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An Industry-Relevant Metal Mixture, Iron Status, and Reported Attention-Related Behaviors in Italian Adolescents

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Background: Exposure to environmental metals has been consistently associated with attention and behavioral deficits in children, and these associations may be modified by coexposure to other metals or iron (Fe) status. However, few studies have investigated Fe status as a modifier of a metal mixture, particularly with respect to attention-related behaviors.

Methods: We used cross-sectional data from the Public Health Impact of Metals Exposure study, which included 707 adolescents (10–14 years of age) from Brescia, Italy. Manganese, chromium, and copper were quantified in hair samples, and lead was quantified in whole blood, using inductively coupled plasma mass spectrometry. Concentrations of Fe status markers (ferritin, hemoglobin, transferrin) were measured using immunoassays or luminescence assays. Attention-related behaviors were assessed using the Conners Rating Scales Self-Report Scale–Long Form, Parent Rating Scales Revised–Short Form, and Teacher Rating Scales Revised–Short Form. We employed Bayesian kernel machine regression to examine associations of the metal mixture with these outcomes and evaluate Fe status as a modifier.

Results: Higher concentrations of the metals and ferritin were jointly associated with worse self-reported attention-related behaviors: metals and ferritin set to their 90th percentiles were associated with 3.0% [$\beta = 0.03$; 95% credible interval (CrI): -0.01, 0.06], 4.1% ($\beta = 0.04$; 95% CrI: 0.00, 0.08), and 4.1% ($\beta = 0.04$; 95% CrI: 0.00, 0.08) higher T-scores for self-reported attention deficit/hyperactivity disorder (ADHD) index, inattention, and hyperactivity, respectively, compared with when metals and ferritin were set to their 50th percentiles. These associations were driven by hair manganese, which exhibited nonlinear associations with all self-reported scales. There was no evidence that Fe status modified the neurotoxicity of the metal mixture. The metal mixture was not materially associated with any parent-reported or teacher-reported scale.

Conclusions: The overall metal mixture, driven by manganese, was adversely associated with self-reported attention-related behavior. These findings suggest that exposure to multiple environmental metals impacts adolescent neurodevelopment, which has significant public health implications. https://doi.org/10.1289/EHP12988

Introduction

Children living in proximity to steel-producing ferroalloy industry are commonly exposed to exogenous metals through inhalation or ingestion, including lead (Pb), manganese (Mn), chromium (Cr), and copper (Cu), through air emissions, dust, and soil.[1] Exposure from ferroalloy industry has been associated with increased metal biomarker concentrations in children in the United States, Brazil, Canada, and elsewhere.[1–6] An expanding literature supports the potential neurotoxic impacts of these metals in children,[7] which is an important public health concern because ferroalloy markets, as well as other markets using metals (e.g., electric vehicle and cell phone batteries), are expected to grow substantially in the coming years.[8,9]

Exposure to individual environmental metals has been consistently associated with inattention and other neurodevelopmental outcomes in the pediatric epidemiology literature.[10–30] Attention, defined as the ability to receive, focus on, and process incoming information, underlies general cognitive abilities[31] and has been associated with learning and overall academic achievement in children.[32] Externalizing behaviors (e.g., impulsivity, aggression, hyperactivity), internalizing behaviors (e.g., withdrawal, anxiety) and emotional dysregulation have similarly been associated with poorer academic performance in reading, writing, and mathematics.[33–35] Thus, attention and attention-related behaviors play a key role in child neurological function and overall well-being.

Attention is modulated primarily by the prefrontal cortex, which undergoes extensive maturation (e.g., pruning, myelination, and refinement of synaptic connectivity) during the adolescent period (10–19 years of age).[36–44] These structural refinements are accompanied by developing attentional function and emotional regulation.[31,45,46] Given the profound changes taking place in the prefrontal cortex during adolescence, this brain region, and the cognitive functions it modulates (e.g., executive function, attention, emotion), may be susceptible to insults from exogenous contaminants like metals in this developmental period. Metals used in ferroalloy industry (e.g., Pb, Mn, Cr, Cu) target and exert their toxic mechanisms, such as oxidative stress and disruption of neurotransmission, in various regions of the brain, including the prefrontal cortex.[36–43] This suggests that attention-related behaviors may be especially susceptible to neurotoxic metals in the dynamic adolescent period.

Because Pb, Mn, Cr, and Cu share several mechanistic pathways for neurotoxicity,[36–43] exposure to a mixture of these metals may have cumulative or interactive effects on the central nervous system. In the Public Health Impact of Metals Exposure (PHIME) study, a cohort of 10- to 14-y-old Italian adolescents living near

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ferroalloy industrial facilities in the province of Brescia, Italy, we previously reported cumulative associations of a metal mixture (Pb, Mn, Cr, and Cu) with visuospatial abilities, as well as interactive associations of components of the mixture (Pb, Mn, and Cu) with verbal intelligence quotient (IQ).[47,48] However, our understanding of joint and interactive associations of metal mixtures in relation to attention-related behaviors in the adolescent period remains nascent.

Emerging epidemiological evidence also indicates that metal neurotoxicity may be modified by iron (Fe) status (e.g., Fe deficiency) owing to similar toxicokinetics and toxicodynamics among Fe and other metals.[36–39,49–52] Fe is an essential nutrient needed for biological functions, such as cellular oxygen transport and neurotransmitter synthesis.[53] Altered Fe status (e.g., decreased ferritin and hemoglobin concentrations) has been associated with attentional deficits, risky behavior, and poor emotional regulation in children.[54–57] When Fe was considered as a modifier of metals exposure in early life (prenatally and in early childhood), adverse associations of multiple metals (Pb, Mn, Cu) were found to be stronger under Fe-deficient conditions for multiple neurodevelopmental outcomes, including off-task behaviors in the classroom that reflect poorer attention function.[58–62] Nearly all prior studies in children quantified metal exposure and Fe status prenatally or in early childhood[63]; yet, adolescents may be more likely to experience Fe deficiency owing to the onset of menstruation, changes in dietary habits, and significant increases in Fe need (up to 25%) to support rapid physical, neurological, and sexual growth and development.[64–68] Therefore, given the increased likelihood of Fe deficiency and the heightened susceptibility to metals neurotoxicity in adolescence, Fe status may be an important modifier of metals toxicity during this developmental window. However, to our knowledge, no studies to date have assessed the modification of a mixture of metals by Fe status in relation to attention-related behaviors in the adolescent period.

The objective of the present study was to estimate associations of an industry-relevant metal mixture (Pb, Mn, Cr, and Cu) with reported attention-related behaviors among adolescents from the PHIME cohort. We further examined the modifying role of Fe status on the metal mixture, where we hypothesized that adverse associations of the metal mixture with reported attention would be stronger among adolescents with lower concentrations of Fe status markers. In exploratory analyses, we assessed the potential for sex-specific associations given prior evidence that metals neurotoxicity in adolescence may vary by sex.[48]

Methods

Study Population

We used cross-sectional data from the PHIME study, a cohort designed to examine associations between metals and neurodevelopment among adolescents living in proximity to ferroalloy industry. Full details of recruitment have been described previously.[69] In brief, we recruited 721 adolescents between 2007–2014 from three regions of Brescia, Italy, with varying degrees of exposure to ferroalloy industry: Garda Lake (GL), with no current or historical ferroalloy industry; Valcamonica (VC), with historical industrial activity that ended in 2001; and Bagnolo Mella (BM), with active industry since 1974. Enrollment of adolescents into the PHIME study occurred in two distinct phases that reflect two waves of funding, although all study protocols remained consistent between study phases. We recruited participants from GL and VC in the first phase of the study (2007–2010). In the second phase of the study (2011–2014), we recruited adolescents from all study sites (GL, VC, BM) and administered selected questions from the Home Observation Measurement of the Environment (HOME) Short Form[70]; the HOME Short Form was not administered in the first phase of the study. Most participants ($n = 410$) were enrolled in the second phase, whereas 311 adolescents were enrolled in the first phase.

Adolescents were eligible for PHIME if they *a*) were 10–14 years of age at the time of enrollment, *b*) were born into families that lived in the study region since the 1970s, and *c*) had lived in the study region since birth. Recruitment was restricted to participants whose families had lived in the study region since the 1970s to capture historical exposures that began during this period. We excluded adolescents who *a*) had a diagnosed neurologic, hepatic, metabolic, endocrine, or psychiatric disease or clinically relevant motor deficits that may have impacted testing, including attention deficit/hyperactivity disorder (ADHD); *b*) used medication with neurologic side effects; *c*) had clinically diagnosed cognitive or behavioral impairment; *d*) had visual deficits without corrective measures; or *e*) had ever received parenteral nutrition. All potential participants were given detailed information on PHIME study protocols and gave informed consent. Study protocols were approved by institutional review boards at the Icahn School of Medicine at Mount Sinai and the University of California, Santa Cruz, as well as the ethical committee of Brescia.

Quantification of Metals and Fe Status

Mn, Pb, Cr, and Cu were quantified in blood and hair samples that were collected cross-sectionally. For the present analysis, we selected blood, which reflects recent exposure, as the primary biomarker for Pb because it is considered a valid measure of Pb body burden and is commonly used in the epidemiology literature.[71] Consensus regarding the optimal biomarker of exposure for Mn, Cr, and Cu is lacking, with prior epidemiological studies typically using blood, hair, or urine.[20,47,58,72,73] We chose hair as the primary biomarker for Mn, Cr, and Cu in this analysis because *a*) hair metal concentrations have been consistently associated with environmental metal exposure[1,74]; *b*) hair has been used widely in the epidemiology literature to measure environmental exposure to metals, including in the current cohort[47,48,72]; *c*) hair metal concentrations (i.e., Mn) have been associated with neurodevelopmental outcomes in prior studies[3,30,47,48,75]; *d*) hair concentrations of these metals reflect exposure from the past several months[76–78]; and *e*) hair had the least missing data for these metals (<6%).

Methods for metal biomarker collection have been described in detail elsewhere.[69,74,79,80] Briefly, hair samples (2–3 cm from the occipital region of the scalp) were collected with stainless steel clippers and extensively cleaned with Triton detergent, nitric acid, and sonication to remove exogenous contamination using previously validated methods.[74,79] Hair samples were then dried and digested in distilled nitric acid. Whole blood (4 mL) was collected using 19-gauge butterfly catheters, and samples were stored in lithium-heparin Sarstedt Monovette Vacutainers. Metals were quantified in hair and blood using magnetic sector inductively coupled plasma mass spectrometry.[69,79,80] Limits of detection (LODs) were determined using repeated measurements of procedural blanks. The analytic LOD for blood Pb was $0.01\ \mu g/dL$; the analytic LODs for hair Mn, Cr, and Cu were 0.02, 0.01, and $0.04\ ng/mL$, respectively. Few measurements ($n = 1$ for hair Mn, $n = 1$ for hair Cr) were below the LOD; samples below the LOD were imputed as the LOD divided by 2.

Three clinically relevant biomarkers were used to assess Fe status: serum ferritin (in nanograms per milliliter), blood hemoglobin (in grams per deciliter), and serum transferrin (in milligrams per deciliter). Hemoglobin was measured in whole blood samples as part of a complete blood count using the Beckman Coulter LH Series hematology analyzer (Beckman Coulter, Inc.). Blood samples were analyzed following collection in a lavender-top tube with ethylenediaminetetraacetic acid anticoagulant. Serum was collected in a red-top tube, and ferritin and transferrin were quantified in serum by immunoassays with the Architect *i*2000*SR* (Abbott Laboratories) and ADVIA immunoassay analyzers (Siemens Healthcare GmbH), respectively. In our main analyses, we selected serum ferritin to characterize Fe status because ferritin is a sensitive measure of Fe deficiency with or without anemia.[81]

Neuropsychological Assessment

Attention and regulatory behaviors were assessed using the Conners Rating Scales (CRS) Self-Report Scale–Long Form (CASS: L), Parent Rating Scales Revised–Short Form (CPRS-R: S), and Teacher Rating Scales Revised–Short Form (CTRS-R: S). The CRS is a questionnaire completed by the participant, the participant's parent, and the participant's teacher, and has been used to clinically assess problematic behaviors and ADHD symptomology.[82] Self-reported scales include ratings for cognitive problems/inattention, hyperactivity, ADHD index, family problems, emotional problems, conduct problems, anger control problems, and three symptoms (inattention, impulsivity, total problems) from the *Diagnostic and Statistical Manual of Mental Disorders* (*DSM*)*-IV*. Parent- and teacher-reported scales include ratings of cognitive problems/inattention, hyperactivity, and the ADHD index, as well as an additional rating for oppositional behavior.

Scores for CRS scales were sex- and age-standardized and expressed as *T*-scores with a mean of 50, standard deviation (SD) of 10, and maximum of 100. Higher scores for each outcome reflect greater reported symptomology and more problematic behaviors. For the present analysis, we *a priori* chose to focus on cognitive problems/inattention, hyperactivity, and the ADHD index from the self-, parent- and teacher-reported scales because these outcomes were measured across all three respondent types. Fourteen participants were missing all CRS data and were therefore excluded from the analyses. The self-reported scales were administered in both phases of the study, whereas the parent and teacher scales were only administered in the second phase of the study. Therefore, sample sizes varied by respondent type: all ($n = 707$) adolescents completed the self-reported scale and, of these 707 adolescents, 396 also had data for the parent-reported scale. A total of 281 adolescents with both self- and parent-reported data also had data for the teacher-reported scale. The sample size for the teacher-reported scale was smaller than the parent-reported scale because fewer teachers completed the CRS for participants in the study.

Measurement of Covariates

Information on covariates was collected by trained study staff using standardized questionnaires administered either in person or over the phone. Sociodemographic information included age (continuous, in years), biological sex (female vs. male), birth order (first, second, third, or higher), area of residence (BM, GL, VC), parental occupation, and parental education level. Socioeconomic status (SES) for each participant was classified as low, medium, or high based on both parental education and occupation, using a previously developed method for Italian populations.[10,83] Specifically, parental education and occupation levels were classified as low, medium, or high based on World Health Organization classifications.[84] An overall index of SES was then constructed based on the classified education and occupation levels according to Table S1. Parental education and occupation levels were queried for both parents, and the highest level of education and occupation for either parent was used to construct the SES index. Ten items from the HOME Short Form, which reflects cognitive stimulation at home, were administered (possible scores: 0–9).[70]

Statistical Analysis

Data imputations. To optimize sample size, we used all available data on each Conners scale; that is, our analytical sample included 707 adolescents for the self-reported outcomes, 396 adolescents for the parent-reported outcomes, and 281 adolescents for the teacher-reported outcomes. There were little missing data (<11% for biomarkers and <3% for covariates; Table S2); the exception was the HOME score, which was administered only in the second phase of the study, representing 53% of study participants. Biomarker data was missing for ~11% of participants who did not provide hair or blood samples. Missing data on exposure and covariates was imputed to the full cohort ($n = 721$) with Monte Carlo Markov Chain (MCMC) multiple imputation using the *mice* package in R.[85,86] Analyses were then restricted to participants with complete outcome data. We ran MCMC imputations for 20 datasets under the assumption that data were missing at random.

Descriptive statistics and data transformations. Summary statistics for biomarkers, Conners scores, and sociodemographic variables were calculated and presented for one randomly selected imputed dataset (Table 1). We examined the distributions of all biomarkers, Fe status markers, potential confounders, and Conners scores. All metal biomarkers, outcomes, and serum ferritin were right-skewed; we natural log-transformed these variables to reduce the influence of outlier points and satisfy modeling assumptions of

Table 1. Descriptive statistics [*n* (%), mean ± SD, or median (25th, 75th percentiles)] for Public Health Impact of Metals Exposure (PHIME) study participants with available data on self-reported, parent-reported, and teacher-reported scales.

Characteristic	Self-report ($N = 707$)	Parent-report ($N = 396$)	Teacher-report ($N = 281$)
Biological sex			
Female	340 (48.1)	187 (47.2)	128 (45.6)
Male	367 (51.9)	209 (52.8)	153 (54.4)
Age (y), mean (SD)	12.3 ± 0.9	12.2 ± 1.0	12.0 ± 0.9
SES index			
Low	165 (23.3)	97 (24.5)	70 (24.9)
Medium	374 (52.9)	212 (53.5)	150 (53.4)
High	168 (23.8)	87 (22.0)	61 (21.7)
HOME score, mean (SD)	6.1 ± 1.7	6.1 ± 1.7	6.1 ± 1.6
Area of residence			
Garda Lake	244 (34.5)	83 (20.9)	55 (19.6)
Valcamonica	253 (35.8)	104 (26.3)	64 (22.8)
Bagnolo Mella	210 (29.7)	209 (52.8)	162 (57.6)
Birth order			
First	355 (50.2)	193 (48.7)	140 (49.8)
Second	271 (38.3)	153 (38.6)	103 (26.7)
Third	70 (9.9)	40 (10.1)	31 (11.0)
Fourth	11 (1.6)	10 (2.5)	7 (2.5)
Conners *T*-scores, median (range)			
Inattention	44 (35, 95)	44 (41, 100)	45 (40, 91)
Hyperactivity	46 (35, 88)	45 (40, 93)	45 (39, 99)
ADHD index	42 (36, 89)	47 (39, 100)	44 (36, 88)
Metal biomarkers, median (25th, 75th %)			
Hair Mn (μg/g)	0.05 (0.08, 0.15)	0.07 (0.04, 0.12)	0.07 (0.04, 0.11)
Hair Cu (μg/g)	9.4 (7.0, 15.1)	9.3 (6.5, 14.8)	11.8 (8.0, 16.0)
Hair Cr (μg/g)	0.05 (0.03, 0.08)	0.04 (0.03, 0.06)	0.04 (0.03, 0.06)
Blood Pb (μg/dL)	1.3 (1.0, 1.9)	1.3 (1.0, 1.8)	1.3 (1.0, 1.8)
Iron biomarkers, median (25th, 75th %)			
Serum ferritin (ng/mL)	27.0 (17.0, 40.0)	32.0 (21.0, 44.0)	32.0 (21.0, 45.0)
Blood hemoglobin (g/dL)	13.7 (13.2, 14.3)	13.8 (13.2, 14.4)	13.7 (13.1, 14.3)
Serum transferrin (mg/dL)	285 (261, 313)	284 (259, 309)	285 (260, 312)

Note: Conners score range: 35–100; HOME score range: 0–9. ADHD, attention deficit/hyperactivity disorder; Cr, chromium; Cu, copper; HOME, Home Observation Measurement of the Environment; Mn, manganese; Pb, lead; SD, standard deviation; SES, socioeconomic status.

normality of residuals. Spearman correlation coefficients were calculated between the metals and Fe status markers and between each of the Conners scales across the 20 imputed datasets using the *miceadds* package in R.[87] All metals and Fe status metrics were z-standardized (centered to the mean and scaled by the SD) to account for varying units across the biomarkers.

Confounder selection. Potential confounders were selected *a priori* based on published literature,[7,30,88,89] and a directed acyclic graph (Figure S1). All models were adjusted for HOME score (continuous), SES (low, medium, high), and serum ferritin (continuous); we did not include age (continuous) or biological sex (male or female) as covariates in statistical models because the Conners T-scores were standardized for these variables.

Bayesian kernel machine regression. We used Bayesian kernel machine regression (BKMR) to investigate associations of the metal mixture (Mn, Pb, Cr, Cu) with Conners outcomes. BKMR is a highly flexible mixtures modeling approach that assumes that individuals with similar exposure profiles have similar health outcomes. The model uses a kernel function that allows for nonlinear associations between exposures and the outcome, pairwise interactions between exposures, and higher-order interactions between exposures (e.g., the interaction of an individual component with multiple other components of the mixture). Joint associations of the overall mixture with the outcome can also be estimated.[90] We specifically included ferritin, a marker of Fe status, in the kernel function to examine potential interactions between the metal mixture (Mn, Pb, Cr, Cu) and Fe status. Therefore, ferritin was considered a component of the mixture in the BKMR models. The BKMR models took the following form:

$$\begin{aligned} Conners\ scale_i &= h(Mn_i,\ Pb_i,\ Cr_i,\ Cu_i,\ Ferritin_i) \\ &\quad + \beta_1 \times SES_i + \beta_2 \times HOME\ score_i + e_i, \end{aligned} \tag{1}$$

where h is the kernel function that allows for interactions between the components of the mixture and nonlinearity, e_i is the error term, and confounders are assumed to have linear associations with the outcome.

For each Conners outcome, we estimated *a*) exposure–response profiles for each mixture component, holding all other components at their medians; *b*) exposure–response profiles for each component at varying percentiles (25th, 50th, and 75th) of a second component while holding the remaining components at their 50th percentiles; *c*) the association for a percentile increase in all components simultaneously, compared with the 50th percentile of all metals and ferritin; and *d*) associations for a given component at its 75th percentile compared with its 25th percentile when other components were held at their 25th, 50th, or 75th percentiles.

We fit BKMR models for each of the 20 imputed datasets and then obtained overall effect estimates and 95% credible intervals (CrIs) by pooling results of all 20 model fits using Rubin's rule with previously developed code.[91] Each model was fit with the default noninformative priors, 40,000 iterations, and a 50% burn in. The component-wise variable selection option was used and posterior inclusion probabilities (PIPs), which reflect the relative importance of each of the exposure variables included in the h function, were estimated. For ease of interpretation, we back-transformed the beta coefficients to percent differences in Conners T-scores for *a*) the joint association of the mixture at the 90th or 10th percentiles compared with the 50th percentile, and *b*) the association of individual components for a change from their 25th to 75th percentiles, holding the rest of the mixture at its 25th, 50th, or 75th percentiles, using the following equation:

$$Percentage\ difference\ in\ Conners\ scores = (e^{\beta} - 1) \times 100. \tag{2}$$

Multivariable linear regression. We next used multivariable linear regression to supplement and aid in the interpretation of findings from BKMR models. There was evidence of nonlinear associations for serum ferritin (U-shape) and hair Mn (inverted U-shape) for several Conners outcomes in the BKMR models. We therefore categorized ferritin and Mn into tertiles for all subsequent linear regression models, whereas Pb, Cr, and Cu were modeled continuously. The middle tertile was set as the reference category for ferritin and Mn. There was no evidence of pairwise or higher-order interactions between the metals or between the metals and ferritin in BKMR models; interaction terms were therefore not included in multivariable linear regression models. Adjusted multivariable regression models were fit for each of the 20 imputed datasets, adjusting for *a priori* selected confounders:

$$\begin{aligned} Conners\ scale &= \beta_0 + \beta_1 \times Mn(tertile\ 1) + \beta_2 \times Mn(tertile\ 3) \\ &\quad + \beta_3 \times Pb + \beta_4 \times Cu + \beta_5 \times Cr + \beta_6 \times Ferritin(tertile\ 1) \\ &\quad + \beta_7 \times Ferritin(tertile\ 3) + \beta_8 \times SES + \beta_9 \times HOME\ score. \end{aligned} \tag{3}$$

We obtained pooled beta estimates (β) and 95% confidence intervals (CIs) by combining the 20 mean and variance estimates using Rubin's rule.[92] From these regression models, beta coefficients for continuous variables (Pb, Cr, Cu) were interpreted as the mean difference in ln-transformed Conners T-scores for a 1-SD increase in ln-transformed metal concentration. We estimated percentage differences in Conners T-scores for a doubling in metals (Pb, Cr, Cu) concentrations as follows:

$$Percentage\ difference\ in\ Conners\ scores = \left(e^{(\ln(2) \times \beta)}\right) - 1 \times 100. \tag{4}$$

Beta coefficients for tertiled variables (Mn, ferritin) were interpreted as the mean difference in ln-transformed Conners T-scores for the first or third tertile of Mn concentrations, compared with the second tertile. We estimated percentage differences in Conners T-scores for the first and third tertiles using Equation 2.

Data-driven selection of Fe status metric. Serum ferritin was used to represent Fe status in the main analyses because it reflects long-term Fe tissue stores and is considered a sensitive measure of Fe status. However, there is a lack of consistency in the markers of Fe status that have been used in the literature, with prior studies in pediatric populations using ferritin, hemoglobin, and serum Fe concentrations to characterize Fe status as a confounder or modifier.[63] Because each Fe status marker reflects different aspects of Fe status with varying degrees of sensitivity, it is therefore plausible that the selection of an Fe status marker may affect the findings from studies considering Fe status as a modifier of metals-induced neurotoxicity.[81,93–95] In a secondary analysis, we used the component-wise variable selection option in BKMR to investigate and identify the Fe status indicator most strongly associated with Conners outcomes. The component-wise variable selection option allows the user to select the most predictive variable (e.g., hemoglobin) among a set of correlated variables (i.e., hemoglobin, ferritin, transferrin) by ranking the relative importance of each variable with the outcome using PIPs.[96]

As a first step, we fit BKMR models with all three Fe status metrics in relation to Conners outcomes, adjusting for potential confounders, to identify the most predictive Fe status metric for each outcome based on PIPs (Equation 5). The Fe status biomarkers included in these models each reflect various aspects of Fe status: *a*) serum ferritin, which reflects circulating Fe stores; *b*) serum transferrin, which transports Fe and reflects Fe availability; and *c*) blood hemoglobin, which reflects functional Fe status.[81] These BKMR models took the following form:

$$\begin{aligned} & \textit{Conners self-reported scale}_i \\ & \quad = h(\textit{Ferritin}_i, \textit{Transferrin}_i, \textit{Hemoglobin}_i) \\ & \quad + \beta_1 \times \textit{SES}_i + \beta_2 \times \textit{HOME score}_i + e_i, \end{aligned} \tag{5}$$

where h represents the kernel function, and e_i is the error term.

These BKMR models were fit for the self-reported outcomes only because the self-reported outcomes had the largest sample size, and because the mixture components (Mn, Pb, Cr, Cu, ferritin) were not materially associated with parent- and teacher-reported outcomes in primary analyses. BKMR models were fit using Equation 5 for five randomly selected imputed datasets. PIPs were estimated for each of the five model fits for the self-reported outcomes, and the Fe status metric with the highest average PIP was selected as the most predictive of the outcomes.

As a second step, we then fit BKMR models for all 20 imputed datasets, as described above in Equation 1, for the self-reported outcomes, replacing ferritin with the selected Fe status marker from Equation 5. Estimates from these 20 fits were pooled using Rubin's rule, as described above.

Sex-stratified analyses. There is evidence in the literature, and in previous studies in this cohort, to suggest that associations between metals and neurodevelopment may be sex-specific, particularly in adolescence, where females are at a higher risk of developing Fe deficiency.[48,97–103] In exploratory models, we stratified data by biological sex and fit BKMR and multivariable linear regression models, as described above, to assess potential effect measure modification by sex.

Sensitivity analyses. Sample sizes varied by Conners respondent type (i.e., for self-, parent-, and teacher-reported scales) and we chose to optimize our sample size for each set of analyses, thus resulting in differing numbers of adolescents in each set of analyses. Given potential differences in covariates between the analytic samples, we conducted a sensitivity analysis restricting the sample to those with outcome data from all three respondents (i.e., self, parent, and teacher; $n = 281$). Because there was a large percentage of participants missing HOME score (47%), we further conducted a sensitivity analysis restricting our sample to participants with complete data for HOME score. We also ran sensitivity analyses for the BKMR models by changing *a*) the default uniform prior to a gamma prior distribution, and *b*) the degree of smoothness of the h function from the default (100) to 50 and 1,000. All analyses were conducted in R (version 3.6.1; R Development Core Team).

Results

Study Participant Characteristics

Sociodemographic characteristics of PHIME study participants were similar for adolescents with complete self-, parent-, and teacher-reported outcomes (Table 1). The mean ± SD age of participants across all reports was 12 ± 1 y, and about half of participants were male and from families of medium SES. The mean ± SD HOME score was 6 ± 2 across all reports.

Median *T*-scores on the Conners outcomes tended to be similar across the self-, parent- and teacher-reported outcomes (Table 1). Biomarker concentrations were also similar, although median Cu concentrations were higher in the teacher-reported sample (11.8 μg/g) than in the self- (9.4 μg/g) and parent-reported (9.3 μg/g) samples. Notably, the median blood Pb concentration in the overall study population (1.3 μg/dL) was below the current US Centers for Disease Control and Prevention reference value of 3.5 μg/dL.[104] Median serum ferritin concentrations were lower in the self-reported sample (27.0 ng/mL) than in the parent- (32.0 ng/mL) and teacher- (32.0 ng/mL) reported samples. Summary statistics were similar for participants with imputed data and complete data (Table S3).

Biomarker concentrations for females and males in the self-reported sample ($n = 707$) tended to be similar (Table S4). However, males had higher median serum ferritin concentrations (30.0 ng/g) than females (24.0 ng/g), which is typical in the adolescent period.[81] In contrast, females had higher median hair Cu concentrations (10.9 μg/g) compared with males (8.5 μg/g), which may relate, in part, to competition of Fe and Cu for uptake (e.g., higher Cu uptake when Fe levels are lower).[105,106]

Spearman correlations among the metals and Fe status indicators were weak (Figure S2). The strongest correlations between metals were observed for Mn-Cr ($r_s = 0.48$) and Mn-Cu ($r_s = 0.31$), whereas the strongest correlation between Fe status indicators was between serum ferritin and transferrin ($r_s = -0.41$). Spearman correlations for the Conners outcomes were highest within the same respondent, especially between inattention and the ADHD index: $r_s = 0.67$ for the self-report, 0.65 for the parent-report, and 0.52 for the teacher-report. Correlations between Conners outcomes across different respondents tended to be low, although correlations between the self- and parent-reports were generally higher ($r_s = 0.06$–0.44) than correlations between parent- and teacher-reports ($r_s = -0.04$–0.29).

Associations of the Metal Mixture with Attention and Behavior

Higher concentrations of all four metals and ferritin were associated with worse (i.e., higher) *T*-scores on the self-reported outcomes (Figure 1). For example, compared with the 50th percentile, the 90th percentile of the mixture was associated with 3.0% ($\beta = 0.03$; 95% CrI: −0.01, 0.06), 4.1% ($\beta = 0.04$; 95% CrI: 0.00, 0.08), and 4.1% ($\beta = 0.04$; 95% CrI: 0.00, 0.08) higher self-reported *T*-scores for the ADHD index, inattention, and hyperactivity, respectively, compared with the 50th percentile. Conversely, lower percentiles of the mixture were beneficially associated with self-reported scales: compared with the 50th percentile, the 10th percentile of the mixture was associated with 5.8% ($\beta = 0.06$; 95% CrI: −0.09, −0.03), 7.7% ($\beta = 0.08$; 95% CrI: −0.13, −0.02), and 7.7% ($\beta = 0.08$; 95% CrI: −0.13, −0.04) lower *T*-scores for the ADHD index, inattention, and hyperactivity, respectively.

The association of the overall mixture with the self-reported outcomes was driven primarily by Mn, which had the highest PIPs for the ADHD index (0.84), inattention (0.86), and hyperactivity (0.95). Based on BKMR models, there was visual evidence of an inverted U-shaped dose response between Mn and self-reported ADHD symptomology, such that Mn was most strongly associated with higher (i.e., worse) scores for the ADHD index at mid-levels of the Mn distribution, when all other metals were fixed at their medians (Figure 2). Similar exposure–response profiles were found for Mn with self-reported inattention and hyperactivity (Figures S3A, S4A, and S5A). Results from adjusted linear regression models were similar to BKMR findings in that low Mn (first tertile) was also associated with 3.3% and 6.2% lower scores on the self-reported ADHD index ($\beta = -0.03$; 95% CI: −0.07, 0.00) and hyperactivity ($\beta = -0.06$; 95% CI: −0.11, −0.02) compared with the middle tertile. However, in contrast to BKMR, high Mn (third tertile) was associated with higher *T*-scores for self-reported inattention, ADHD index, and hyperactivity (e.g., ADHD index: 3.7% increase, $\beta = 0.04$; 95% CI: 0.00, 0.07) (Table S5).

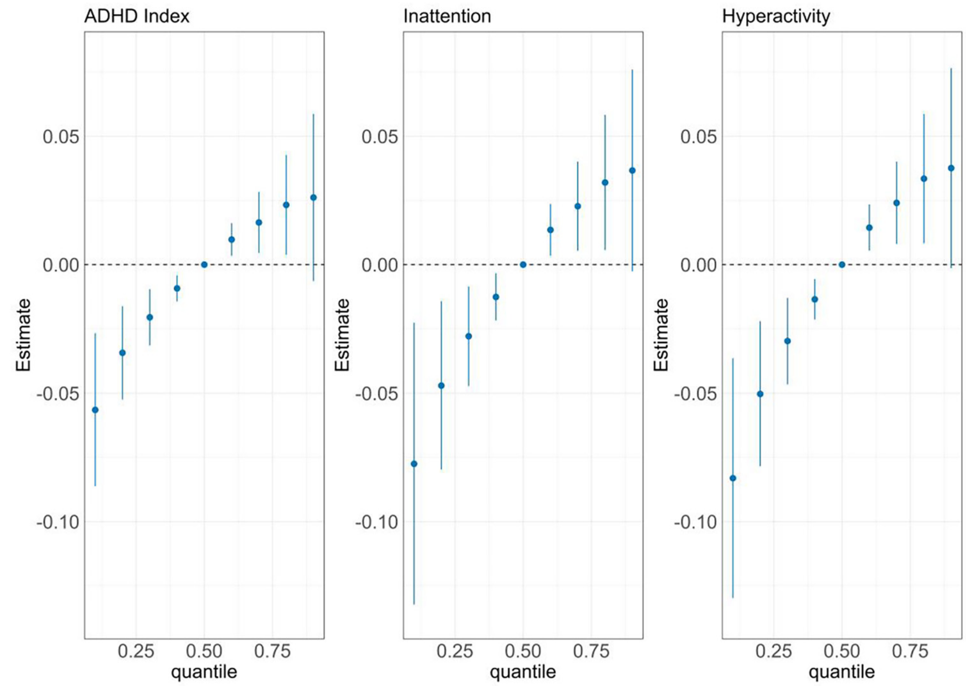

Figure 1. Joint associations (95% CIs) of the overall mixture (Pb, Mn, Cr, Cu, and ferritin) with self-reported ADHD index, inattention, and hyperactivity at increasing percentiles (10th, 20th, 30th, 40th, 60th, 70th, 80th, and 90th) of all metals and ferritin, compared with the medians. Metal and ferritin concentrations were ln-transformed and z-standardized. Conners T-scores were ln-transformed. Models were adjusted for SES and HOME score ($n = 707$). Associations were estimated from BKMR models fit for 20 imputed datasets and pooled using Rubin's rule. Numerical estimates and 95% CrIs are provided in Excel Table S1. Note: ADHD, attention deficit/hyperactivity disorder; BKMR, Bayesian kernel machine regression; CrI, credible interval; Cr, chromium; Cu, copper; HOME, Home Observation Measurement of the Environment; Mn, manganese; Pb, lead; SES, socioeconomic status.

PIPs for Pb, Cr, and Cu for the self-reported outcomes were low relative to Mn, and there was limited evidence that these metals were associated with self-reported attention-related behaviors in BKMR models (Figure 2; Figures S3–S5). This was mostly consistent with linear regression models, although increasing Pb concentrations were associated with 1.3% higher T-scores for self-reported hyperactivity in regression models only (per 1-SD increase: $\beta = 0.02$; 95% CI: 0.00, 0.04). There was no evidence of pairwise or higher-order interactions between the metals or between the metals and ferritin in BKMR models for the self-reported outcomes (Figures S3–S5).

There was limited evidence that the mixture or its components were associated with the parent-reported (Figures S4–S8) or teacher-reported (Figures S9–S11) outcomes in BKMR models. There was also no indication of pairwise or higher-order interactions among any of the metals or ferritin for the parent- and teacher-reported outcomes (Figures S6–S11). However, in linear regression models, the third tertile of Mn, compared with the second tertile, was associated with higher T-scores for the ADHD index, inattention, and hyperactivity for both parent-reported (Table S6) and teacher-reported (Table S7) scales. These associations were strongest for hyperactivity ($\beta = 0.07$; 95% CI: 0.03, 0.10) and inattention ($\beta = 0.04$; 95% CI: −0.01, 0.09) for the parent- and teacher-reported scales, respectively.

Sex-Stratified Analyses

In sex-stratified models, lower percentiles of the mixture were associated with lower (i.e., better) T-scores for the self-reported outcomes in both males and females, compared with when the mixture was held at its 50th percentile. For example, the 10th percentile of the mixture was associated with 4.9% ($\beta = -0.05$; 95% CrI: −0.09, −0.01) and 5.8% ($\beta = -0.06$; 95% CrI: −0.12, −0.01) lower T-scores for the ADHD index among males and females, respectively. However, at higher percentiles of the mixture, positive (i.e., adverse) associations were stronger among females: the 90th percentile of the mixture was associated with 3.0% ($\beta = 0.03$; 95% CrI: −0.02, 0.08), 6.2% ($\beta = 0.06$; 95% CrI: −0.01, 0.12), and 5.1% ($\beta = 0.05$; 95% CrI: −0.01, 0.10) higher T-scores for self-reported inattention, ADHD index, and hyperactivity, respectively, compared with the 50th percentiles (Figure 3A). The associations were null among males (e.g., ADHD index: $\beta = 0.00$; 95% CrI: −0.04, 0.04) (Figure 3B).

The inverted U-shaped dose response of Mn with the self-reported scales tended to be more evident in males compared with females (Figure S12–S17). Other components of the mixture, including ferritin, were not materially associated with the self-reported outcomes in either sex.

Sex-stratified findings for both parent- and teacher-reported outcomes tended to be similar across the sexes (Figures S18–S23). However, there was some evidence that Cu was associated with lower (i.e., better) scores on the parent-reported ADHD index only in males (Figure 4): When the mixture was held at its 50th percentile, an increase in Cu from its 25th to 75th percentiles was associated with 3.0% ($\beta = 0.03$; 95% CrI: −0.06, 0.01) lower ADHD T-scores, and this association was null in females ($\beta = 0.00$; 95% CrI: −0.03, 0.04).

Component-Wise Variable Selection for Fe Status Metric

In secondary analyses, we used the BKMR component-wise variable selection option to select the Fe status marker most predictive of each of the three self-reported outcomes. PIPs obtained from BKMR fits for five randomly selected multiply imputed datasets are shown in Figure 5. Transferrin had the highest mean PIP for inattention (0.75), hyperactivity (0.45), and the ADHD index (0.85) and was therefore used as the Fe status indicator (replacing ferritin) in subsequent BKMR models.

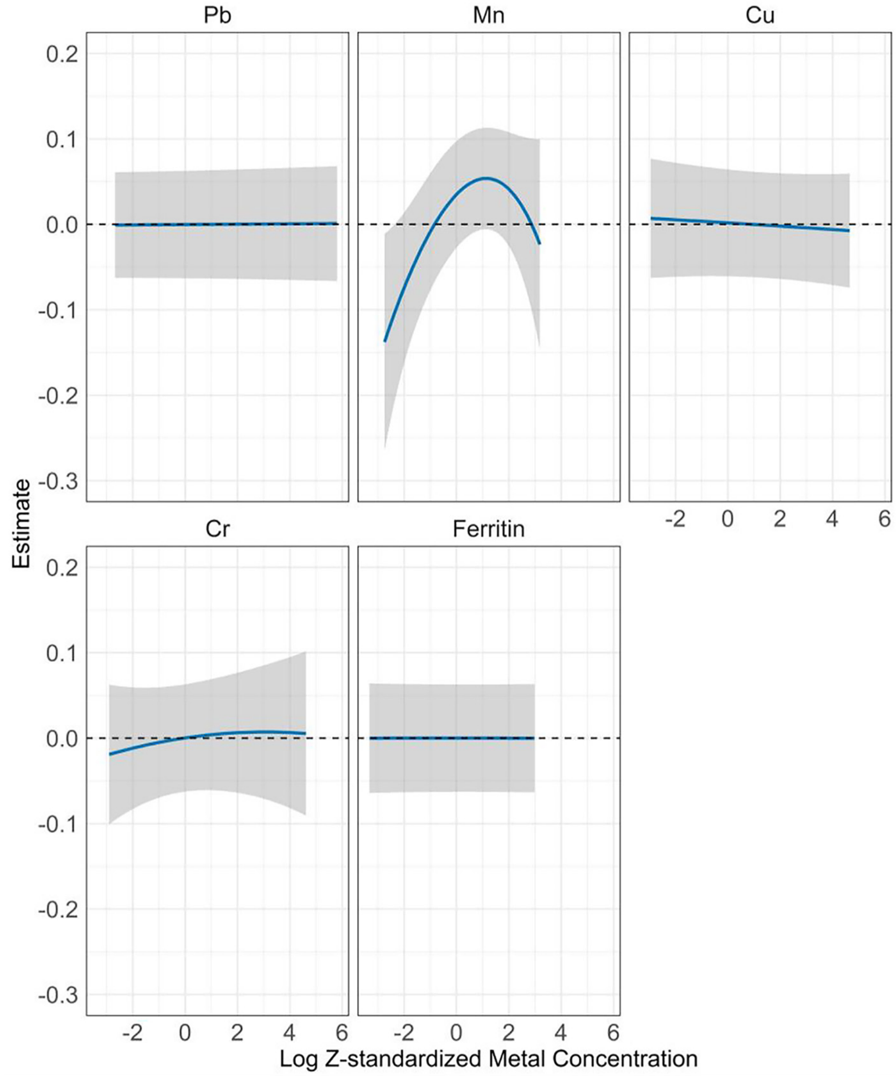

Figure 2. Exposure–response profiles from BKMR models for each metal and ferritin with the self-reported ADHD index for participants in the Public Health Impact of Metals Exposure (PHME) cohort ($n = 707$). Gray shading represents 95% credible intervals. Metals are ln-transformed and z-standardized. Conners T-scores are ln-transformed. Associations are adjusted for HOME score and SES; T-scores are sex- and age-standardized. BKMR models were fit for 20 imputed datasets and pooled using Rubin's rule. Numerical estimates are provided in Excel Table S2. Note: ADHD, attention deficit/hyperactivity disorder; BKMR, Bayesian kernel machine regression; Cr, chromium; Cu, copper; HOME, Home Observation Measurement of the Environment; Mn, manganese; Pb, lead; SES, socioeconomic status.

However, using transferrin as the marker of Fe status did not materially impact our main findings (Figures S24–S26), and there was also no evidence that transferrin modified the association of the mixture with any of the self-reported scales.

Sensitivity Analyses

We ran several sensitivity analyses to examine the robustness of our findings. First, we restricted the dataset to participants with outcome data available from all three respondents (self, parent, and teacher; $n = 281$). Findings in this subset of participants were generally similar to main results (Figures S27–S32) although the association of Mn with self-reported inattention was attenuated (Figure S27). In addition, we observed a negative (beneficial) association between Cu and self-reported hyperactivity that was not present in the main results (from multivariable linear regression, $\beta = -0.04$; 95% CI: -0.07, 0.00). Second, we restricted our sample to participants with complete data on HOME score ($n = 377$); findings from these sensitivity analyses were similar to our main findings (Table S8). Finally, our findings did not materially differ in sensitivity analyses in which we changed the default prior uniform distribution to a gamma distribution or the default smoothness of the h function (100) to 50 or 1,000 (Figures S33–S35).

Discussion

In this cross-sectional analysis of data from Italian adolescents, we found that the mixture of metals and iron status (Pb, Mn, Cr, Cu, and ferritin) was jointly associated with adverse self-reported attention-related behaviors, including inattention, ADHD symptomology, and hyperactivity. A nonlinear association of Mn drove the adverse association of the overall mixture with self-reported attention: at the middle of its distribution, Mn was associated with worse scores for self-reported attention, compared with low and high Mn. There was no evidence that Fe status modified the associations of the metal mixture, or any of its components, with self-reported attention-related behaviors in BKMR or linear regression models.

We previously reported adverse joint associations of the metal mixture (Pb, Mn, Cr, Cu) with verbal IQ and visuospatial abilities

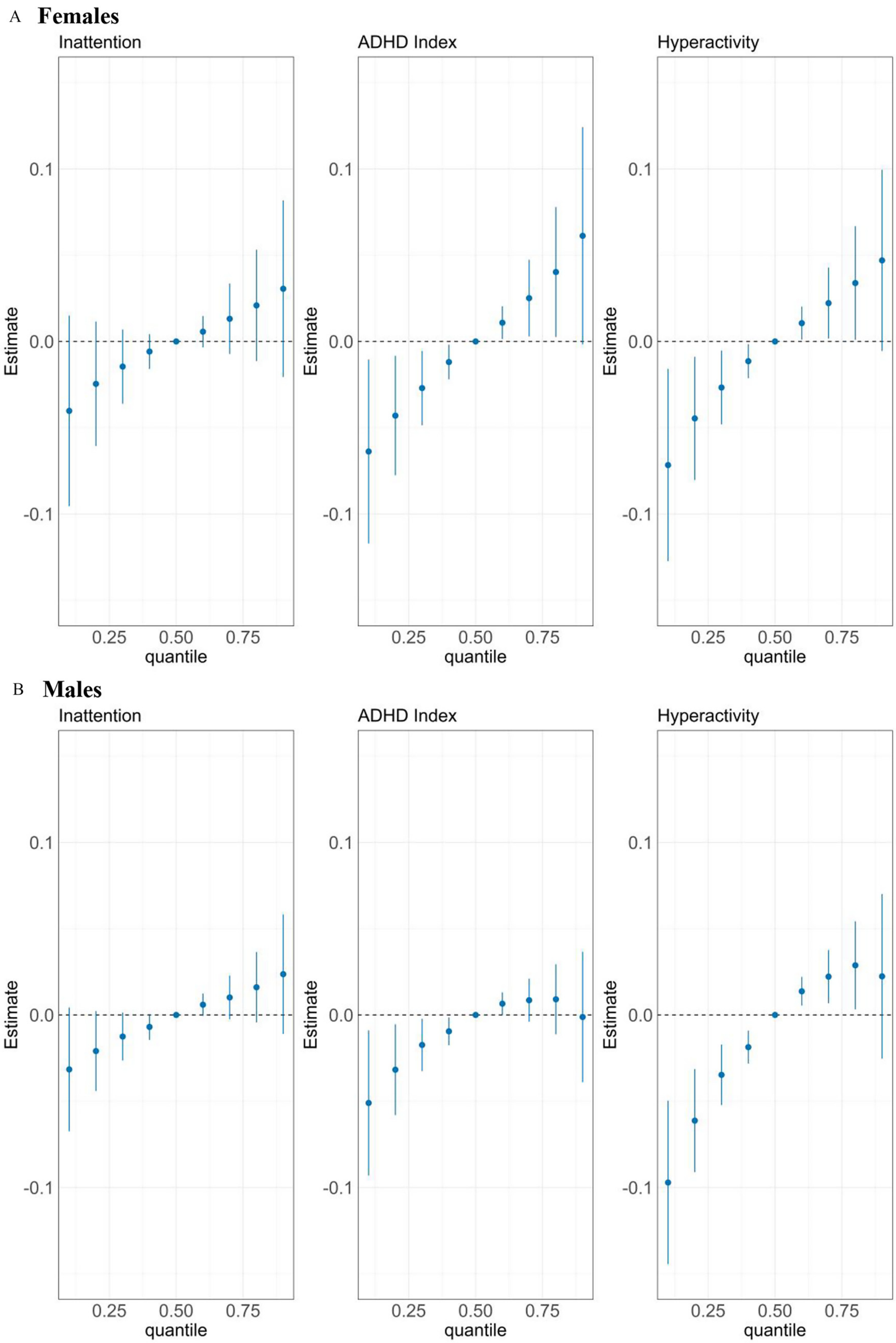

Figure 3. Joint associations (95% CrIs) of the overall mixture (Pb, Mn, Cr, Cu, ferritin) with self-reported ADHD index, inattention, and hyperactivity among (A) females ($n = 340$) and (B) males ($n = 367$) at increasing percentiles (10th, 20th, 30th, 40th, 60th, 70th, 80th, and 90th) of all metals and ferritin, compared with the medians. Metal and ferritin concentrations were ln-transformed and z-standardized. Conners T-scores were ln-transformed. Models were adjusted for SES and HOME score. Associations were estimated from BKMR models fit for 20 imputed datasets and pooled using Rubin's rule. Numerical estimates and 95% CrIs are provided in Excel Table S3. Note: ADHD, attention deficit/hyperactivity disorder; BKMR, Bayesian kernel machine regression; CI, credible interval; Cr, chromium; Cu, copper; HOME, Home Observation Measurement of the Environment; Mn, manganese; Pb, lead; SES, socioeconomic status.

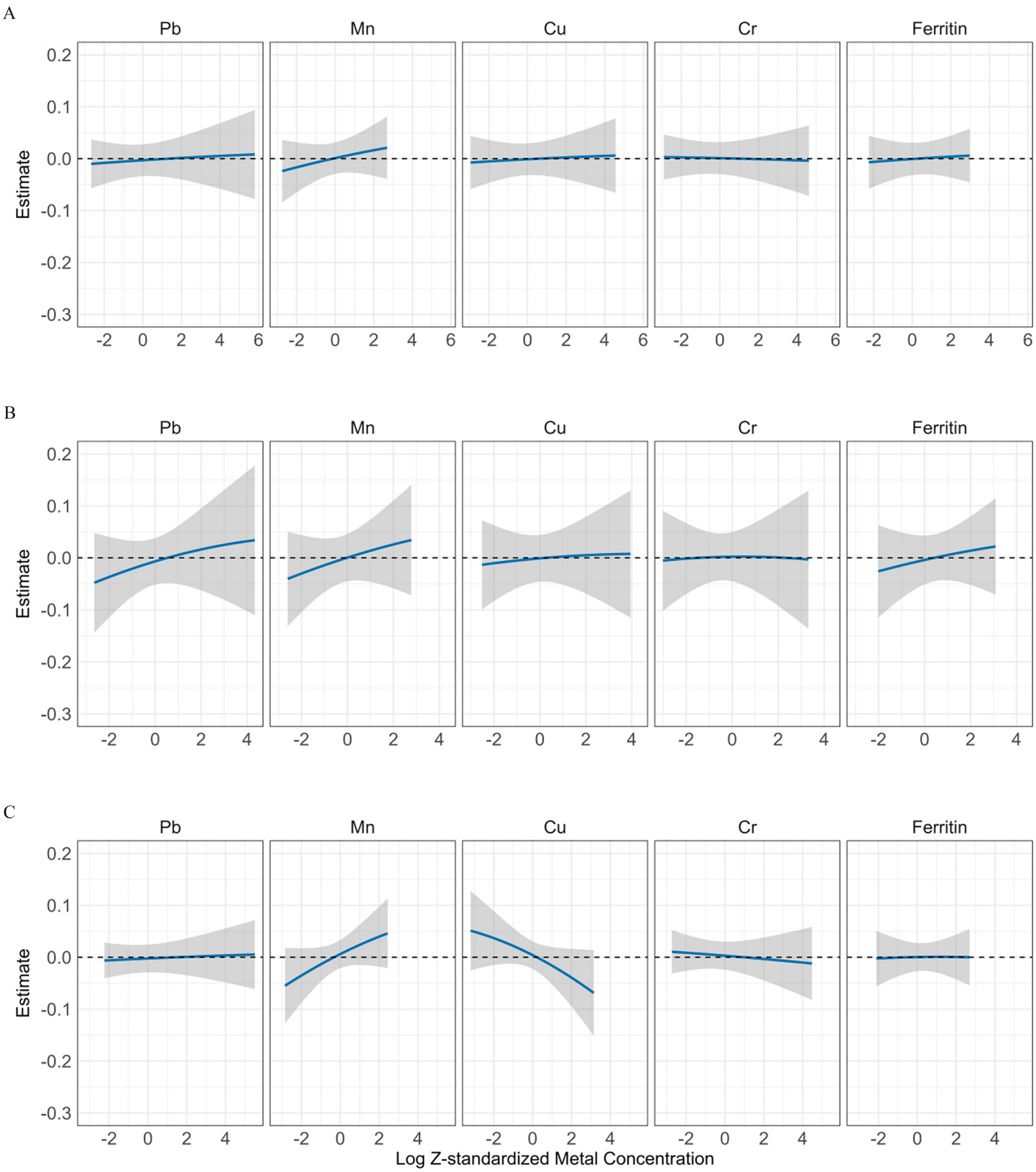

Figure 4. Exposure–response profiles from BKMR models for each metal and ferritin with the parent-reported ADHD index among (A) the full cohort ($n = 396$), (B) females ($n = 187$), and (C) males ($n = 209$). Gray shading represents 95% credible intervals. Metals are ln-transformed and z-standardized, and outcomes are T-scored. Associations are adjusted for HOME score and SES; T-scores are sex- and age-standardized. BKMR models were fit for 20 imputed datasets and pooled using Rubin's rule. Numerical estimates are provided in Excel Table S4. Note: ADHD, attention deficit/hyperactivity disorder; BKMR, Bayesian kernel machine regression; Cr, chromium; Cu, copper; HOME, Home Observation Measurement of the Environment; Mn, manganese; Pb, lead; SES, socioeconomic status.

in the PHIME cohort. Notably, concurrent increases in concentrations of Pb, Mn, and Cr were associated with lower verbal IQ scores when Cu was held at its 10th percentile, suggesting that the mixture was jointly associated with adverse cognitive function.[47] The same metal mixture (Pb, Mn, Cr, Cu) was also associated with worse scores for visuospatial abilities in females, which was similarly driven by Mn.[48] Studies in other cohorts have observed adverse joint associations of mixtures that included Mn, Pb, and Cr or Cu with verbal fluency, cognitive indices, inhibition, and affectivity,[107–111] and many of these joint associations were similarly driven by Mn.[107,109] Taken together, these data indicate that concurrent exposure to metal mixtures is associated with adverse neurodevelopment, and that Mn plays a critical role in the impacts of the overall mixture.

When considered as an individual exposure, Mn measured in various biomarkers (hair, nails, or blood) during childhood or in environmental samples (e.g., water) has been associated with poorer scores on assessments of attentional abilities, as well as

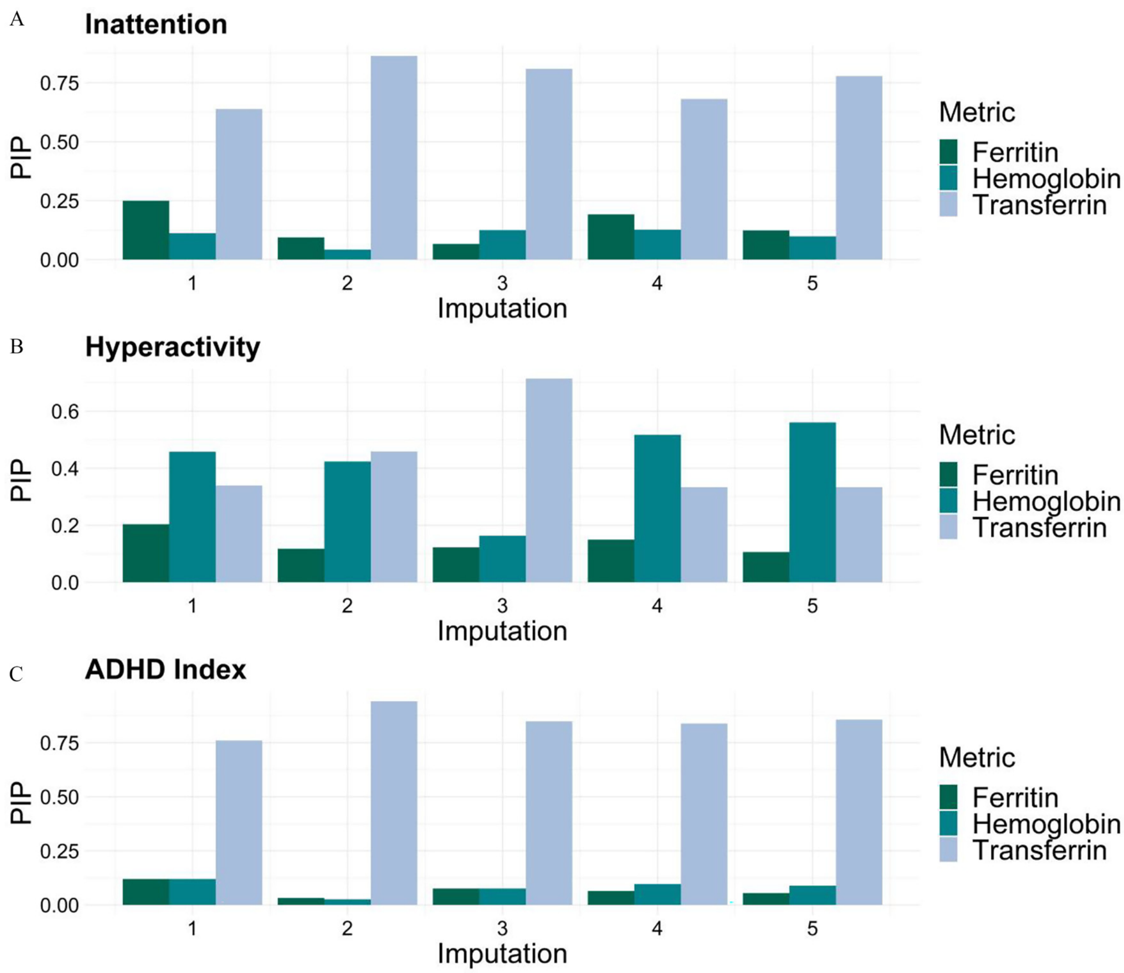

Figure 5. PIPs for ferritin, transferrin, and hemoglobin for self-reported (A) inattention, (B) hyperactivity, and (C) the ADHD index across BKMR models fit using the component-wise variable selection option for the five randomly selected imputed datasets. Numerical estimates are provided in Excel Table S5. Note: ADHD, attention deficit/hyperactivity disorder; BKMR, Bayesian kernel machine regression; PIPs, posterior inclusion probabilities.

social and self-control measures (e.g., internalizing and externalizing behaviors) on continuous performance tests (CPT), the Child Behavior Checklist (CBCL), the CRS, and subtests of the Wechsler Intelligence Scale for Children (WISC) that measure attention or working memory (e.g., digits, coding).[21–30] Although studies in the adolescent period are sparse, evidence from animal models supports epidemiologic findings of adverse Mn associations with attention: postnatal oral Mn exposure has been consistently linked with poorer attention and increased hyperactivity in rats.[112–115] These cognitive decrements in rats were accompanied by increased Mn concentrations in brain tissues,[112] as well as altered concentrations of neurotransmitters (dopamine, norepinephrine, serotonin) and neurotransmitter transporters or receptors (D1 and D2 receptors, norepinephrine transporters) in the prefrontal cortex.[113–115]

Nonmonotonic dose responses for Mn have been reported previously in both animal and human studies,[73,99,111,112] and may reflect the role of Mn as both an essential nutrient and neurotoxicant. However, the adverse association of Mn with self-reported attention-related behaviors only at the middle part of the Mn distribution was generally unexpected because, typically, the lowest and highest concentrations of essential nutrients are associated with worse cognitive development.[73] Consistent with our findings, one other study observed nonlinear associations of cord blood Mn with cognitive development scores from the Bayley Scales of Infant Development (BSID) in early childhood, such that Mn was adversely associated with neurodevelopment only at the middle part of its distribution.[111] We cannot rule out the possibility that the exposure–response profile observed for Mn was driven by influential values because BKMR can be sensitive to outliers,[90,116] although there was limited evidence of outlier Mn observations in this study. Because Mn has been consistently associated with adverse attention-related behaviors in children at higher concentrations,[21–30] the inverted U-shaped dose response observed in the present study was unexpected and should be interpreted with caution, especially because this dose response was only observed in BKMR models. Further studies are needed to better understand the role of dose, duration, and timing of exposure in relation to the dose–response curve for Mn neurotoxicity.

In our data, there was little evidence that Cu was associated with the CRS outcomes in the full cohort. Although there is a limited epidemiology literature on Cu for attention-related behaviors, findings from the small number of prior studies are inconsistent. Among four case-control studies on ADHD diagnosis and Cu, two reported positive associations, and two reported null associations.[19,117–119] Interestingly, we did observe negative (i.e., protective) associations between Cu and the parent-reported ADHD index only in males. This finding may reflect the role of Cu as an essential nutrient, where both deficiency and excess can lead to neurotoxicity.[43,120] As a nutrient, Cu is needed to support the formation and maintenance of myelin, long-term potentiation, and catecholamine synthesis.[120,121] Beneficial associations of Cu with neurodevelopment have been reported in this cohort and elsewhere for other cognitive domains (e.g., learning, memory, cognitive indices),[96,122] supporting the beneficial association we observed in the present analysis. We have also previously reported an inverted U-shaped dose response of Cu with IQ in PHIME, such that Cu was only beneficial for cognitive function at the middle of its distribution.[47] This suggests that Cu may act as a beneficial nutrient for neurodevelopment only within a specific range of concentrations. The sex-specific protective association we observed in this study among males may be partly due to lower Cu concentrations in males (median $= 8.5$ μg/g) than females (median $= 11.6$ μg/g), where the lower concentrations in males may still be within a range that is beneficial for neurologic function.

Although Pb did not materially contribute to the association of the overall mixture, there was evidence of a modest association between increasing blood Pb concentrations and higher scores for self-reported hyperactivity. Pb has been associated with adverse neurodevelopmental outcomes previously in the PHIME cohort[10] and elsewhere,[11–16] which is consistent with known neurotoxic mechanisms of Pb (e.g., disruption of protein kinase C activation).[38,41] Cr was not associated with attention-related behavior either as part of the mixture or individually. Of the two cross-sectional studies that quantified associations between Cr and attention in Spanish children, one reported null findings,[123] and the other found inconsistent associations that varied by Cr biomarker.[20] Where urinary Cr was associated with worse attention measured on the Behavioral Assessment and Research System (BARS) CPT, selective attention test (SAT), and reaction time test (RTT), hair Cr was associated with worse performance only on the RTT, and better performance on the SAT.[20]

We included serum ferritin, an indicator of early Fe deficiency, in the metal mixture to examine both joint associations and modification of the mixture by Fe status. Ferritin did not significantly contribute to associations with attention-related behavior in this study individually, as an effect modifier, or as a component of the mixture. In contrast, two previous studies considered Fe (measured in cord blood or placental tissue at birth) as a component of a metal mixture in relation to neurodevelopment. One of these studies found that a metal mixture, driven primarily by Fe, lithium, and aluminum, was jointly associated with decreased odds of cognitive delay,[124] whereas the second observed adverse effects of a joint increase in a metal mixture on behavior, although Fe did not contribute heavily to this association.[108] Several studies also reported Fe as a modifier of other individual metals, which we did not see in our data. For example, some research has found that the negative association of Pb with neurodevelopment (e.g., attention) is stronger under low Fe conditions or among children with the hemochromatosis (HFE) gene mutation.[60,61,125] Similarly, prenatal Mn and Cu have been associated more strongly with adverse neurodevelopment among children whose mothers had low hemoglobin or serum Fe in pregnancy.[58,59,62] We also previously identified an interaction between ferritin and Cu for verbal learning and memory in the PHIME cohort, such that Cu was more strongly associated with better cognition at higher percentiles of ferritin.[122] It is possible that we did not observe modification by Fe status in the present analysis because of the narrower range of Fe levels in this analytic sample, in which there was limited evidence of Fe deficiency or excess. However, findings from previous studies indicate that Fe status likely contributes to the neurotoxicity of individual metals and their mixtures[63] and should continue to be considered in future analyses.

There is a lack of consensus in the Fe status indicators used for epidemiological studies of metals neurotoxicity to date, with studies using hemoglobin, ferritin, and serum Fe, among other measures, to quantify Fe status.[63] Hemoglobin has commonly been used in neurodevelopment studies because it is clinically relevant and the presence of anemia is a component of chronic Fe deficiency.[59,60,62,125–131] But hemoglobin is a less sensitive measure of Fe deficiency, with concentrations being reduced only in the last phase of Fe deficiency, when anemia develops.[81] Therefore, hemoglobin may not be an optimal metric for environmental epidemiology research where subtle health effects are postulated, especially in populations where the prevalence of anemia is low. In our exploratory analysis in which we used a data-driven approach to select the Fe marker most strongly associated with CRS scales, transferrin (a marker of Fe availability) was selected as the most predictive Fe status marker, rather than ferritin (a marker of circulating Fe and early Fe deficiency) or hemoglobin (a marker of functional Fe status). We hypothesize that transferrin may have been selected as the most predictive Fe status marker because of its role in supporting oligodendrocyte function,[132] although the inclusion of transferrin (rather than ferritin) in the models did not materially affect our findings. These exploratory findings nonetheless suggest that the selection of an appropriate Fe status marker in neurodevelopmental studies may be specific to the population under study and may depend on the extent of Fe deficiency or excess. Therefore, future studies of metal–neurodevelopmental associations that examine Fe status as a confounder or effect modifier should carefully consider the Fe status marker that is most relevant for their population.

There was limited evidence of sex-specific associations in exploratory sex-stratified analyses. However, we did find that the joint association of the mixture at its 90th percentile with the self-reported outcomes was stronger in females, which was driven primarily by Mn. Stronger adverse associations of Mn and metal mixtures with neurodevelopment among females have been previously reported in the literature,[48,133] and may reflect differences in brain plasticity between sexes, including dendritic length and spine density,[134–136] or gender differences in reporting of inattentive behavioral symptomology.[137]

One of the main limitations of this analysis was the reliance upon self-, parent-, and teacher-reported data for the assessment of attention-related behaviors. These measures are subjective and likely reflect differences in attention-related behaviors in various settings, possibly explaining why associations between metals and the scales were not always consistent across CRS respondent types, particularly because correlations between scales across the three reports (self, parent, and teacher) were weak. Child attention may differ in the home vs. school environments; for example, children may be expected to have better attention and executive function in the school environment.[138,139] Parents and teachers may also have different perspectives on a child's cognitive ability because parents and teachers interact with children in unique ways. Parent- and teacher-reports tend to have better internal consistency and test–retest reliability than the self-reported scales,[140] although the self-reported scales were shown to be more strongly correlated with objective measures of attention (e.g., CPT commission and omission scores) in adolescent populations than the parent- and teacher-reports.[139] Therefore, the self-reported scales are still a relevant metric for attention-related behaviors for this age group.[139,141] However, because our sample size differed across the self-, parent-, and teacher-reports, differences in findings across these reports cannot be attributed strictly to differences in attention-related behaviors in varying environments.

Our analysis was cross-sectional, which precluded our ability to establish temporality between the exposures and outcomes, as well as between the Fe status markers, exposures, and outcomes. It is possible that we did not measure Fe status or metal exposures in the relevant exposure window in relation to neurodevelopmental outcomes, and this could, in part, explain why we did not observe modification by Fe status in this study. Future studies with longitudinal designs are needed to better understand the interplay between Fe status and metals. The PHIME cohort was also comprised of healthy adolescents with minimal evidence of Fe deficiency; this is another possible explanation for why we did not observe modification by Fe status. We were unable to address potential confounding by early life (e.g., prenatal) metal exposures in this analysis, which have been consistently associated with neurodevelopmental outcomes in this cohort and elsewhere.[97–99,109,142–146] We were also not able to control for other coexposures (e.g., nickel, arsenic) or dietary intakes of metals that may be relevant for neurodevelopmental outcomes.[147] The limited sample size for parent- and teacher-reported outcomes reduced the statistical power and precision of the findings, particularly for sex-stratified results. It should also be noted that the beta coefficients estimated in this study were small in magnitude and therefore reflect subclinical effects, rather than clinical decrements. Finally, we were not able to control for pubertal status in this population, which may affect functional Fe status and the uptake and deposition of environmental metals.[148–150]

This study also has several strengths. This study is among the first to assess modification of associations between a complex metal mixture and neurodevelopment by Fe status. Because PHIME has multiple measures of Fe status, we were able to *a priori* select a sensitive Fe status marker (ferritin) for use in our main analyses and then employ a data-driven approach to identify the most predictive Fe status marker for further exploratory analyses. Another strength is the focus on the adolescent period, a critical yet understudied developmental window for cognition. Adolescence is an especially important developmental period to consider for attention-related behaviors because of the rapid maturation of the prefrontal cortex at this age.[151] Last, we used a state-of-the-art mixtures approach to quantify associations of the complex mixture with neurodevelopment and grounded those findings in standard regression models, allowing for a relative comparison of our findings across statistical methods.

In summary, we found that the mixture, driven by Mn, was adversely associated with attention-related behaviors in adolescence. These findings suggest that metal exposure in adolescence may be detrimental for attention-related behavior, especially when considered as a complex mixture, which has significant public health ramifications.

Acknowledgments

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