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Associations of Prenatal First Trimester Essential and Nonessential Metal Mixtures with Body Size and Adiposity in Childhood.

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Background: Prenatal nonessential metals may contribute to postnatal adiposity, whereas essential metals may have metabolic benefits. We evaluated joint and individual associations between prenatal metals and childhood adiposity.

Methods: We measured concentrations of six nonessential (arsenic, barium, cadmium, cesium, lead, and mercury) and four essential (magnesium, manganese, selenium, and zinc) metals in first trimester maternal blood from a prebirth cohort. We collected anthropometric measures in early childhood, mid-childhood, and early adolescence including subscapular+tricep skinfold thickness (mm) (N = 715–859), waist circumference (cm) (N = 717–882), and body mass index (BMI) (z-score) (N = 716–875). We measured adiposity in mid-childhood and early adolescence using bone densitometry total- and trunk- fat mass index (kg/m^2) (N = 511–599). We estimated associations using adjusted quantile g-computation and linear regression.

Results: The nonessential metal mixture was associated with higher total (β = 0.07, 95% CI = 0.01, 0.12) and trunk fat mass index (β = 0.12, CI = 0.02, 0.22), waist circumference (β = 0.01, CI = 0.00, 0.01), and BMI (β = 0.24, CI = 0.07, 0.41) in mid-childhood, and total fat mass index (β = 0.07, CI = 0.01, 0.14), and BMI (β = 0.19, CI = 0.02, 0.37) in early adolescence. The essential metal mixture was associated with lower early adolescence total-(β = –0.11, CI = –0.17, –0.04) and trunk- fat mass index (β = –0.13, CI = –0.21, –0.05), subscapular+tricep skinfold thickness (β = –0.02, CI = –0.03, –0.00), waist circumference (β = –0.003, CI = –0.01, –0.00), and BMI (β = –0.16, CI = –0.28, –0.04). Cadmium and cesium were individually associated with childhood adiposity at different timepoints.

Conclusions: Prenatal first-trimester essential metals were associated with lower childhood adiposity, whereas nonessential metals were associated with higher adiposity into adolescence.

Keywords: adiposity; embryonic and fetal development; environmental health; metals; nutrients

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Childhood adiposity is increasing in prevalence[1] and associated with adult comorbidities,[2,3] including a higher risk of mortality.[4] The Developmental Origins of Health and Disease (DOHaD) hypothesis postulates that exposures encountered in utero influence later disease susceptibility and outcomes including adiposity and obesity risk.[5,6] In support of this hypothesis, a number of prenatal factors have been associated with childhood adiposity including higher prepregnancy body mass index (BMI),[7] gestational weight gain,[7] in utero nutrient availability,[8] and environmental exposures.[9] Endocrine-disrupting chemicals (EDCs), including certain metals, may act as obesogens, disrupting metabolic processes at various developmental stages including critical windows of fetal development.[10]

Exposure to metals during pregnancy is ubiquitous,[11] and some metals cross the placental barrier and accumulate

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Because of the sensitive nature of the data collected for this study, requests to access the data set from qualified researchers trained in human subject confidentiality protocols may be sent to Project Viva at project_viva@hphc.org and to the corresponding author. Data can be accessed with the appropriate permission from the Project Viva study team following the study policies (https://www.hms.harvard.edu/viva/).

The authors report no conflicts of interest.

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in fetal tissue.[12,13] For example, mercury is found in higher concentrations in cord blood, compared to maternal blood,[14] whereas cadmium accumulates in the placenta altering biological pathways and function.[15] Conversely, essential metals at therapeutic levels facilitate biochemical and physiologic processes[16] and may mitigate the adverse effect of toxic metals by acting as antioxidants.[17]

Prior experimental and observational studies have tested single exposures and found associations between individual prenatal nonessential and essential metals and postnatal metabolic outcomes.[18–29] Two recent observational studies evaluated associations between metal mixtures and obesity in adults.[30,31] One cross-sectional study found that a circulating concentration of a heavy metal mixture was associated with obesity and its related chronic conditions,[31] whereas the other found that a nonessential metal mixture was associated with higher BMI, prospectively.[30] We aimed to expand on prior literature in adults and examine the relationship between maternal first-trimester metal concentration and postnatal metabolic disease, hypothesizing that prenatal mixtures of essential metals would be associated with lower body size and adiposity, whereas nonessential metals would be associated with higher body size and adiposity in childhood and adolescence.

METHODS

Study Population and Design

During 1999-2002, we recruited pregnant participants into Project Viva during their first prenatal visit at Atrius Harvard Vanguard Medical Associates, a multispecialty group practice in eastern Massachusetts.[32] Eligibility criteria included English fluency, gestational age <22 weeks, singleton pregnancy, and plans to remain in the study area during gestation. Children were seen at in-person visits from birth to adolescence.

Of 2128 mother-infant pairs, 28 participants were enrolled more than once owing to multiple pregnancies. We used data from the first enrollment for this analysis (N = 2100). We measured first-trimester metals in maternal red blood cells among 1407 participants with available samples. Mercury was not measured in 17 of these participants. Of the 1390 mother-infant pairs with all prenatal metal concentrations, 999 mother-infant pairs had at least one of the body size and adiposity measures of interest and were included in analyses (eAppendix, eFigure 1 http://links.lww.com/EDE/B978).

Mothers provided written informed consent at recruitment and all postnatal follow-up visits. Children provided verbal assent beginning in mid-childhood. The Institutional Review Board of Harvard Pilgrim Health Care reviewed and approved all study protocols.

Maternal First-Trimester Metal Concentrations in Red Blood Cells

We have described metal measurements previously.[33] Briefly, upon recruitment (median = 10 weeks gestation), a blood sample was obtained from participants, centrifuged at 2000 rpm for 10 minutes at 4°C, and the red blood cell pellet was stored at –70°C before analysis. Erythrocyte concentrations of all metals, except mercury, were measured using triple quadrupole inductively coupled plasma mass spectrometry (ICP-MS) on a single run (Agilent 8800 ICP-MS-QQQ, Agilent Technologies, Inc., Delaware, USA) in MS/MS mode with appropriate cell gases and internal standards. Mercury concentrations were analyzed using a Direct Mercury Analyzer 80 (Milestone Inc.).

Quality control (QC) measures were previously described,[33] and they included calibration verification, procedural blanks, and repeated analysis of 2% of samples. Intra-day coefficients of variation (CVs) were calculated using in-house QC pools at three different concentrations before and after every 10 samples (N = 7). Intra-day CVs were <5% for analytes included in the analysis, except for selenium (<10%). Inter-day CV was <15%, except for concentrations near the limit of detection (LOD).

We report metal concentrations in ng/g. We selected metals with an intra-class correlation >0.70 for duplicates and >90% detection frequency, including one nonessential metalloid (arsenic), five nonessential metals (barium, cadmium, cesium, lead, and mercury), and four essential metals (magnesium, manganese, selenium, and zinc) (eAppendix, eTable 1 http://links.lww.com/EDE/B978). We refer to all elements, including metal(loid)s, as metals. Metal concentrations below the LOD were given the value (LOD/[square root of 2]). Metal concentrations were $\log_2$-transformed in the analyses.

Child Body Size Measures

Trained research assistants obtained body size measurements during the early childhood, mid-childhood, and early adolescence research visits. We measured height to the nearest 0.1 cm using a calibrated stadiometer (Shorr Productions, Olney, Maryland) and weight to the nearest 0.1 kg using a calibrated scale (early childhood: Seca model 881, Seca Corp., Hanover, MD; mid-childhood and early adolescence: Tanita model TBF-300A, Tanita Corporation of America, Inc., Arlington Heights, IL). We computed each child's BMI as (weight in kg/[height in meters])^2 and calculated age- and sex-adjusted BMI z-score using World Health Organization reference data. We measured the sum of subscapular and triceps skinfold thicknesses to the nearest 0.1 mm using Holtain calipers (Holtain Ltd, Crosswell, Wales) and summed the two thicknesses. We measured waist circumference to the nearest 0.1 cm using a Hoechstmass measuring tape (Hoechstmass Balzer GmbH, Sulzbach, Germany).

Child Adiposity Measures

Trained research assistants administered dual-energy X-ray absorptiometry (DXA) scans (Hologic model Discovery A) during the mid-childhood and early adolescence visits. A single trained research assistant checked scans for positioning, movement, and artifacts, and defined body regions for analysis. We calculated DXA total fat mass index and DXA trunk fat mass index as ([total fat mass or trunk fat mass in kg]/[height in meters]).^{2}

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Covariate Assessment

Mothers self-reported demographic characteristics, including race–ethnicity, at study enrollment, through a brief interview and questionnaire. We abstracted the child's assigned sex at birth from the interview at the time of delivery. We used directed acyclic graphs to select model confounders based on prior literature about covariates. These included maternal pre-pregnancy age,[34] pre-pregnancy BMI, calculated from self-reported weight and height,[7,34] education (<college or college graduate), annual household income ($70,000 per year or less, >$70,000 per year),[35,36] self-identified race–ethnicity (Asian, Black, Hispanic, white, or more than one race–ethnicity),[34,35,37] smoking status (never, former, or during pregnancy),[34] parity (nulliparous or >1),[38] and child assigned sex at birth (female or male). We included maternal race–ethnicity as a covariate because differences between categories are observed in both metal exposure[37] and childhood adiposity,[34,35] which we think could be from shared differences in unmeasured variables, such as environmental racism and marginalization.

Statistical Analysis: Metal Mixtures

We used quantile g-computation[39] to estimate the association between each body size and adiposity measurement and a one-quantile simultaneous increase in all metals in a specified mixture.[30] We examined associations between the entire metal mixture, the nonessential metal mixture (arsenic, barium, cadmium, cesium, lead, and mercury), as well as the essential metal mixture (magnesium, manganese, selenium, and zinc) and each of the outcomes, adjusting for covariates and $\log_2$-transformed metals not included in the mixture. In sensitivity analyses, we examined associations between the metal mixture and adiposity outcomes, stratified by child sex.

Statistical Analysis: Multivariable Analyses for Individual Metals

We used multivariable linear regression models to test associations between individual first-trimester metal concentrations and body size and adiposity measures at each of the time points during childhood, adjusting for covariates. As an exploratory analysis, we checked for statistical interactions between each metal and child sex, using multivariable linear regression models with a multiplicative term. When an interaction term was statistically significant at $\alpha < 0.05$, we examined associations between the individual metals and outcomes, stratified by child sex.

In addition, we considered several sensitivity analyses in obtaining the finalized models. We conducted analyses controlling for the timing of outcome measurement as a covariate (child age in months), but this did not improve the precision of estimates, and so we did not include this in the main models, as it could be on the causal pathway. We considered models in which the metal exposure was not $\log_2$ transformed. Model diagnostics were similar, and we chose the models in which metals were $\log_2$ transformed to minimize the influence of extreme influential points. This approach also allows for comparing doubling of metal concentrations across the outcomes, independent of metal range.

In addition, we considered accounting for pubertal onset. However, we did not adjust for this because (1) prenatal metal exposure would not be impacted by pubertal onset, and (2) pubertal onset may be on the causal pathway. Future research will consider if metals alter pubertal timing. We also considered generalized estimating equations for longitudinal models, but we chose linear regression for individual metals because a longitudinal mean model would have assumed the same effect across time, and there is evidence that associations differed by timepoint of collection. Finally, we performed complete case analyses among participants with exposure/outcome data at all timepoints (N = 385) for quantile g-computation models, because the number of participants who contributed data at each visit was different. Although some observations differed, we believe this from the lower sample size and not differences in the study population of those who were complete case and those who were not, as the distributions of exposures, outcomes, and covariates were not different (eAppendix, eTables 2–4 http://links.lww.com/EDE/B978).

We report estimated associations and 95% confidence intervals (95% CIs) for all analyses. All analyses were conducted in R (version 4.1.0; R Development Core Team).

RESULTS

Population Characteristics and Metal Distributions

Table 1 describes the participant characteristics (N = 999) included in any of the analyses. Most participants were white (74%), had at least a college education (72%), never smoked (69%), and had normal prepregnancy BMI (60%). Median concentrations of all essential metals were within reference ranges, and median concentrations of cadmium and lead were similar to those in whole blood from 1999 to 2000 subset of female National Health and Nutrition Examination Survey (NHANES) participants (eAppendix, eTable 1 http://links.lww.com/EDE/B978).[40] Blood metal concentrations in our study population were mildly to moderately correlated and reported previously.[33]

Body Size and Adiposity Distributions

The mean (standard deviation [SD]) of child age at each visit was 3.3 years (0.33 years) at the early childhood visit, 8.0 years (0.84 years) at the mid-childhood visit, and 13 years (0.95 years) at the early adolescence visit (Table 1). The mean (SD) of each body size and adiposity measure increased across visit time points for all measures, except for BMI z-score, which decreased (Table 2). Correlations

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TABLE 1. Sample Characteristics of Mother-Child Pairs from the Project Viva Cohort with Metal Measurements Who Were Included in at Least One of the Body Size and Adiposity Analyses (N = 999[a]).

Demographic Characteristics		*N* (%) or Mean (Standard Deviation)
Maternal age (years), mean (SD)		33 (4.6)
Maternal pre-pregnancy body mass index (kg/m²), n (%)		
	Underweight (<18.5)	27 (3)
	Normal (18.5–<25.0)	595 (60)
	Overweight (25.0–<30.0)	228 (23)
	Obese (≥30.0)	149 (15)
Maternal race/ethnicity, n (%)		
	Asian	42 (4)
	Black	114 (11)
	Hispanic	65 (7)
	More than one race/ethnicity	42 (4)
	White	736 (74)
Maternal education, n (%)		
	Less than college	275 (28)
	College graduate	724 (72)
Household income, n (%)		
	≤$70,000 per year	371 (37)
	>$70,000 per year	628 (63)
Maternal smoking, n (%)		
	Never	689 (69)
	Former	200 (20)
	During pregnancy	110 (11)
Parity, n (%)		
	Nulliparous	479 (48)
	One or more	520 (52)
Child sex, n (%)		
	Male	526 (53)
	Female	473 (47)
Child age (years), mean (SD)[b]		
	Early childhood visit	3.3 (0.33)
	Mid-childhood visit	8.0 (0.84)
	Early adolescence visit	13 (0.95)

[a]Data was complete for all participants.
[b]Early-childhood visit based on 883 observations; mid-childhood visit based on 750 observations; early adolescence visit based on 717 observations.

TABLE 2. Body Size and Adiposity Measurement Distributions, Mean and Standard Deviations (SDs), Among Children from the Project Viva Cohort With Prenatal First Trimester Metal Concentrations who were Included in the Respective Body Size and Adiposity Analyses (N = 999 overall)

Outcome	Sample Size	Early Childhood Mean (SD)	Sample Size	Mid-Childhood Mean (SD)	Sample Size	Early Adolescence Mean (SD)
SS + TR(mm)	859	17 (4.4)	747	20 (10)	715	28 (14)
Waist circumference (cm)	882	52 (3.7)	748	60 (8.5)	717	73 (12)
Body mass index (z-score)	875	0.79 (1.0)	745	0.56 (1.2)	716	0.53 (1.2)
DXA total fat mass index (kg/m^2)	—	—	599	4.4 (2.0)	511	6.4 (3.2)
DXA trunk fat mass index (kg/m^2)	—	—	599	1.5 (0.89)	511	2.4 (1.5)

DXA, dual-energy x-ray absorptiometry; SD, standard deviation; SS + TR, sum of skinfold and tricep thicknesses.

among outcome measurements at the different time points are reported in eAppendix, eFigure 2 http://links.lww.com/EDE/B978. Mid-childhood and early adolescent outcomes were moderately to highly correlated ($r > 0.5$), whereas early childhood measures were modestly correlated with later outcome measures.

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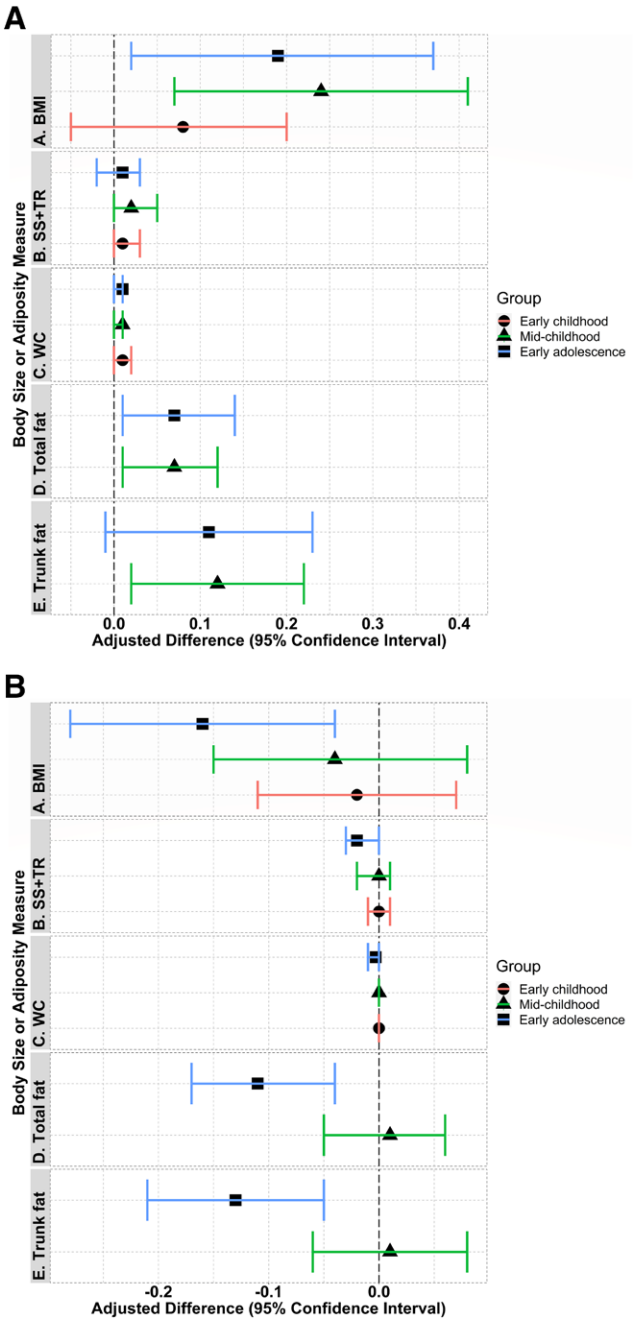

FIGURE 1. Differences and 95% bootstrap confidence intervals, estimated with quantile g-computation, for body mass index (BMI; z-score), sum of skinfold and tricep thicknesses (SS + TR; mm), waist circumference (WC; cm), dual-energy X-ray absorptiometry (DXA) total fat mass index (total fat; kg/m^2), and DXA trunk fat mass index (trunk fat; kg/m^2) for a one quartile increase in (A) nonessential metals (arsenic, barium, cadmium, cesium, lead, and mercury), conditional on covariates (maternal age, pre-pregnancy BMI, race/ethnicity, education, household income, smoking, parity, and child sex) and essential metals, and (B) essential metals (magnesium, manganese, selenium, and zinc), conditional on covariates and nonessential metals. Figure corresponds to Table 3, essential and nonessential metal rows. Figure is available in color online.

Table 3. Quantile g-computation Estimates for Difference in Each Outcome for a One Quartile Increase in Metal Mixtures

Metal[a]	Difference in DXA Total Fat Mass Index (kg/m^2) (β [95% Bbootstrap CI])		Difference in DXA Trunk Fat Mass Index (kg/m^2) (β [95% Bootstrap CI])		Difference in Sum of Subscapular and Tricep Skinfold Thicknesses (mm) (β [95% bootstrap CI])		Difference in Waist Circumference (cm) (β [95% bootstrap CI])		Difference in Body Mass Index (z-score) (β [95% Bootstrap CI])	
	Mid-childhood (N = 599)	Early adolescence (N = 511)	Mid-childhood (N = 599)	Early adolescence (N = 511)	Early childhood (N = 859)	Mid-childhood (N = 747)	Early childhood (N = 882)	Mid-childhood (N = 748)	Early childhood (N = 875)	Mid-childhood (N = 745)	Early adolescence (N = 716)
All metals[b]	0.10	0.00	0.12	−0.03	0.02	0.05	0.29	0.02	0.05	0.20	0.04
	(0.01, 0.18)	(−0.06, 0.06)	(0.01, 0.23)	(−0.28, 0.22)	(−0.02, 0.07)	(−0.03, 0.13)	(−0.22, 0.79)	(−0.00, 0.05)	(−0.08, 0.19)	(0.03, 0.37)	(−0.15, 0.23)
Essential	0.01	−0.11	0.01	−0.13	−0.00	−0.00	−0.00	−0.00	−0.02	−0.04	−0.16
metals[c]	(−0.05, 0.06)	(−0.17, −0.04)	(−0.06, 0.08)	(−0.21, −0.05)	(−0.01, 0.01)	(−0.02, 0.01)	(−0.00, 0.00)	(−0.00, 0.00)	(−0.11, 0.07)	(−0.15, 0.08)	(−0.28, −0.04)
Nonessential	0.07	0.07	0.12	0.11	0.01	0.02	0.01	0.01	0.08	0.24	0.19
metals[d]	(0.01, 0.12)	(0.01, 0.14)	(0.02, 0.22)	(−0.01, 0.23)	(−0.00, 0.03)	(−0.00, 0.05)	(−0.02, 0.03)	(0.00, 0.01)	(−0.05, 0.20)	(0.07, 0.41)	(0.02, 0.37)

[a]Metals were $\log_2$-transformed in the analyses.
[b]Quantile g-computation model assessed difference in each outcome for one quartile increase in all metals (arsenic, barium, cadmium, cesium, lead, magnesium, manganese, mercury, selenium and zinc), conditional on the covariates maternal age, prepregnancy body mass index, race/ethnicity, education, household income, smoking status, parity, and child sex.
[c]Quantile g-computation model assessed difference in each outcome for one quartile increase in essential metals (magnesium, manganese, selenium and zinc), conditional on the covariates maternal age, pre-pregnancy body mass index, race/ethnicity, education, household income, smoking status, parity, child sex, and the nonessential metals.
[d]Quantile g-computation model assessed difference in each outcome for one quartile increase in nonessential metals (arsenic, barium, cadmium, cesium, lead and mercury), conditional on the covariates maternal age, pre-pregnancy body mass index, race/ethnicity, education, household income, smoking status, parity, child sex, and the essential metals.
CI indicates confidence interval; DXA, dual-energy x-ray absorptiometry.

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Associations Between Metal Mixtures and Body Size and Adiposity Measures

Higher levels of the prenatal nonessential metal mixture were associated with higher total fat mass index (β = 0.07 kg/m^2 per quartile, 95% CI = 0.01, 0.12), trunk fat mass index (β = 0.12 kg/m^2, CI = 0.02, 0.22), waist circumference (β = 0.01, CI = 0.00, 0.01), and BMI z-score (β = 0.24, CI = 0.07, 0.41) in mid-childhood, and higher total fat mass index (β = 0.07 kg/m^2, CI = 0.01, 0.14) and BMI z-score (β = 0.19, CI = 0.02, 0.37) in early adolescence (Figure 1 and Table 3). Complete case analyses (N = 348) were similar but with lower precision and reported in eAppendix, eFigure 3 http://links.lww.com/EDE/B978. In stratified analyses by child sex, higher levels of prenatal nonessential metals were associated with a higher sum of subscapular and triceps skinfold thicknesses, waist circumference, and BMI z-score in mid-childhood and higher total fat mass index, trunk fat mass index, sum of subscapular and triceps skinfold thicknesses, and BMI z-score in early adolescence in males, but not females (eAppendix, eTable 5 http://links.lww.com/EDE/B978).

Higher prenatal essential metals, as a mixture, were associated with lower total fat mass index (β = –0.11 kg/m^2, CI = –0.17, –0.04), trunk fat mass index (β = –0.13 kg/m^2, CI = –0.21, –0.05), sum of subscapular and triceps skinfold thicknesses (β = –0.02 mm, CI = –0.03, –0.00), waist circumference (β = –0.003 cm, CI = –0.01, –0.00), and BMI z-score (β = –0.16, CI = –0.28, –0.04) in early adolescence (Figure 1 and Table 3). Complete case analyses are reported in eAppendix, eFigure 3 http://links.lww.com/EDE/B978. In stratified analyses by child sex, higher prenatal essential metals were associated with lower total fat mass index, trunk fat mass index, sum of subscapular and triceps skinfold thicknesses, waist circumference, and BMI in males, but not females (eAppendix, eTable 5 http://links.lww.com/EDE/B978). When an association was observed between either the essential or nonessential metal mixture and an outcome in the pooled analyses, weights representing the proportion of the positive and negative associations for each metal are reported in eAppendix, eFigure 4 http://links.lww.com/EDE/B978 and eAppendix, eFigure 5 http://links.lww.com/EDE/B978, respectively.

The overall mixture, combining essential and nonessential metals, was associated with higher total fat mass index (β =0.10 kg/m^2, CI = 0.01, 0.18), trunk fat mass index (β =0.12 kg/m^2, CI = 0.01, 0.23), and BMI z-score (β =0.20, CI = 0.03, 0.37) in mid-childhood (Table 3). However, we observed the association between the overall mixture and total fat mass index and BMI z-score in mid-childhood in males, but not females (eAppendix, eTable 5 http://links.lww.com/EDE/B978), and no association between the overall mixture and trunk fat mass index in mid-childhood in stratified analyses.

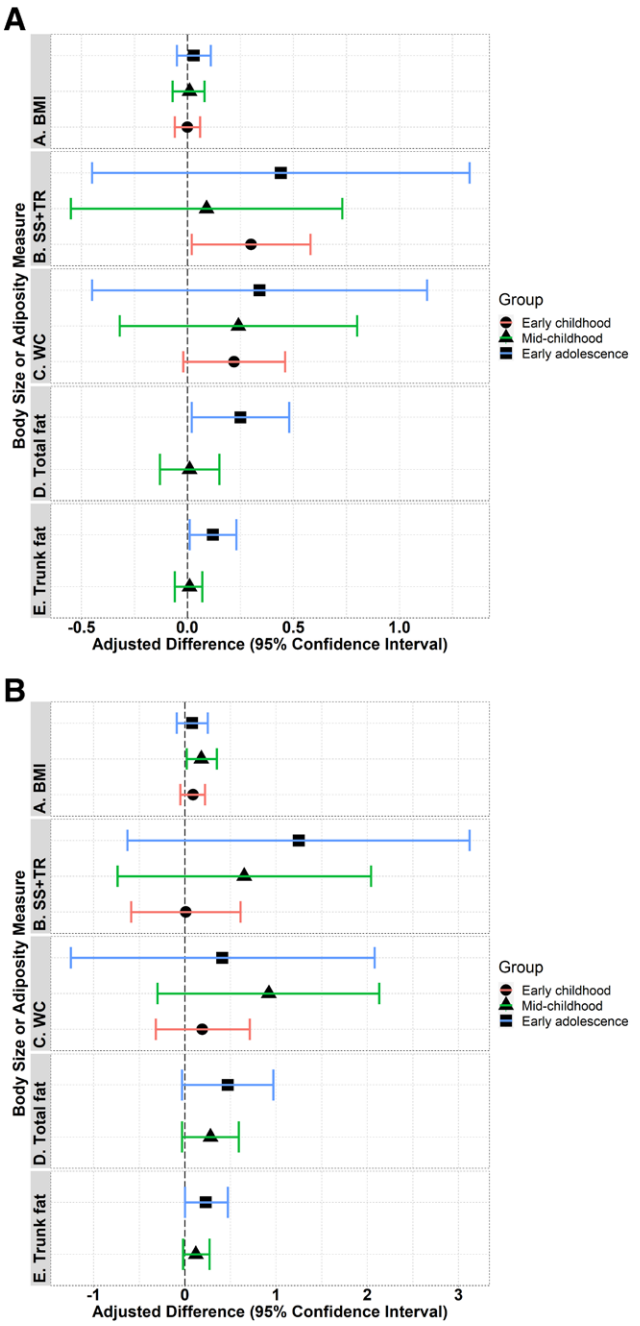

FIGURE 2. Multivariable linear regression estimates and 95% confidence intervals for associations of first trimester red blood cell concentrations of (A) cadmium and (B) cesium individually with body mass index (BMI; z-score), sum of skinfold and tricep thicknesses (SS+TR; mm), waist circumference (WC; cm), dual-energy X-ray absorptiometry (DXA) total fat mass index (total fat; kg/m^2), and DXA trunk fat mass index (trunk fat; kg/m^2), conditional on covariates (maternal age, prepregnancy BMI, race/ethnicity, education, household income, smoking, parity, and child sex). Figure corresponds to Table S6, cadmium and cesium rows. Figure is available in color online.

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Associations Between Individual Metals and Body Size and Adiposity Measures

Individually, a doubling in prenatal cadmium was associated with greater sum of subscapular and triceps skinfold thicknesses in early childhood (β = 0.30 mm, 95% CI = 0.02, 0.58), and higher total- (β = 0.25 kg/m^2, CI = 0.02, 0.48) and trunk- fat mass index index (β =0.12 kg/m^2, CI = 0.01, 0.23) in early adolescence. A doubling in prenatal cesium was associated with greater BMI z-score in mid-childhood (β = 0.18, CI = 0.02, 0.35) and trunk fat mass index in early adolescence (β = 0.23 kg/m^2, CI = 0.00, 0.47) (Figure 2 and eAppendix, eTable 6 http://links.lww.com/EDE/B978). The linear regression estimates generally agreed with the multipollutant models, as cadmium largely drove the association between nonessential metals and total fat mass index in early adolescence, and cesium largely drove the association between nonessential metals and BMI z-score in mid-childhood (eAppendix, eFigure 5 http://links.lww.com/EDE/B978). We observed no further associations for other individual metals (eAppendix, eTable 6 http://links.lww.com/EDE/B978). However, we observed interactions between prenatal metals and sex for several metal–outcome relationships (eAppendix, eTable 7 http://links.lww.com/EDE/B978). Associations between higher cadmium and higher total fat mass index, trunk fat mass index, and sum of subscapular and triceps skinfold thicknesses, and BMI z-score were present in early adolescence in males, but associations in females were in the opposite direction and imprecise (eAppendix, eTable 7 http://links.lww.com/EDE/B978). There was an association between greater magnesium and higher early childhood BMI in males, but not females, as well as an association between higher zinc and lower total fat mass index in mid-childhood in females, but not males (eAppendix, eTable 7 http://links.lww.com/EDE/B978).

DISCUSSION

In this prebirth cohort study examining associations between prenatal metal mixtures and childhood adiposity, we observed associations between a mixture of first-trimester nonessential metals and higher adiposity and a mixture of essential metals with lower adiposity. Most consistently, the prenatal nonessential metal mixture was associated with greater measures of adiposity and body size in mid-childhood, and the essential metal mixture was associated with lower adiposity and body size in early adolescence. We hypothesize that differences observed at each timepoint could reflect prenatal programming that becomes evident at different developmental periods. Sex-stratified analyses showed magnitude and direction of associations between the metal mixtures and body size and adiposity measures similar to the observed overall associations mostly among males, but not females.

We analyzed associations between mixtures of nonessential and essential metals, based on prior mechanistic evidence. The primary mechanism of action for individual metals includes the generation of reactive oxygen species, depletion of the antioxidant glutathione, and bonding to sulfhydryl groups,[41] leading to inflammation, oxidative stress, and genotoxicity.[42] Oxidative stress may mediate health effects related to EDCs.[43] In experimental studies, prenatal arsenic alters gene pathways related to hormone signaling, prenatal cadmium influences hormone synthesis, and lead alters the physiologic activity of hormones.[44] In vivo evidence suggests that perturbation of the hypothalamic dopaminergic system, endoplasmic reticulum stress, impaired adipogenesis, and adipocytokines secretion might serve as potential mechanisms underlying the association of nonessential metals such as arsenic, cadmium, lead, and mercury with adiposity.[42,45–47] In contrast, essential metals at therapeutic levels play an important role in biochemical and physiologic processes[16] and act as antioxidants,[17] and thus we think they could potentially mitigate the mechanistic processes that drive metabolic disruption.

Few studies have examined associations between metal mixtures and adiposity. A recent cross-sectional study examined blood and urinary markers of 18 heavy metals among U.S. adults from NHANES. An increase in an environmental risk score, a summary of main effects and pairwise interactions between metals, was associated with higher BMI, skinfold thickness, and total body fat.[31] Another observational study measured concentrations of 16 nonessential and essential metals in the toenails of participants from the Sister Study (ages 35–74 years) and assessed their associations with BMI measured on average 5.2 years after baseline.[30] Apart from cobalt, no single metal was strongly related to BMI in individual metal models. The nonessential mixture was associated with higher BMI, whereas the essential metal mixture was suggestively associated with lower BMI.[30] Another prospective cohort study in Mexico City, Mexico, measured 11 metals in maternal second-trimester whole blood, as well as BMI, percent body fat, and other cardiometabolic biomarkers in their children (4–6 years).[48] Prenatal levels of selected metals were associated with lower cardiometabolic risk in the children, and associations with essential metals were greater than nonessential metals.[48] No other study to our knowledge has examined associations between prenatal metal mixtures and childhood adiposity.

Prior studies have examined the associations between prenatal individual metals and child adiposity. In a prospective cohort study of pregnant participants and their children in North Carolina (N = 324), first-trimester red blood cell cadmium was associated with higher childhood obesity at 5 years and growth trajectories, independent of arsenic, lead, and smoking status.[22] Similarly in our study, first-trimester red blood cell cadmium was associated with higher total- and trunk-fat mass index in early adolescence, as well as a higher sum of subscapular and triceps skinfold thicknesses in early childhood. Another prospective cohort study in Greece assessed associations between second-trimester urinary cadmium and repeated weight and height measurements from

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birth to childhood, including waist circumference, skinfold thickness, and other cardiometabolic and immune biomarkers (4 years) (N = 515).[25] The study observed no associations between urinary cadmium and body size measurements during early childhood. However, in this study, higher cadmium was associated with slower weight trajectory from age 3 months to 4 years, as well as slower height trajectory in females only.[25]

We hypothesized that prenatal metals may have sex-specific impacts on the development of adiposity in childhood. Consistently, we observed sex-specific relationships between cadmium and adiposity in early adolescence. A prior mouse study found that gestational cadmium exposure from drinking water was associated with higher body weight and perigonadal fat weight in female offspring, compared to unexposed controls at gestational day 18. On postnatal day 1, both males and females were heavier than controls, whereas the mean weight and perigonadal fat weight of females, but not males, in the exposed group was higher after postnatal day 90 and on postnatal day 120, respectively.[49] A birth cohort study in Mexico City, Mexico (N = 185) found that higher third-trimester urinary cadmium was associated with lower BMI z-score, waist circumference, and the sum of subscapular and triceps skinfold thicknesses in female, but not male children (mean age = 10 years).[27] In our study, first-trimester red blood cell cadmium was associated with a higher total fat mass index, trunk fat mass index, sum of subscapular and triceps skinfold thicknesses, and BMI in early adolescence in males, but not females. Cadmium was associated with lower body size and adiposity measures in females in early adolescence, but these associations were imprecise. Differences across the findings in these studies could be owing to different species, experimental vs. observational study designs, windows of exposure, biologic matrices, or false positives from multiple testing.

Another prospective cohort study examined whether first-trimester cadmium was associated with birthweight and cord blood DNA methylation at regulatory sequences of imprinted genes (N = 319) in Durham, North Carolina.[50] Higher cadmium was associated with lower birthweight, as well as higher offspring methylation at the *PEG3* differentially methylated region in females only, a paternally expressed imprinted gene that encodes a zinc finger protein that facilitates p53/c-*myc*-mediated apoptosis. The association between cadmium and *PEG3* methylation was limited to participants with first-trimester zinc levels in the top tertile.[50] Fetal development is a susceptible period of rapid epigenetic reprogramming, and both essential and nonessential metal exposure was shown to influence DNA methylation at birth and in childhood.[51] Programming of metabolic-related genes could influence metabolic trajectories, particularly in periods of accelerated growth.[52] We observed the strongest associations in mid-childhood and adolescence, supporting this hypothesis.

Strengths of this study include large sample sizes, longitudinal repeated outcome measures during childhood, and blood metals measured in participants during the first trimester of pregnancy. Metals measured in red blood cells are good indicators of overall metal burden for most metals. For example, blood lead binds to red blood cells[53] and is the primary biomarker for recent lead exposure.[54] Red blood cell magnesium is the preferred measure for magnesium deficiency, compared with serum or plasma.[55] Blood cadmium is the most valid marker of recent exposure,[56] and a useful measure of long-term exposure among nonsmokers.[53]

A limitation of this study is that participants were all recruited from the greater Boston, Massachusetts area, were majority white with high education and incomes, and all had health care at recruitment, limiting generalizability to the population of all pregnant persons in the United States. For example, median blood metal distribution may be shifted higher in predominantly ethnic minority communities owing to environmental racism.[57–59] We also did not control for multiple comparisons, as we focused on estimation and consistency of associations. Residual confounding is possible from co-exposure to other chemicals or dietary factors not included in our study, or the granularity for socioeconomic status, income, and race–ethnicity. Our race–ethnicity classifications are limited owing to the inherit limitations of race as a social construct and limited ethnic group self-reported categories, and the variable is only used in this study as a proxy for unmeasured experiences of racism and marginalization.

Our research warrants further investigation related to the mechanisms by which prenatal metal mixture concentrations in the first trimester are associated with body size and adiposity during childhood and whether these associations persist into adulthood conferring cardiometabolic risks. The results could inform future clinical care for the reduction of nonessential metal exposure during pregnancy and monitoring essential metal intake prenatally.

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