA visual-motor subtest score (−3.01 points, 95% CI: −5.55, −0.47) (Table 3), driven by Ba and Cs (Figure S6). This was not consistent with the null association observed between the neurotoxic mixture and WRAVMA visual-motor in early childhood (0.41, 95% CI: −1.11, 1.93) (Table 3).

In mid-childhood, the nutrient mixture was associated with higher KBIT-II nonverbal IQ, though the CI included the null (1.31 points, 95% CI: −0.83, 3.45) (Table 3). The association between the nutrient mixture and KBIT-II verbal IQ in mid-childhood, which assesses receptive vocabulary (as well as expressive vocabulary) was null (−0.07 points, 95% CI: −1.73, 1.60), suggesting the association observed between the nutrient mixture and receptive vocabulary in early childhood, as measured by the PPVT-III, did not persist into mid-childhood.

Individual Exposure Associations from Multivariable Linear Regression

Plots of point estimates and 95% CIs for associations per doubling of individual metals and micronutrients with early childhood cognitive test scores are provided in Figure 1, and corresponding numeric estimates are provided in Table S3. For associations between neurotoxic metals and early childhood cognition, As and Cd exhibited positive associations with early childhood visual-motor subtest score (mean difference per doubling of As: 0.72 points, 95% CI: 0.15, 1.28; Cd: 1.08 points, 95% CI: 0.34, 1.82). To investigate residual confounding by

Table 2. Distributions and characteristics of maternal first-trimester (~10 wk) erythrocyte concentrations of metals or plasma concentrations of vitamin B12 and folate in the Project Viva cohort, Boston, Massachusetts, USA ($n = 900$).

Exposure	Blood component used for measurement	Units	GM (GSD)	Minimum	10th percentile	25th percentile	Median	75th percentile	90th percentile	Maximum	Limit of detection	Percent detected	ICC	Laboratory reference range[a]	Clinically deficient[b] mothers [*n* (%)]
Arsenic (As)	Erythrocytes	ng/g	0.772 (2.7)	0.0964	0.164	0.43	0.833	1.55	2.55	34.1	0.153	90	0.89	<8	—
Barium (Ba)	Erythrocytes	ng/g	3.64 (2.3)	0.291	1.44	2.04	3.16	6.12	11.7	59.2	0.412	99	0.82	—	—
Cadmium (Cd)	Erythrocytes	ng/g	0.365 (2.1)	0.0402	0.169	0.268	0.38	0.542	0.805	6.94	0.0569	95	0.93	<2	—
Cesium (Cs)	Erythrocytes	ng/g	2.56 (1.4)	0.782	1.66	2.05	2.57	3.21	3.9	10	0.0587	100	0.90	<15	—
Copper (Cu)	Erythrocytes	ng/g	563 (1.2)	256	460	512	564	617	679	3,970	1.85	100	0.64	520–900	—
Mercury (Hg)	Erythrocytes	ng/g	3.06 (3.3)	0.00004	0.825	1.7	3.28	6.54	11.6	117	0.300	92	0.92	<10	—
Magnesium (Mg)	Erythrocytes	ng/g	41,100 (1.2)	19,100	33,500	36,900	40,900	45,900	51,300	76,600	4.15	100	0.73	39,000–59,000	—
Manganese (Mn)	Erythrocytes	ng/g	16.1 (1.6)	0.298	10.8	13.1	16.2	20.4	25.3	42.9	0.422	100	0.83	9–33	—
Lead (Pb)	Erythrocytes	ng/g	18.2 (1.5)	4.41	11.1	13.7	17.8	23.8	31.1	90.8	0.0746	100	0.96	<50	—
Selenium (Se)	Erythrocytes	ng/g	250 (1.2)	98.4	199	222	248	279	318	1,090	1.73	100	0.77	160–490	—
Zinc (Zn)	Erythrocytes	ng/g	10,300 (1.2)	4,630	8,340	9,290	10,400	11,600	12,600	22,200	8.74	100	0.75	7,200–13,000	—
Vitamin B12	Plasma	pg/mL	483 (1.5)	106	307	381	483	596	765	4,000	30	99	—[c]	118–438	6 (1%)
Folate	Plasma	ng/mL	21.2 (1.8)	7.08	11.4	14	18.9	28.5	50.8	360	2.0	100	—[c]	2.6–15	0 (0%)

Note: —, no data; GM, geometric mean; GSD, geometric standard deviation; ICC, intraclass correlation coefficient across blinded technical replicates.

[a]Clinical laboratory reference ranges (https://newsite.doctorsdata.com/Red-Blood-Cell-Elements) for erythrocyte metal concentrations are specific to adult women (17–99 y of age). Reference ranges for vitamin B12 and folate are specific to pregnant women's first-trimester serum, obtained from Abbassi-Ghanavati et al.[23]

[b]Clinically sufficient ranges for essential metal concentrations in erythrocytes are unknown. The Institute of Medicine identified a clinical cutoff for serum vitamin B12 deficiency at 200 pg/mL and for serum folate deficiency at 3 ng/mL.[24]

[c]Assays for vitamin B12 and folate were run in singleton according to manufacturer instructions. The assay for Vitamin B12 has day-to-day imprecision values for concentrations of 203, 481, and 1,499 pg/mL of 7.6%, 4.4%, and 3.2%, respectively. The assay for folate has day-to-day imprecision values for concentrations of 7.6, 14.3, and 19.2 ng/mL of 3.9%, 3.1%, and 2.0%, respectively. Both assays are approved by the U.S. Food and Drug Administration for clinical use.

fish consumption, we conducted additional analyses stratifying mother–child pairs above vs. below the median maternal fish consumption level, with adjustment for continuous fish consumption in each model. The positive association noted for As was attenuated in mothers consuming below the median fish consumption of 1.47 servings/week (0.73 points, 95% CI: −0.36, 1.83) in comparison with above (1.61 points, 95% CI: 0.18, 3.03).

Associations were observed between several nutrient mixture components and early childhood cognitive test scores (Figure 1; Table S3). Though estimates were imprecise, the essential metals Cu and Zn were generally associated with higher scores on all WRAVMA subtests. Higher PPVT-III score was observed in relation to the essential metals Mg (4.11 points; 95% CI: 0.87, 7.35), Se (3.45 points; 95% CI: 0.42, 6.48), Zn (3.44 points; 95% CI: 0.06, 6.83), and Cu (3.35 points; 95% CI: −0.06, 6.76), supporting results from quantile g-computation where, in a joint model with mutual adjustment for nutrient components, Zn and Se drove the association between the nutrient mixture and PPVT-III score (Figure S5).

Plots of point estimates and 95% CIs for associations of individual metal and micronutrient exposures with mid-childhood cognitive test scores are provided in Figure 2, and corresponding numeric estimates are provided in Table S4. Cu was associated with higher WRAML2 picture memory score (1.04 points; 95% CI: 0.15, 1.92) and total WRAML2 score (1.30 points; 95% CI: 0.01, 2.59). Mn was associated with higher KBIT-II nonverbal IQ (2.38 points; 95% CI: 0.51, 4.24) and higher KBIT-II verbal IQ (1.30 points; 95% CI: −0.14, 2.74). In contrast, Mg was associated with lower KBIT-II nonverbal IQ (−4.27 points; 95% CI: −8.77, 0.24) and Zn was associated with lower KBIT-II verbal IQ (−4.76 points; 95% CI: −8.36, −1.15). The nutrient mixture components were not associated with WRAVMA visual-motor subtest scores in mid-childhood.

Associations were observed between several individual neurotoxic mixture components and mid-childhood cognitive test scores (Figure 2; Table S4). Ba was associated with lower WRAVMA visual-motor subtest score (−1.27 points, 95% CI: −2.29, −0.25), consistent with the relatively large negative weight estimated from quantile g-computation analyses (Figure S6). The negative association between Cs and WRAVMA visual-motor subtest score was of similar magnitude, though less precise (−1.49 points; 95% CI: −4.16, 1.17). Ba was also associated with slightly lower KBIT-II nonverbal IQ, though the estimate was imprecise (−0.70 points; 95% CI: −1.70, 0.30). The neurotoxic metals were not consistently associated with WRAML2 visual memory scores.

Effect Measure Modification by Child Sex

In sex-stratified quantile g-computation models, there was little statistical evidence for effect measure modification (Table S5). Qualitatively, more negative associations for the neurotoxic mixture, and stronger positive associations for the nutrient mixture, were observed in females in comparison with males for most cognitive tests. Among females, positive associations were observed between the nutrient mixture and PPVT-III score in early childhood (2.58 points; 95% CI: 0.39, 4.77), driven by Mg (Figure S7), whereas the association was weaker among males (0.92 points; 95% CI: −1.26, 3.09). Among males, the neurotoxic mixture was negatively associated with WRAVMA visual-motor subtest score in mid-childhood (−3.98 points; 95% CI: −7.64, −0.33), driven by Ba (Figure S8). The association in females was consistent in direction but weaker (−0.72 points; 95% CI: −4.26, 2.82).

Table 3. Quantile g-computation estimates of adjusted mean difference in early- (∼3 y) or mid- (∼8 y) childhood cognitive test scores assessing visual-motor ability (WRAVMA), receptive vocabulary (PPVT-III), visual memory (WRAML2), fluid intelligence (KBIT-II nonverbal), or crystallized intelligence (KBIT-II verbal) associated with a quartile increase in first-trimester (∼10 wk) maternal blood concentration of neurotoxic (As, Ba, Cd, Cs, Hg, Pb) or nutrient (Cu, Mg, Mn, Se, Zn, vitamin B12, folate) mixtures. The analytic sample for these analyses included $n = 900$ mother–child pairs from the Project Viva cohort, Boston, Massachusetts. Quartile values for each mixture component are provided in Table 2.

	Adjusted[a] mean difference in cognitive test score (95% CI)	
Cognitive test	Neurotoxic mixture (As, Cd, Cs, Ba, Hg, Pb)	Nutrient mixture (Cu, Mg, Mn, Se, Zn, vitamin B12, folate)
Early childhood		
Overall visual-motor (WRAVMA summary)	0.52 (−1.03, 2.07)	0.02 (−1.35, 1.39)
Visual-motor (WRAVMA)	0.41 (−1.11, 1.93)	0.55 (−0.80, 1.90)
Fine-motor (WRAVMA)	−0.21 (−1.68, 1.26)	−0.21 (−1.52, 1.09)
Visual-spatial (WRAVMA)	0.90 (−0.97, 2.77)	0.10 (−1.53, 1.72)
Receptive vocabulary (PPVT-III)	0.11 (−1.66, 1.88)	1.58 (0.06, 3.10)[b]
Mid-childhood		
Overall visual memory (WRAML2 total)	0.04 (−0.63, 0.72)	−0.02 (−0.60, 0.56)
Design memory (WRAML2)	0.01 (−0.41, 0.43)	−0.01 (−0.38, 0.35)
Picture memory (WRAML2)	0.03 (−0.43, 0.49)	0.01 (−0.39, 0.41)
Visual-motor (WRAVMA)	−3.01 (−5.55, −0.47)[b]	−0.93 (−3.15, 1.29)
Fluid intelligence (KBIT-II non-verbal)	−0.48 (−2.94, 1.99)	1.31 (−0.83, 3.45)
Crystallized intelligence (KBIT-II verbal)	−0.23 (−2.16, 1.70)	−0.07 (−1.73, 1.60)

Note: As, arsenic; Ba, barium; Cd, cadmium; CI, confidence interval; Cs, cesium; Cu, copper; Hg, mercury; KBIT-II, Kaufman Brief Intelligence Test, 2nd edition; Mg, magnesium; Mn, manganese; Pb, lead; PPVT-III, Peabody Picture Vocabulary Test, 3rd edition; Se, selenium; WRAML2, Wide Range Assessment of Memory and Learning, 2nd edition; WRAVMA, Wide Range Assessment of Visual Motor Abilities; Zn, zinc.

[a]Quantile g-computation models adjusted for maternal intelligence (PPVT-III at early-childhood visit; KBIT-II composite at mid-childhood visit), prepregnancy body mass index, first-trimester fish consumption, education, self-reported race/ethnicity, age at enrollment, smoking, annual household income, and child sex.

[b]95% CI excludes the null value (0); $n = 834$ mother–child pairs were included across early-childhood analyses, and $n = 705$ mother–child pairs were included across mid-childhood analyses.

Statistical Interaction between Mixture Components in BKMR Models

For each mixture component, visual inspection of concentration–response functions revealed consistent shape across quantiles of each of the other components (Figures S9–S14), providing no qualitative evidence of interaction among the analyzed metals and micronutrients. The 10th, 50th, and 90th percentiles for each exposure are provided in Table 2.

Sensitivity Analyses

Neurotoxic and nutrient mixture point estimates and CIs from quantile g-computation models were minimally changed after adjustment for maternal hematocrit, abnormal plasma sample conditions (hemolysis or lipemia), and maternal first-trimester Prudent diet score (Figure S15). Numeric estimates are provided in Table S6.

Discussion

We analyzed longitudinal data from a U.S. prebirth cohort, Project Viva, to study the individual and joint associations of early pregnancy (∼10 wk) blood concentrations of 13 metals and micronutrients with child cognitive test scores in early (∼3 y) and mid-childhood (∼8 y). We found that a nutrient mixture of five essential metals (Cu, Mg, Mn, Se, and Zn) and two micronutrients (vitamin B12 and folate) was associated with higher scores on a test of receptive vocabulary (PPVT-III) in early childhood, with the association driven by Zn and Se. A neurotoxic mixture of six nonessential metals (As, Ba, Cd, Cs, Hg, and Pb) was associated with lower visual-motor ability (WRAVMA) in mid-childhood, which was driven by Ba and Cs. However, associations were largely null for the other nine cognitive tests examined across early and mid-childhood. Traditional single-exposure analyses supported findings from mixture analyses. Visual inspection of BKMR model pairwise interaction plots did not show evidence of interaction among any pair of exposures. Classically neurotoxic metals including As, Cd, Hg, and Pb were not associated with cognition in this cohort with high folate levels. There was some evidence of stronger associations in females in comparison with males.

This study is among the first to estimate the joint effects of exposure to a mixture of nutrients in early pregnancy on child cognition in an observational setting, whereas most previous studies have focused on combined effects of neurotoxic metals.[13,30,31] Preliminary analyses regarding linearity of effects informed our parameterization of quantile g-computation models for the effect of increasing concentrations of all included nutrients simultaneously by one quartile. Such joint effects may be more informative for public health action because supplementation recommendations, such as via prenatal multivitamin tablets, typically involve multiple nutrients. In addition, studying the joint effects of nonessential metals may be informative as to the impacts of interventions to improve water or food quality or to reduce industrial emissions or contamination.[32] Previous studies have found maternal blood concentrations of essential and nonessential metals are related to proximity to industrial sources and diet,[33] and thus changes to the environment or diet are likely to affect circulating concentrations of multiple metals in pregnant persons. It is notable that a limitation of quantile g-computation is that its estimates do not apply to those in the uppermost quantile, as concentrations above the highest quantile are, by definition, not observed in the data used to generate the estimate. Use of traditional g-computation to examine the impacts of specific hypothetical interventions to modulate the observed exposure distribution can retain interpretability in the entire sample by positing counterfactual shifts in the distribution, while also, more importantly, providing useful information for potential public health interventions. For example, Keil et al. simulated the impact on birth weight of reduction in exposure to six airborne metals via decommissioning three coal-fired power plants, in Milwaukee, Wisconsin.[34] We did not use traditional g-computation because it is not clear in the literature how an intervention on environmental contaminant sources or diet might be expected to affect erythrocyte concentrations of metals as measured in our study.

In addition, we note that the interpretability of the quantile g-computation estimates depends on support for joint exposure shifts in the observed data, indicated by correlations among the exposures. Because metals and micronutrients were weakly correlated in the present study (see "Results" section), reported quantile g-computation estimates are derived intervention parameters that extrapolate beyond the observed data and smooth over sparsity in the data, a necessary cost for estimating joint effects in the real world.

The observed associations were relatively small in magnitude: for most tests, the standard deviation is ∼10–15 points, and in

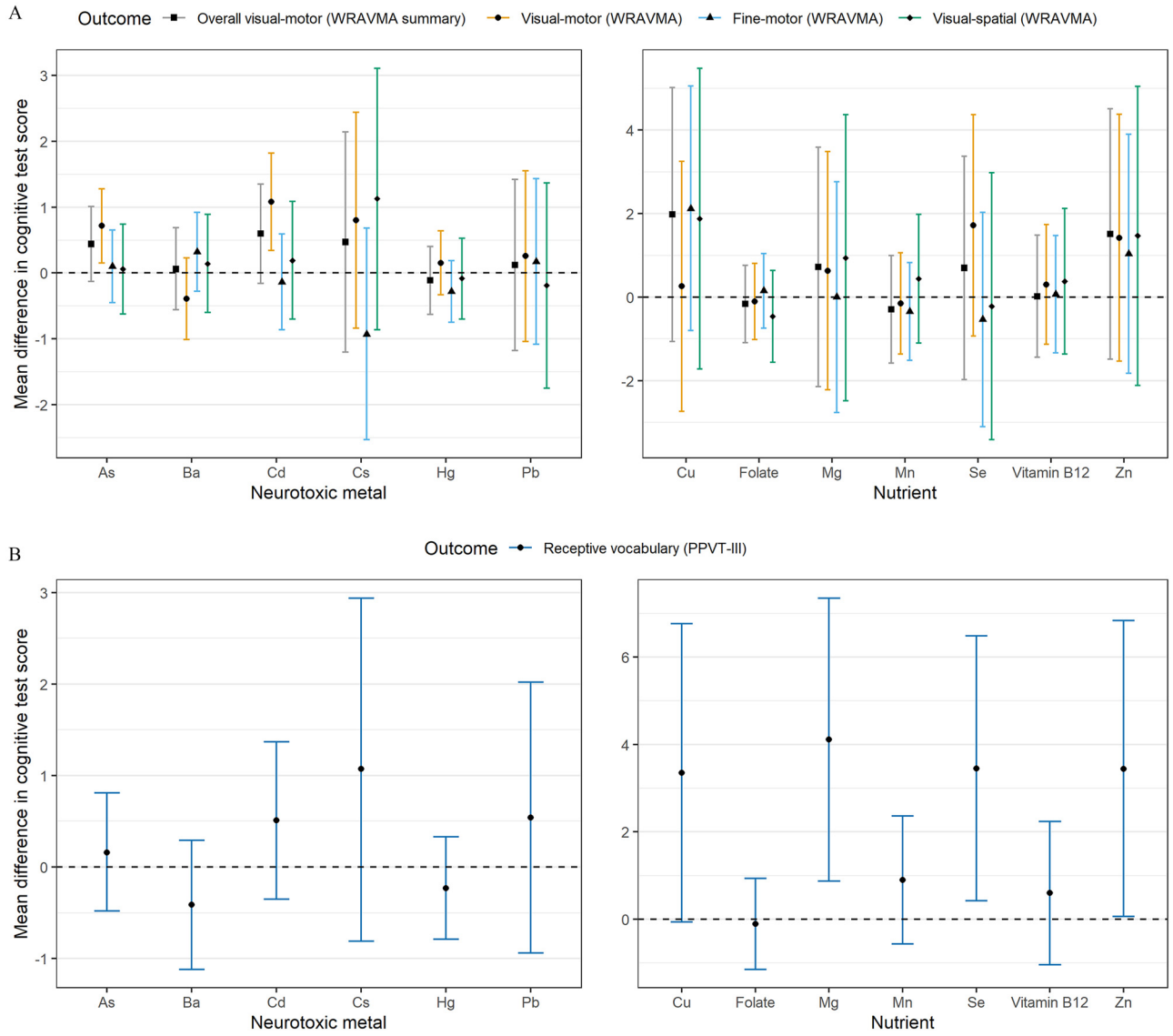

Figure 1. Multivariable linear regression estimates (black shapes) and 95% CIs (colored bars) of adjusted mean difference in early childhood (~3 y) cognitive test scores assessing (A) visual-motor ability (WRAVMA) or (B) receptive vocabulary (PPVT-III) associated with a doubling in maternal first-trimester (~10 weeks) blood concentration of individual metals or nutrients. The analytic sample for these early childhood cognition analyses included $n = 834$ mother–child pairs from the Project Viva cohort in Boston, Massachusetts, USA. Metals were measured in erythrocytes, and vitamin B12 and folate were measured in plasma. Models adjusted for maternal PPVT-III score at early childhood visit, prepregnancy body mass index, first-trimester fish consumption, education, self-reported race/ethnicity, age at enrollment, smoking, annual household income, and child sex. Models were not adjusted for coexposure to other metals or micronutrients because of collinearity and for comparability with previous studies. Corresponding numeric estimates are provided in Table S3. Note: As, arsenic; Ba, barium; Cd, cadmium; CI, confidence interval; Cs, cesium; Cu, copper; Hg, mercury; Mg, magnesium; Mn, manganese; Pb, lead; PPVT-III, Peabody Picture Vocabulary Test, 3rd edition; Se, selenium; WRAVMA, Wide Range Assessment of Visual Motor Abilities; Zn, zinc.

quantile g-computation analyses, the largest association entailed on average a 3-point difference in score per quartile increase in a mixture. However, small shifts in the distribution of cognitive function can entail large increases in numbers of clinically significant cases in populations,[35] and for this reason ubiquitous exposures, such as metals, with small effects are expected to attribute significant public health burdens.

We found that several essential metals drove a positive association between the nutrient mixture and receptive vocabulary in early childhood, with highest and similarly sized contributions from Zn and Se. Prenatal Zn supplementation has been studied in several randomized controlled trials for improving pregnancy and newborn outcomes,[36] because it is known that severe prenatal Zn deficiency (maternal whole blood Zn <56 μg/dL) has teratogenic consequences. In a systematic review, Ota et al. concluded there was insufficient evidence for an effect of prenatal Zn supplementation on cognitive function in the child,[36] based on several trials with null results, including one conducted in the United States among women with plasma Zn levels below the median of the reference range.[37] In our study, Zn appeared to be positively associated with receptive vocabulary around 3 y of age but negatively associated with crystallized intelligence around 8 y of age, in a cohort of generally good health and high SES. Note that we measured metals in erythrocytes, precluding comparisons with levels in other cohorts where metals were measured in other components of blood or other biological media. Observational evidence of the neurodevelopmental consequences of observed maternal Zn concentrations from other studies is inconclusive but suggests weak positive or negative associations.[38]

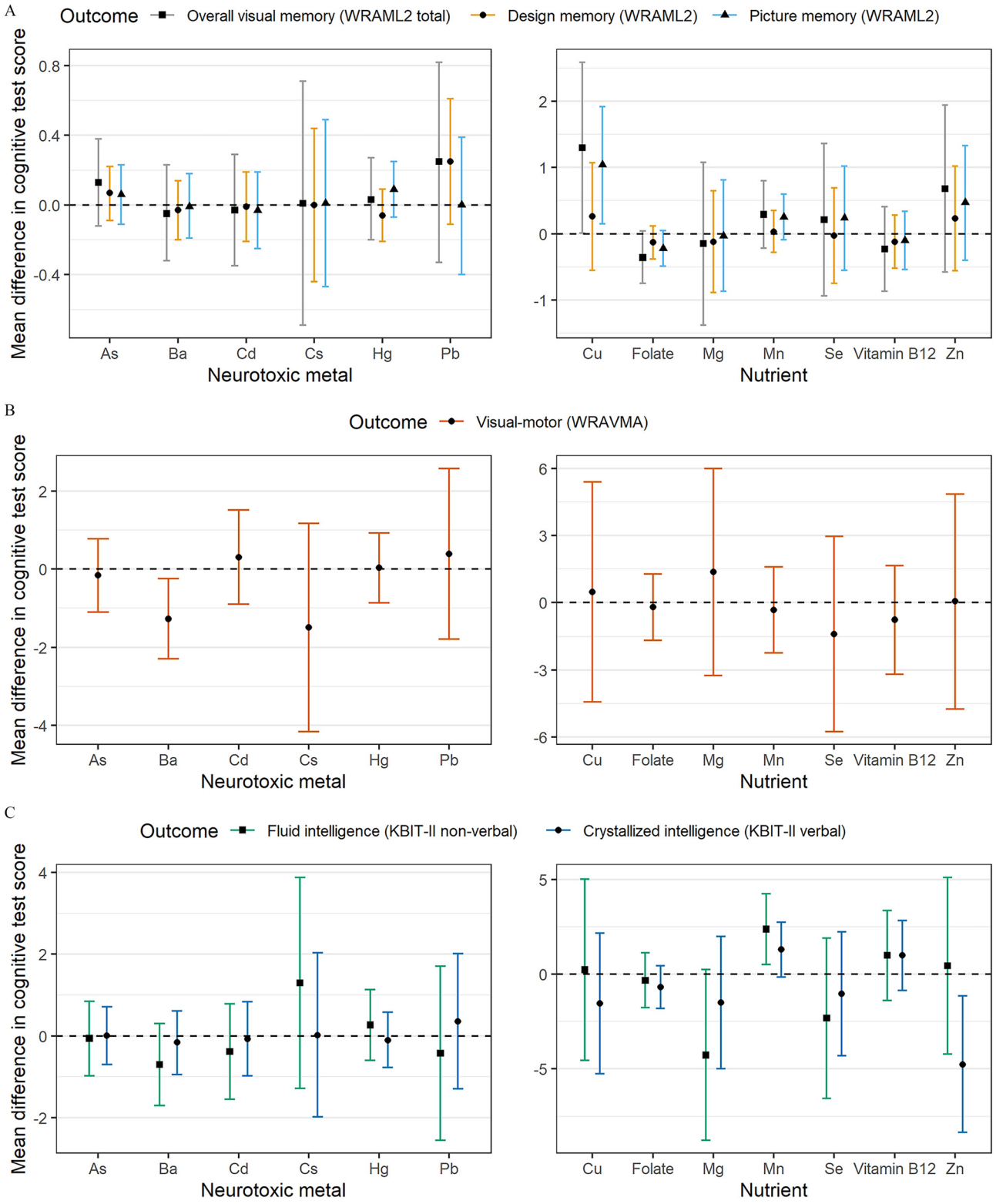

Figure 2. Multivariable linear regression estimates (black shapes) and 95% CIs (colored bars) of adjusted mean difference in mid-childhood (~8 y) cognitive test scores assessing (A) visual memory (WRAML2), (B) visual-motor ability (WRAVMA), or (C) fluid intelligence (KBIT-II nonverbal) or crystallized intelligence (KBIT-II verbal) associated with a doubling in maternal first-trimester (~10 wk) blood concentration of individual metals or nutrients. The analytic sample for these mid-childhood cognition analyses included $n = 705$ mother–child pairs from the Project Viva cohort in Boston, Massachusetts, USA. Metals were measured in erythrocytes, and vitamin B12 and folate were measured in plasma. Models adjusted for maternal KBIT-II composite score at mid-childhood visit, prepregnancy body mass index, first-trimester fish consumption, education, self-reported race/ethnicity, age at enrollment, smoking, annual household income, and child sex. Models were not adjusted for coexposure to other metals or micronutrients because of collinearity and for comparability with previous studies. Corresponding numeric estimates are provided in Table S4. Note: As, arsenic; Ba, barium; Cd, cadmium; CI, confidence interval; Cs, cesium; Cu, copper; Hg, mercury; KBIT-II, Kaufman Brief Intelligence Test, 2nd edition; Mg, magnesium; Mn, manganese; Pb, lead; Se, selenium; WRAML2, Wide Range Assessment of Memory and Language, 2nd edition; WRAVMA, Wide Range Assessment of Visual Motor Abilities; Zn, zinc.

We observed a positive linear association between first-trimester Se and receptive vocabulary in early childhood, but no association with mid-childhood cognition, consistent with a previous Project Viva study of second-trimester erythrocyte Se and cognition.[39] Skröder et al. observed positive associations between maternal erythrocyte Se at 30 wk and language comprehension at age 1.5 y in a prospective study of 750 Bangladeshi children.[40] In a prospective study of 410 Polish mother–child pairs, first-trimester maternal plasma Se concentration was associated with higher language development at 2 y of age, as well as better motor development at 1 y of age.[41] However, an excess of Se may be harmful. For example, in a prospective study of 490 mother–child pairs in the Spanish Childhood and Environment study (INMA), Amorós et al. observed an inverted U-shaped relationship between maternal Se measured in serum around 10 wk gestation and child verbal and memory abilities at 5 y.[42] Further research is needed to characterize the shape of the relationship between prenatal Se and cognition, though our findings align with these previous studies in suggesting beneficial impacts on verbal abilities in early life.

Maternal first-trimester plasma concentrations of vitamin B12 and folate had largely null associations with cognition in this cohort. It is well-established that adequate folic acid intake during pregnancy helps prevent neural tube defects, and vitamin B12 may confer a similar benefit, potentially by making folate more bioavailable. However, it is less clear whether benefits of these nutrients for brain development extend to improved cognition in the child and at what concentrations. In contrast with our study, a previous analysis of 1,210 mother–child pairs in Project Viva found a positive association between early pregnancy estimated total folic acid intake (inclusive of diet and supplements) measured by FFQ and receptive vocabulary (PPVT-III).[43] A recent review of the effect of prenatal folate levels on neurodevelopment found that most studies of folic acid intake and neurodevelopmental outcomes showed positive associations, whereas about half of studies that examined folate status in maternal blood in relation to neurodevelopment showed positive findings.[44] Folic acid intake is moderately to strongly correlated with plasma folate concentration, though plasma concentrations are more indicative of folate that reaches the fetus, suggesting some form of recall bias or confounding may explain the discrepancy in findings. Timing of exposure may also explain our null result. A recent trial found improved language abilities and neuronal function among 119 Irish children whose mothers received folic acid supplements in the second and third trimester, in comparison with placebo, suggesting folic acid may have neurodevelopmental benefits in later pregnancy.[45]

Two nonessential metals, Ba and Cs, drove the association between the neurotoxic metal mixture and visual-motor function in mid-childhood. Food, drinking water, and air are the major sources of Ba and Cs, though exposure levels from these sources are typically thought to be too low to be of concern for human health; however, animal and epidemiological studies of their developmental effects are scarce.[46,47] A recent study of 308 mother–infant pairs in the PRogramming of Intergenerational Stress Mechanisms (PRISM) cohort found Ba and Cs were the primary drivers of a positive association between a nonessential metal mixture and fear response, supporting our findings, though assessing child behavior rather than cognition.[48] However, other epidemiological studies have observed null associations between these metals and neurodevelopment.[49] Ba exposure from industrial sources is likely to increase as the uses of this element expand, including in shale gas development, suggesting it deserves greater attention and study as a potential developmental toxicant.[50] In contrast to WRAVMA visual-motor subtest scores in mid-childhood, scores for the same test in early childhood were not associated with the neurotoxic mixture. This difference may be because of analytic biases such as unmeasured negative confounding or age-related variability in test sensitivity, as suggested by scores between the time points being weakly correlated ($r = 0.15$), and biological mechanisms by which adverse cognitive effects from early life exposures may be delayed,[51] such as insufficient maturity of the relevant brain structures or delayed changes in gene expression.[52] As and Cd were positively associated with visual-motor subtest scores in early childhood, even after adjustment for fish consumption and overall diet in sensitivity analyses. For As, this association was attenuated among mothers with fish consumption levels below the median vs. above the median. This difference could be explained by residual confounding caused by measurement errors in fish consumption among mothers consuming higher levels of fish. Without complete control for confounding by fish consumption, total erythrocyte As may act as a biomarker for fatty acids and other nutrients in fish essential to neurodevelopment, resulting in the observed positive association.[53]

Although no safe level of blood Pb in children has been found,[54] first-trimester erythrocyte Pb was not associated with cognition in our study. Project Viva is comprises a high SES and insured population, factors that might buffer Pb neurotoxicity.[55] Alternatively, the timing of Pb exposure might play a role in differential findings, because previous studies have observed associations in later pregnancy in comparison with early pregnancy,[54] including a Project Viva study finding that second-trimester erythrocyte Pb at levels similar to those in the present study were associated with reduced executive function in mid-childhood.[56] Furthermore, emerging animal and human evidence supports the notion that certain nutrients, including folate, calcium, iron, and Zn, may protect against Pb-induced neurotoxicity by preventing the accumulation of Pb or exerting antioxidant effects.[57]

Folic acid fortification of grains and cereals became required by federal law in 1998 to prevent folate insufficiency and consequent neural tube defects. Serum folate levels in this cohort were higher than those observed in the general population of reproductive-age women in the United States between 1999 and 2016, according to a previous study of blood folate levels in the National Health and Nutrition Examination Survey (NHANES), a representative survey of the U.S. noninstitutionalized population.[58] However, similar to Project Viva, <1% of the general population was folate-deficient during that time. In Project Viva, it was estimated that in the first trimester, most mothers consumed more than the recommended 400 μg of folic acid per day through diet and supplements.[59] Because Project Viva mothers were recruited between 1999 and 2002, the enactment of the fortification law and prenatal supplementation with folic acid, which was done by 94% of Project Viva mothers,[59] likely account for the high folate levels in this cohort. This and other nutritional factors in this cohort may have contributed to the null results observed for Pb and other neurotoxic metals. This hypothesis is supported by recent epidemiological evidence identifying protective effects of perinatal folic acid intake against the neurotoxicity of early-life air pollution exposure.[60] Thus, our results may not be generalizable to U.S. women for whom folate levels are typically lower and particularly not to countries where folate deficiency during pregnancy has been reported to be common, such as Bangladesh.[61]

A variety of mechanisms may underlie the effects of prenatal metal and micronutrient exposures on neurodevelopment. Some circulating metals easily pass through the placenta to reach the fetus, where they may accumulate, interact with, and influence the success of critical developmental processes throughout pregnancy. Laboratory studies suggest essential metals such as Zn and Cu promote synaptic efficiency, neurotransmitter production, myelination, and the mTOR signaling pathway, which affect

structural brain development.[62] The mechanism by which vitamin B12 and folate affect neurodevelopment is well established: Both are methyl donors in the production of S-adenosylmethionine (SAM), a molecule that supplies methyl groups to a variety of methylation-dependent reactions in neurodevelopment, including the production of neurotransmitters and synaptic plasticity. The mechanisms of neurotoxic heavy metals such as Pb have been corroborated in multiple animal and human investigations: Oxidative stress causes altered neurotransmission and destruction of astrocytes, cells that support neurons.[63] The potential mechanisms of neurotoxicity from Ba and Cs are unknown, though it is known that Ba accumulates in the placenta and is toxic to neonates at high doses,[46,50] and prenatal Cs exposure was observed in a previous study to cause reduced brain weight in mice.[64]

Several mechanisms for sex-specific neurotoxicity have been suggested and observed in animal and human studies, including differences in metal metabolism, interactions with sex-specific hormones, and differences in brain development.[65] Recently, it was observed that a prenatal mixture of neurotoxic metals had a stronger effect on fear responses in girls in comparison with boys, driven by Ba and Cs, in line with our findings.[48] Sex-specific effects of essential metals have been observed as well: Skröder et al. observed stronger associations between maternal Se and psychomotor development at 1.5 y among girls in comparison with boys,[40] consistent with our study. Conversely, Amorós et al. showed a stronger negative effect of maternal Cu in boys.[66]

Several limitations in our study are worth noting. Residual confounding, particularly by metal sources that may directly affect cognition via nonmetal-dependent pathways, such as systemic inflammation caused by ambient air pollution exposure, is possible because this was an observational study.[67] Our choice to not adjust for coexposure to essential metals in mixture models for nonessential metals, and vice versa, may result in residual confounding by upstream factors common to nonessential and essential metals, such as diet[33] and genetic factors that affect metal metabolism.[68] However, this confounding as captured by the complementary mixture is likely to be minimal, given weak correlations among the metals and modest associations with the outcomes for a few metals. In addition, adjustment for coexposures that share a source with the target exposure can induce bias: Weisskopf et al.[25] note that coexposure adjustment causes collider stratification bias when there is unmeasured confounding unique to any of the individual mixture components. They instead recommend directly adjusting for common sources of the target exposure and coexposures. Our results were robust to adjustment for maternal first-trimester Prudent diet index in sensitivity analyses, directly capturing a major source of both nutrients and neurotoxic metals in this cohort.[33]

Blood biomarkers are not ideal for some metals, though they may be better suited for other metals.[33,69] For example, we used total As concentrations measured in erythrocytes, which includes nontoxic organic arsenicals found in fish. Metabolites of As measured in urine would better represent inorganic As exposure.[70] However, blood Pb, and in particular erythrocyte Pb, is sensitive for recent (weeks or months) Pb exposure, because most blood Pb is bound to erythrocytes, and erythrocytes are degraded roughly every 90 d, representing roughly the first trimester for most Project Viva mothers. In addition, previous studies analyzing prenatal Ba and Cs blood biomarkers have observed significant associations with cognition in diverse cohorts, suggesting blood may be a toxicologically relevant media for these elements.[71–73] Although no single media is perfect for all metals, we had erythrocytes available with sufficient quantity to measure multiple exposures simultaneously. However, findings must be interpreted in light of this important limitation in exposure assessment for each element we studied.[70] We did not adjust findings for multiple testing. Multiple testing adjustment is appropriate when the aim is null hypothesis testing, hypotheses are mutually independent, and a global null hypothesis is plausible.[29] However, the relationships examined are not independent of each other given the relatedness of the cognition measures, and many previous studies have demonstrated relationships between prenatal metal concentrations and child cognition, suggesting that the global null is unlikely.

In addition, estimation of the magnitude and precision of effects is more relevant for public health policy and practice. Therefore, we focus our interpretations on point estimates and width of CIs, while acknowledging that it is possible, as in any study, that some findings are due to chance. Although we aimed to study associations of first-trimester metal exposure with cognition, we note that confounding by exposure later in pregnancy is possible, as metal concentrations are typically correlated throughout pregnancy, though are expected to shift with biological changes during this period, such as metabolic changes and plasma volume expansion.[74] Few previous studies measured metals in erythrocytes, challenging our ability to compare concentrations in this cohort to others to assess generalizability of the exposure levels, though notably our concentrations were within a set of publicly available laboratory reference ranges. In a sample of 750 Bangladeshi mother–child pairs recruited between 2001 and 2003, levels of maternal prenatal erythrocyte Pb were much higher than those in Project Viva (median $\sim 90\ \mathrm{ng/g}$ vs. geometric mean $18.2\ \mathrm{ng/g}$, respectively),[40] which is expected, given high concentrations in dietary and other sources found in the region.[75] Generalizability of the Project Viva study population is limited because it is largely non-Hispanic White with high SES. In addition, in comparison with those lost to follow-up or missing data, the analytic sample was composed of mothers who were more likely to be White and college-educated and have higher income, and they were less likely to smoke during pregnancy. Selection on these factors further limits generalizability but should not cause structural selection bias[76] (a form of collider stratification bias) because, even if these factors drove selection, they were controlled for in the analyses. In addition, the homogeneity of this cohort has the benefit of implicitly restricting the levels of potential unmeasured confounders, thus potentially reducing confounding bias.

In conclusion, we found that first-trimester maternal erythrocyte concentrations of certain essential (Cu, Mg, Mn, Se, and Zn) and nonessential (Ba and Cs) metals were associated with measures of receptive vocabulary and visual-motor ability in early and mid-childhood, though findings were null for most of the cognition measures examined. It is notable that, in this folate-replete cohort, classically neurotoxic metals (As, Cd, Hg, Pb) were not associated with cognitive function. Within an environmental mixtures framework, we showed that joint modulation of multiple metals was associated with cognitive outcomes, with certain metals driving the associations. Metal exposure is ubiquitous, but the effects of low-level exposure, particularly during the vulnerable period of pregnancy when many neurodevelopmental processes occur rapidly, are poorly characterized. Additional studies are needed to clarify inconsistent findings in the literature and establish the timing and dose–response relationships between these metals and neurodevelopment, as well as the nutrient levels that might mitigate heavy metal neurotoxicity. Furthermore, accounting for coexposure to multiple metals and examining joint effects can help the field of metal neurotoxicology move toward modeling the benefits of potential interventions to optimize the metal and micronutrient profile of pregnant persons.

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