Prenatal and postnatal mercury exposure and blood pressure in childhood

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Abstract

Elevated blood pressure in childhood is an important risk factor for hypertension in adulthood. Environmental exposures have been associated with elevated blood pressure over the life course and exposure to mercury (Hg) has been linked to cardiovascular effects in adults. As subclinical vascular changes begin early in life, Hg may play a role in altered blood pressure in children. However, the evidence linking early life Hg exposure to altered blood pressure in childhood has been largely inconsistent. In the ongoing New Hampshire Birth Cohort Study, we investigated prenatal and childhood Hg exposure at multiple time points and associations with blood pressure.
measurements in 395 young children (mean age 5.5 years, SD 0.4). Hg exposure was measured in children’s toenail clippings at age 3 and in urine at age 5–6 years, as well as in maternal toenail samples collected at ~28 weeks gestation and 6 weeks postpartum, the latter two samples reflecting early prenatal and mid-gestation exposures, respectively. Five measurements of systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial pressure (MAP) were averaged for each child using a standardized technique. In covariate-adjusted linear regression analyses, we observed that a 0.1 µg/g increase in child toenail Hg at age 3 or a 0.1 µg/L urine Hg at age 5–6 were individually associated with greater DBP (toenail β: 0.53 mmHg; 95% CI: −0.02, 1.07; urine β: 0.48 mmHg; 95% CI: 0.10, 0.86) and MAP (toenail β: 0.67 mmHg; 95% CI: 0.002, 1.33; urine β: 0.55 mmHg; 95% CI: 0.10, 1.01). Neither early prenatal nor mid-gestation Hg exposure, as measured by maternal toenails, were related to any changes to child BP. Simultaneous inclusion of both child urine Hg and child toenail Hg in models suggested a potentially stronger relationship of urine Hg at age 5–6 with DBP and MAP, as compared to toenail Hg at age 3. Our findings suggest that Hg exposure during childhood is associated with alterations in BP. Childhood may be an important window of opportunity to reduce the impacts of Hg exposure on children’s blood pressure, and in turn, long-term health.

Keywords
Mercury; Children’s health; Blood pressure; Cohort; New Hampshire

1. Introduction

Cardiovascular disease (CVD) remains a growing public health issue across the globe, accounting for nearly a third of all deaths worldwide (World Health Organization, 2017). High blood pressure (BP) is one of the leading risk factors for CVD and a number of studies have suggested that adverse cardiovascular changes, including elevated BP, begin early in childhood (Magnussen et al., 2013). Increases in BP in children have been associated with future hypertension risk as well as coronary heart disease mortality, left ventricular hypertrophy, and atherosclerosis, as measured by carotid intima-media thickness (Magnussen et al., 2013; Hao et al., 2017; Koskinen et al., 2019; Flynn, 2019). Together, these studies underscore the need to identify potentially modifiable risk factors for elevated BP in children.

Environmental contaminants have emerged as key contributors to many chronic diseases, including CVD, and may be equally important to the development of CVD as recognized risk factors, such as smoking, diet, and exercise (Cosselman et al., 2015). Mercury (Hg) is a ubiquitous environmental contaminant that has been associated with adverse cardiovascular effects in adults, including cardiovascular mortality, acute myocardial infarction, coronary heart disease, carotid atherosclerosis and elevated BP (Salonen et al., 2000; Valera et al., 2009; Virtanen et al., 2005; Vupputuri et al., 2005; Karagas et al., 2012; Roman et al., 2011). A small number of epidemiological studies have investigated Hg’s effects on BP elevations in childhood. One of the first studies to investigate this found that prenatal methylmercury (MeHg) measured in cord blood was associated with higher BP at age 7 among children in the Faroe Islands, a population with very high levels MeHg exposure from the consumption
of whale meat as part of traditional dietary practices (Sorensen et al., 1999). A study among residents of the Seychelles Islands, a population with high levels of MeHg exposure primarily from heavy fish consumption also found that prenatal MeHg measured in maternal hair samples was associated with greater BP at age 15 years, but this association was primarily limited to boys (Thurston et al., 2007). In the CASPIAN study of Iranian adolescents, serum Hg was cross-sectionally associated with higher BP (Poursafa et al., 2014). In contrast, at least two large prospective studies with low to moderate levels of exposure examined maternal whole blood or erythrocyte Hg during pregnancy and childhood BP and found inconsistent or no associations (Gregory et al., 2016; Kalish et al., 2014). Other studies have observed effects of Hg on children’s heart rate variability, another cardiovascular risk factor, without concurrent effects on BP (Grandjean et al., 2004; Valera et al., 2011; Valera et al., 2012). The present evidence of Hg’s influence on children’s cardiovascular health remains uncertain and differences across studies could be due to variability in exposures, matrices tested and underlying differences across populations. Further, few have accounted for the child’s consumption of fish and seafood, which may result in greater exposures to both MeHg but also cardioprotective nutrients that may have beneficial effects on children’s BP. Moreover, the majority of studies to date have focused on Hg exposures at a single point in time, primarily the prenatal period, without information on Hg exposures during other critical windows of development.

To our knowledge, the relative impacts of environmentally relevant Hg exposure levels during multiple critical windows of development on childhood BP have not been investigated in US populations. In this study of children enrolled in the New Hampshire Birth Cohort, we utilized biomarkers of Hg exposure collected at four time points to investigate the effects of early and mid-gestation prenatal and early childhood and mid-childhood Hg exposures on children’s BP.

2. Methods

The New Hampshire Birth Cohort:

The New Hampshire Birth Cohort (NHBCS) is an ongoing cohort study, with over 2000 women enrolled since 2009. The NHBCS recruits 18–45 year-old pregnant women receiving prenatal care at study clinics, as previously described (Gilbert-Diamond et al., 2011). Women were enrolled at about 24–28 weeks gestation if they reported using water from a private well at their residence since their last menstrual period and were not planning to move prior to delivery. Only singleton births were included in the study. Cohort mothers and their children are followed-up regularly throughout early childhood, including during an in-person assessment scheduled after the child’s fifth birthday. All protocols were approved by the Dartmouth College Institutional Review Boards. Participants provided written, informed consent upon enrollment.

Questionnaires and Medical Record Review:

Mothers enrolled in the study completed a detailed medical history and lifestyle questionnaire upon enrollment at ~24–28 weeks gestation, which ascertained sociodemographic factors (age, race/ethnicity, marital status, education), reproductive
history (previous pregnancies, complications, birth outcomes), and health history. Women were asked about habits, including tobacco and alcohol use, along with their home water source and water consumption. At two weeks postpartum, mothers were sent a follow-up questionnaire to obtain additional information about pregnancy, delivery and changes in key exposures. Participants also consented to a medical record review, which allowed additional information to be recorded about prenatal infections, medication use, birth outcomes and delivery details, and general health of the women and their infants after birth. Pediatric medical records were reviewed over the course of follow-up to obtain additional child health information, such as diagnosis of cardiovascular disorders. Participants were contacted by telephone at regular intervals to obtain updated participant information via questionnaires. At an in-person follow-up visit scheduled at 5–6 years of age, mothers filled out additional questionnaires to update sociodemographic information, as well as day-of-visit information about their child’s health, recent medication use, and other lifestyle characteristics. A validated semi-quantitative food frequency questionnaire of the child’s eating habits was administered by mail at age 3 and again at the time of the 5–6 year in-person assessment (Hunsberger et al., 2015).

Child follow-up assessment:

During the 5–6 year in-person follow-up assessment, each child’s height and weight were measured by a trained study staff member using a Seca 213 stadiometer and Seca 763 medical digital scale. As previously described, a set of blood pressure measurements was obtained, after the child had been quietly seated for a minimum of 5 min (Farzan et al., 2018). Briefly, a blood pressure cuff of appropriate size was fitted to the child’s arm, and the arm was supported to comfortably rest near heart level. Children were instructed to refrain from talking or moving during the measurements to minimize variability (Flynn et al., 2017). Five measurements were obtained at 1-minute intervals using an automated oscillometric instrument (GE Carescape Dinamap V100). Systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial pressure (MAP) were recorded and averaged across the 5 measurements for analyses. Mothers were asked to refrain from giving their child cold medications and sugary and/or caffeinated foods or beverages within 12 h prior to the assessment. Any other chronic medication use was also recorded.

Only children who had participated in the child BP assessment at the time of this analysis and who had available urine metal analyses at 5–6 years and/or toenail metal analyses at age 3 were included in the current study. At the time of this analysis, 395 children had both valid blood pressure measurements obtained prior to their 7th birthday and child urine and/or toenail metals analyses. A subset of this sample also had available maternal prenatal (n = 301) and/or postpartum (n = 322) toenail metals measurements at the time of analysis. Two children with a medically diagnosed cardiovascular disorder (e.g. congenital heart defect, arrhythmia) were excluded prior to analyses.

2.1. Metals assessment in urine and toenail clippings

Child urine samples were collected prior to the in-person assessment and if a sample was not available, they were offered the option to provide a sample at the time of the study visit. For ease of collection, urine was obtained in a toilet “hat”, and then transferred to a pre-labeled,
acid-washed urine specimen cup provided to the participants. Urine samples were stored upright at 4 °C, and processed within 24 h of collection, after which they were aliquoted and stored at −80 °C. Child urine samples were analyzed for a panel of trace metals by high-performance liquid chromatography (HPLC) inductively coupled plasma mass spectrometry (ICP-MS), including total Hg, lead (Pb), cadmium (Cd), arsenic (As), and selenium (Se). Analysis of As species was also performed to determine levels of arsenite (iAs\textsuperscript{III}), arsenate (iAs\textsuperscript{V}), monomethylarsonic acid (MMA), dimethylarsinic acid (DMA) and arsenobetaine (AsB) (Hartiala et al., 2012; Juonala et al., 2005; Raitakari et al., 2003); a form of As found in fish and seafood (Knoflach et al., 2009). For this study, AsB was used as an indicator of fish and seafood consumption in sensitivity analyses.

Toenail samples were collected from both mother and child. Maternal toenail samples were collected upon enrollment at approximately 24–28 weeks gestation and again at approximately 6 weeks postpartum. Child toenail samples were collected at approximately 36 months of age. Collection protocols were the same for both children and mothers, such that participants were asked to remove any nail polish, clip a normal amount of toenail from each toe after bathing and place clippings into a small collection envelope. Samples were washed and then sonicated in solutions of acetone and 1% Triton X-100 with each step followed by deionized water rinses and sonication, and then dried before low-pressure microwave digestion. Toenail samples were analyzed for a panel of elements, including Hg, using ICP-MS (Emeny et al., 2019). Hg was detected at levels above the limit of detection in 37% of child urine samples (instrument detection limit = 0.02 µg/L), 27% of child toenails (instrument detection limit = 0.05 µg/g), 72% of maternal gestational week 24–28 toenails and 90% of maternal 6-week postpartum toenail samples, with a detection limit of 0.05 µg/g. Individuals with values that fell below the limit of detection (LOD) were assigned the instrument detected values.

Statistical Analysis: All analyses were performed using SAS 9.4, except where noted. We computed frequencies (percentages) and/or means (standard deviations) of characteristics of mothers and children to examine the distributions of all variables, including exposure biomarker measurements. Spearman’s rank correlations were computed between 1) all urine and toenail Hg concentrations measured from child and maternal samples, 2) other key metals (lead (Pb), arsenic (As), cadmium (Cd), and selenium (Se) in child samples and 3) between child Hg measures and fish/seafood intake variables and urinary AsB. Using each of the exposure measures on a continuous scale, we examined their associations with child SBP, DBP and MAP using linear regression models. Models were fit separately for each of the exposure biomarkers (maternal prenatal toenail Hg, maternal postpartum toenail Hg, child 3-year toenail Hg, child 5–6 year urine Hg) to explore single exposures within each of these time windows in relation to each of the three BP measures. In secondary analyses, we examined a series of models additionally including either Pb or total As (excluding AsB), measured in the same matrix (urine or toenails) to account for co-exposures that have been previously associated with greater BP in children (Farzan et al., 2018; Gump et al., 2005; Hawkesworth et al., 2012; Sanders et al., 2018; Zhang et al., 2012). Similarly, we also conducted analyses where both child toenail Hg at age 3 and urine
Hg at age 5–6 were included together in linear regression models to examine the relative contribution of each exposure biomarker, which represent different developmental windows. All model estimates were scaled to either a 0.1 µg/g increase or a 0.1 µg/L increase in Hg exposure, in models of toenail or urine Hg, respectively. We expressed our results as a 0.1 µg/g or 0.1 µg/L change given that this was both an easily interpretable unit of change that was similar to a 1 standard deviation change for each of these biomarkers (child urine Hg at age 5–6 years SD: 0.119 µg/L, child toenails Hg at age 3 years SD: 0.087 µg/g) and was scaled to overall level of the exposures in this population.

We tested a number of covariates that could potentially influence child BP based on a priori considerations, and final models included child age, sex, height, weight, maternal smoking during pregnancy, maternal educational attainment, birth weight, and gestational age.

Urinary dilution was also accounted for in models of urine Hg by including urine specific gravity as a covariate. Because fish and seafood consumption can be a source of Hg exposure as well as cardioprotective nutrients, we also explored these measures as potential confounders by restricting analyses to participants who had provided diet data for either the 3-year or 5–6 year time point and examining effect estimates before and after adjustment for fish and seafood intake. Additional adjustment for urinary AsB, a proxy indicator of fish or seafood intake, was also explored in models presented in supplementary analyses.

Given the relatively low percentage of children with detectable levels of Hg in either toenails or urine, we performed two analyses to further examine these exposure variables. First, we evaluated Hg exposures categorically, by determining the median level of Hg among those with detectable levels, then modeled each Hg exposure as a three-level variable (below limit of detection (reference category), detectable Hg level below the median, and detectable Hg level above the median). We also performed a sensitivity analysis excluding any participants with levels of Hg below the limit of detection to evaluate whether trends were similar to those observed in the full sample.

We also considered the possibility of effect modification by child sex and birth weight, given previous literature indicating potential Hg-related differences in children’s blood pressure by each of these factors (Sorensen et al., 1999; Thurston et al., 2007). We evaluated each of these factors by inspecting stratum specific estimates and by including a multiplicative interaction term in the multivariable regression models and assessing its statistical significance at p < 0.05.

3. Results

3.1. Population characteristics

A total of 395 children from the NHBCS with both child blood pressure measurements at age 5–6 years and either 3-year toenail metals (n = 290) and/or 5–6 year urine metals (n = 363) were included in these analyses (Table 1). At the time of assessment, children were, on average, 5.5 (SD: 0.4, range 5.00–6.93) years of age, 112.6 (SD: 5.0) centimeters tall and weighed 20.8 (SD: 3.6) kilograms. Our sample consisted of slightly more girls than boys (51.4% versus 48.6%, respectively). Children had a mean weight of 3340.7 (SD: 550.3)
grams at birth and were born at 38.9 (SD: 1.7) gestational weeks. Mothers of the children in this study sample were 30.9 (SD: 4.6) years of age upon enrollment in the study, and 69% reported having at least a college education. Mean maternal BMI was 26.3 (SD: 5.9) kg/m². A small proportion of women reported smoking during pregnancy (6.4%).

3.2. Biomarkers of Hg exposure

Multiple biomarkers of Hg exposure were assessed in both mothers and children, representing exposures at different critical windows of development. Mean Hg in child urine at the time of the in-person assessment at age 5–6 years was 0.071 (SD: 0.119) µg/L and ranged from 0.0003 to 0.986 µg/L. Hg in child toenails at age 3 years averaged 0.055 (SD: 0.087) µg/g and ranged from 0.0001 to 0.923 µg/g (Table 1). In this study population, Hg was assessed in maternal toenails collected at approximately 24–28 weeks gestation (n = 301) and again at 6 weeks postpartum (N = 322), reflecting Hg exposure ~6 to 12 months prior to collection, i.e. the periconceptional to early prenatal and mid-prenatal periods, respectively (Karagas et al., 2000; Slotnick and Nriagu, 2006). Maternal prenatal and postpartum toenail Hg was similar across time points, with average levels of 0.129 (SD: 0.139, range: 0.0013–0.9742) µg/g and 0.128 (SD: 0.157, range: 0.0001–1.444) µg/g, respectively.

We examined Spearman’s correlations between each of the biomarkers of Hg exposure (Tables S2) and found a strong correlation between maternal prenatal and postpartum levels of toenail Hg (\(\rho = 0.82, p < 0.0001\)). Weaker, but statistically significant, correlations were observed between child toenail Hg at age 3 years and both maternal prenatal (\(\rho = 0.30, p < 0.0001\)) and postpartum (\(\rho = 0.29, p < 0.0001\)) toenail samples. We observed a modest but statistically significant correlation between child toenail Hg at 3 years of age and urine Hg at 5–6 years of age (\(\rho = 0.19, p < 0.01\)). No statistically significant correlations were observed between child urine Hg and maternal toenail Hg measured at either the prenatal or postpartum time point.

3.3. Associations of multiple Hg exposure biomarkers and child blood pressure

In linear regression models adjusted for child age, sex, height, weight, birth weight, gestational age, maternal education, and urine specific gravity (in models with urine biomarkers only), we found that child Hg exposure was associated with statistically significant greater DBP and MAP (Table 2). We observed that a 0.1 µg/L increase in child urine Hg was associated with 0.48 mmHg (95% CI: 0.10, 0.86; \(p = 0.01\)) greater DBP and 0.55 mmHg (95% CI: 0.10, 1.01; \(p = 0.02\)) greater MAP. Similarly, a 0.1 µg/g increase in child toenail Hg was associated with 0.53 mmHg (95% CI: −0.02, 1.07; \(p = 0.06\)) greater DBP and 0.67 mmHg (95% CI: 0.002, 1.33; \(p = 0.049\)) greater MAP. Positive associations with SBP were observed with Hg in both 5–6 year urine (\(\beta: 0.51, 95\% \text{ CI: } 0.13, 1.16; p = 0.12\)) and in 3-year toenails (\(\beta: 0.81, 95\% \text{ CI: } −0.16, 1.78; p = 0.10\)), but neither reached statistical significance. Neither maternal prenatal toenail Hg nor postpartum toenail Hg was associated with any changes to child BP at age 5–6 years.
3.4. Critical time periods of exposure

Our findings that both toenail Hg at age 3 and urine Hg at age 5–6 years were similarly associated with greater child DBP and MAP, prompted the question of whether Hg exposure at one of these time points may be more strongly related to these outcomes. To investigate, we included both child toenail Hg and child urine Hg together within the same linear regression models, adjusted for covariates (Table 3). We found that when both toenail Hg and urine Hg were included in the same model, the effect estimates for toenail Hg with DBP and MAP remained positive, but were attenuated and no longer statistically significant (DBP $\beta$: 0.33, 95% CI: 0.26, 0.92; MAP $\beta$: 0.42 95% CI: 0.31, 1.15), while the associations between urine Hg and DBP were slightly stronger than those observed in the primary analyses (DBP $\beta$: 0.56, 95% CI: 0.11, 1.00) and remained similar for MAP ($\beta$: 0.56, 95% CI: 0.01, 1.11), suggesting a potentially stronger relationship between urine Hg and DBP and MAP, as compared to toenail Hg. Although these two biomarkers of exposure were weakly correlated (Tables S1), we found no evidence of multi-collinearity, as the variance inflation factor for both exposure variables remained low (below 1.5 for all models).

3.5. Co-exposures with other metals

Given that Pb and As previously have been associated with greater BP in children (Farzan et al., 2018; Gump et al., 2005; Zhang et al., 2012), we examined correlations of these and other elements potentially related to child BP with Hg in both child urine and toenails, but did not observe any statistically significant correlations with Hg exposure (Tables S2). We found that inclusion of either toenail Pb or As measured at age 3 years as a potential confounder did not appreciably alter the association between child’s toenail Hg and DBP or MAP (Tables S3). Likewise, inclusion of urine Pb or As in the models did not appreciably alter the associations of child Hg with DBP or with MAP (Tables S3).

3.6. Categorical analysis of detectable versus non-detectable Hg exposure values

In our population, a relatively large proportion of children had toenail and urine Hg values that were below the limit of detection. To examine these relationships among those with detectable Hg values, we performed a sensitivity analysis removing all individuals with Hg values below the limit of detection. We observed that both toenail Hg and urine Hg remained positively associated with DBP and MAP, albeit with reduced statistical precision (Tables S4). In further analyses, we categorized Hg exposure variables as three levels to compare individuals with detectable Hg levels (either above or below the median of detectable values) to those below detection limit (Tables S5). When categorized in this manner, urine Hg remained positively associated with DBP and MAP, with stronger associations among individuals with levels of Hg that were above the median of detectable values (DBP $\beta$: 0.14, 95% CI: 0.03, 0.26; MAP $\beta$: 0.16, 95% CI: 0.02, 0.31) as compared to individuals with non-detectable values. Similar results were observed with categorical toenail Hg levels (Tables S5).

3.7. Effect modification by sex and birth weight

In exploratory analyses, we tested for effect modification by child sex and birth weight in stratified models, given previously published observations (Sorensen et al., 1999; Thurston...
et al., 2007). When we stratified our models by sex, we observed that the relationship between urine Hg and all measures of BP were stronger among boys, particularly for MAP (Boys $\beta$: 1.01, 95% CI: 0.16, 1.87, $p = 0.02$; Girls $\beta$: 0.36, 95% CI: 0.20, 0.92, $p = 0.21$) (Tables S6). Similar trends were observed with toenail Hg in sex-stratified analyses, but were not statistically significant (Tables S7). However, tests for interaction were not statistically significant.

In models stratified by median birth weight, we observed that the relationship between urine Hg and BP appeared to be stronger among children with below median birth weights, particularly for DBP (Below median $\beta$: 0.72, 95% CI: 0.16, 1.28, $p = 0.01$; Above median $\beta$: 0.23, 95% CI: 0.29, 0.74, $p = 0.44$), as compared to children with above median birth weights (Tables S8). In birth weight-stratified models where toenail Hg was the exposure variable, greater effect sizes were observed for Hg and SBP and MAP among children with below median birth weights, as compared to children with above median birth weights (Tables S9). Again, tests for interaction were not statistically significant with either exposure biomarker.

3.8. Role of fish and seafood consumption

Given that Hg exposure commonly results from fish and seafood consumption, we examined correlations between each of the child biomarkers of Hg exposure and frequency of fish and seafood consumption collected at the same time point as each of the exposure biomarkers (Tables S10). At age 3, we observed moderate but statistically significant correlations between toenail Hg and reported frequency of intake of non-fried fish and seafood ($\rho = 0.40$, $p \leq 0.0001$) and total grams of consumption ($\rho = 0.44$, $p \leq 0.0001$). At ages 5–6 correlations between urine Hg and both reported frequency of intake ($\rho = 0.16$, $p \leq 0.01$) and total grams of consumption ($\rho = 0.16$, $p \leq 0.01$) were more modest. We also observed statistically significant correlations between AsB and all fish and seafood consumption variables.

We also performed a sensitivity analysis to adjust for reported fish and seafood consumption patterns at age 3 and ages 5–6 (Tables S11). As not all participants provided dietary information, we first restricted to individuals who completed a food frequency questionnaire at age 3 ($n = 262$) or age 5–6 ($n = 196$). Inclusion of fish and seafood consumption did not appreciably alter observed associations between toenail Hg at age 3 years and DBP (with fish adjustment $\beta$: 0.57, 95% CI: −0.02, 1.15; without fish $\beta$: 0.60, 95% CI: 0.03, 1.17) or MAP (with fish adjustment $\beta$: 0.57, 95% CI: 0.14, 1.29; without fish $\beta$: 0.55, 95% CI: 0.14, 1.25). Similarly, inclusion of fish and seafood consumption at age 5–6 years did not qualitatively alter the observed associations between urine Hg and DBP and MAP (Tables S11). We also performed an analysis including arsenobetaine, a urinary biomarker of fish and seafood consumption, in lieu of reported consumption to avoid a reduction in sample size at age 5–6 (Tables S12). In these analyses, the inclusion of arsenobetaine did not appreciably alter the associations between urine Hg and DBP ($\beta$: 0.45, 95% CI: 0.07, 0.83) or MAP ($\beta$: 0.52, 95% CI: 0.07, 0.98), which remained statistically significant.
4. Discussion

In a prospective cohort of New Hampshire children, we observed an association between childhood exposure to Hg and elevated DBP and MAP at 5–6 years of age. These associations were consistently observed with Hg measured in toenail samples collected at 3 years of age, as well as in urine samples collected at 5–6 years of age. Similar positive trends were observed for SBP, but with limited statistical precision. We did not observe any relation between maternal prenatal exposures, as measured by toenail clippings representing both the perinatal/early prenatal and mid-gestation periods, and any measure of child BP. Models that simultaneously included both child biomarkers, urine Hg at age 5–6 and toenail Hg at age 3, suggested that urine Hg was more strongly associated with DBP and MAP that were taken contemporaneously. Inclusion of co-exposures to Pb and As, which have been previously associated with child BP, did not influence the observed associations between Hg and BP (Farzan et al., 2018; Hawkesworth et al., 2012; Sanders et al., 2018; Zhang et al., 2012). Our results suggest that childhood is a critical window for Hg exposures to influence child BP, which could translate to effects on long-term cardiovascular health as children grow older.

Elevated BP in childhood has been linked to cardiovascular risk factors later in adulthood (Lauer and Clarke, 1989; Berenson et al., 1998; Berenson et al., 1989; Li et al., 2003; Davis et al., 2001; Hartiala et al., 2012; Juonala et al., 2005; Raitakari et al., 2003; Knoflach et al., 2009; Chen and Wang, 2008; Theodore et al., 2015). Although early increases in BP may have negligible effects on a child’s immediate health, these early effects may become magnified over time. There is evidence that child BP is predictive of adult BP (Chen and Wang, 2008; Theodore et al., 2015) and a number of studies have observed associations between childhood BP and later life CVD risk factors, including atherosclerosis, hypertension and metabolic disease (Lauer and Clarke, 1989; Berenson et al., 1998; Berenson et al., 1989; Li et al., 2003; Davis et al., 2001; Hartiala et al., 2012; Juonala et al., 2005; Raitakari et al., 2003; Knoflach et al., 2009; Chen and Wang, 2008; Theodore et al., 2015). Nonetheless, BP changes observed in this study are relatively small and whether changes of such magnitude are of clinical significance remains to be determined. Further, there is relatively limited research on whether early life and/or childhood exposures influence BP in childhood and/or adulthood. During sensitive periods in utero and in early life, toxic exposures may have deleterious health effects that often remain undetected until later in life (Barker et al., 1993; Landrigan et al., 2005; Boekelheide et al., 2012). While a number of studies have examined the contribution of either prenatal or childhood Hg exposures to child BP, few studies have examined the relative contribution of Hg exposures reflecting multiple time periods in utero and in early childhood in relation to BP in children (Gump et al., 2005; Hawkesworth et al., 2012; Zhang et al., 2012). In our study, we observed that exposure biomarkers collected at age 3 years and concurrently with blood pressure assessment at age 5–6 years, were positively associated with elevated DBP and MAP, whereas prenatal exposure to Hg, in either early gestation or mid-gestation, were not related to child BP. While pregnancy is often thought to be one of the most sensitive windows for exposures and prenatal Hg exposure has been associated with deleterious effects on other
health outcomes, such as neurodevelopment, our results suggest that childhood may be a crucial period for Hg effect’s on cardiovascular health.

We also accounted for co-exposures to Pb and As, which have been observed to influence child BP in previous studies (Farzan et al., 2018; Gump et al., 2005; Hawkesworth et al., 2012; Sanders et al., 2018; Zhang et al., 2012). These other metals were not correlated with Hg, and models that accounted for possible co-exposure with either Pb or As did not affect the observed associations between Hg in child toenails or urine with DBP and MAP. Interestingly, inclusion of both biomarkers of child Hg exposure in the same models suggested that child urine Hg measured at the same time as the 5–6 year BP measurement may be a stronger predictor of DBP and MAP than child toenail Hg. These data, together with our finding of a weak correlation between urine Hg at age 5–6 and toenail Hg at age 3, suggest that inorganic Hg, which is the predominant Hg form in urine, may be associated with BP along with MeHg, the predominant Hg form in toenails (Emeny et al., 2019; Maserejian et al., 2008; Berglund et al., 2005). Alternatively, these results could also indicate that more recent exposures are more strongly related to child BP. This will need to be determined in future studies with multiple concomitant measures.

We examined sex-specific effects in our study, as previous work among adolescents living in the Seychelles found a stronger association between prenatal MeHg and DBP among boys as compared to girls (Thurston et al., 2007). In our study, we observed similar effects in sex-stratified analyses. Child urine Hg levels were more strongly associated with elevated MAP among boys than among girls, as well as with marginally significant elevations in DBP and SBP. However, these results must be interpreted with caution, as our estimates lacked statistical precision and these trends were not observed in analyses with child toenail Hg at age 3. Others also have observed sex-specific effects of prenatal Pb exposure on BP in children, which could suggest differential susceptibility by sex to the cardiovascular effects of metal exposures (Farzan et al., 2018; Zhang et al., 2012). Thus, further investigation of sex-specific effects are warranted.

We also performed analyses stratified by birth weight. Previous work from the Faroe Islands observed stronger associations between prenatal MeHg and SBP and DBP at age 7 among children with lower birth weights, in analyses stratified by median birth weight (Sorensen et al., 1999). With limited statistical power, we also observed that higher child urinary Hg measured at age 5–6 was associated with greater DBP and MAP among children who weighed less than the median at birth as compared to children whose birth weight was at or above the median. Similar trends were observed in birth-weight stratified analyses with toenail Hg at age 3, although with stronger associations for SBP and MAP, but not DBP. These findings suggest that children who weighed less than their peers at birth may be more susceptible to the cardiovascular effects of Hg exposure during childhood, although larger studies are needed. Indeed, birth weight, which was not independently related to BP in our study (data not shown), is related to later life metabolic and cardiovascular disease, but appears to have a complex relationship with BP that is being explored in children (Edvardsson et al., 2012).
We observed consistent associations between both toenail Hg at age 3 and urine Hg at 5–6 years with both DBP and MAP. Our effect estimates for SBP were also suggestive of Hg-related increases in SBP measures but did not achieve statistical significance. Though the exact mode(s) of action underlying Hg’s potential cardiovascular effects has not been well characterized, it is possible that Hg exposure could preferentially disrupt mechanisms that regulate peripheral vascular resistance and ventricular relaxation during diastole, which in turn would lead to greater impacts on DBP and MAP than SBP, but to date the role of Hg in these processes is unknown. In general, mechanistic studies have pointed to oxidative stress and the generation of reactive oxygen species, leading to endothelial dysfunction, as a key pathway for Hg toxicity, while others have indicated that inorganic Hg can also accumulate in the kidney and impair renal tubular regulation, which may also increase BP (Farina et al., 2011; Farina et al., 2011; Genchi et al., 2017; de Marco et al., 2010; Grotto et al., 2009; Clarkson and Magos, 2006). Further work is needed to understand the potential mechanisms underlying Hg’s impact on BP particularly early in childhood, as well as the clinical relevance of such early BP changes on long-term health.

There are likely a number of sources of Hg exposure in this population (Risher and De Rosa, 2007; Counter and Buchanan, 2004). Diet, particularly consumption of fish and seafood, is likely a primary source of exposure to MeHg, a highly absorbable and toxic organic form of Hg. As expected, fish and seafood consumption were correlated with Hg levels in both child toenails and urine samples. Fish and seafood also contain other contaminants and potentially beneficial nutrients that could have confounded our results. However, accounting for fish and seafood consumption in our population did not appreciably alter our results in analyses restricted to those with dietary information. Similarly, when we adjusted for AsB as a urinary biomarker of fish and seafood consumption, it also did not influence our results. Exposures to inorganic Hg are also common (Risher and De Rosa, 2007). Around the home, elemental and inorganic Hg are found in household goods, such as compact fluorescent light bulbs and some older thermometers, as well personal care products, particularly skin lightening creams, and in some herbal remedies and ritualistic products (Risher and De Rosa, 2007). Exposure to elemental Hg also can occur through dental amalgams, also known as silver fillings, which are made up of approximately 40–50% Hg and are a likely source of exposure among US children (Maserejian et al., 2008; Geier et al., 2012; Woods et al., 2007; Dunn et al., 2008). While we did not have sufficient information on dental amalgams in the children included in our analysis, in more recently collected data in a subset of 32 children who reported having a cavity, half of those who had the cavity filled reported receiving a silver filling. Thus, it is possible that dental amalgams represent a potential source of Hg exposure for children in our study population. However, as exposure measures were currently unavailable for these children, we were unable to determine whether silver fillings influenced levels of Hg biomarkers.

In this study, we examined two different matrices, urine and toenails, as biomarkers of child Hg exposure and observed positive associations for both of these biomarkers primarily with DBP and MAP. Increased toenail concentrations have been associated with sources of both elemental and MeHg exposure, with approximately 10% representing inorganic Hg exposure and 90% of toenail Hg from MeHg exposure related to fish and seafood consumption (Rees et al., 2007). MeHg is incorporated into toenails and toenail clippings are thought to
represent exposures occurring approximately 6–12 months prior, however, as this depends on rate of toenail growth, the window of exposure for children may be more proximal to the sample collection date, as compared to adults (Karagas et al., 2000; Slotnick and Nriagu, 2006). Further, while toenails generally reflect levels of MeHg, urine Hg primarily contains inorganic Hg (Flynn et al., 2017; Maserejian et al., 2008; Berglund et al., 2005). Urinary Hg reflects levels of Hg in the kidney and total body burden of inorganic Hg at steady state, with peak levels of urine Hg occurring 1–3 weeks after exposure (Barregard, 1993; Barregård et al., 1996; Barregard et al., 1992). However, evidence from experimental models and human biomarker studies suggests that some MeHg may be demethylated to inorganic Hg, thus contributing to urine Hg levels, and that Hg demethylation may be modulated in part by intestinal microbiota (Berglund et al., 2005; Guo et al., 2018; Li et al., 2019; Takanezawa et al., 2019; Uchikawa et al., 2016). Given the statistically significant correlations we observed between fish intake and both urine and toenail Hg levels, we suspect that urine Hg levels may reflect some fraction of demethylated MeHg from fish and seafood consumption, which has been observed in a previous analysis of predictors of children’s urinary Hg levels (Levy et al., 2004). Further, although MeHg is thought to be more toxic and has been more commonly investigated in relation to health outcomes, inorganic Hg exposures may also be important. In a recent study from the New Hampshire Birth Cohort, investigators found that both maternal toenail Hg, a biomarker of MeHg, and the presence of maternal dental amalgams, a measure of elemental Hg exposure, were associated with children’s risk of respiratory infections, allergy and atopy in the first year of life (Emeny et al., 2019). Their findings suggest that both MeHg and elemental Hg were associated with the immune-related outcomes of interest. Therefore, while the two biomarkers used in this study may reflect different forms of Hg, our results suggest that both inorganic Hg and MeHg may contribute to child BP changes.

Of relevance to this work is a recent systematic review and meta-analysis that summarized the association between Hg and BP in adults across populations, as well as across biomarkers and levels of exposure (Hu et al., 2018). The authors found an overall positive association between mercury and blood pressure in adults, despite substantial heterogeneity among studies. However, they only identified positive relationships between inorganic and total Hg with BP in subgroup analyses and no association with MeHg. Further, dose was observed to be an important determinant of effect on BP, with effects primarily observed at higher levels of exposure. In comparison to our work, the levels of exposure in our study population of children would be considered quite low, as compared to levels of exposure observed to influence BP in adults in this meta-analysis. Moreover, it is important to consider that children may be more sensitive to the effects of Hg at lower levels of exposure, which may in part explain the differences observed between our work and this meta-analysis, and these potential differences in susceptibility among children versus adults should be further explored.

Our study has a number of strengths. We assessed Hg exposure using several different biomarkers, including maternal toenail samples from two time points representing the periconceptional/early prenatal and mid-prenatal periods, as well as child toenails at age 3 and child urine at age 5–6, each representing different developmental windows of susceptibility. Our study utilized carefully collected child BP data and detailed information on potential
covariates, including fish and seafood consumption at two time points in childhood. We also explored the potential contribution of other metals exposures at each childhood time point. The limitations of our study must also be noted. Our analyses were based on two different exposure matrices, toenails and urine, obtained from mothers and children at multiple time points, which each have different integration periods and reflect different proportions of inorganic Hg versus MeHg. Urine was only obtained at the 5–6 year in-person child visit, while only toenails were collected at the 3-year time point, therefore we cannot compare the effects of Hg exposure in these two matrices at the same point in time. However, we observed relatively consistent associations between Hg exposure and DBP and MAP across both time points, which supports the reliability of our results. We also cannot rule out the possibility of unmeasured confounding in our analyses. Our rural northern New England study population is predominately of European ancestry, which may limit the generalizability of our results to other populations. Further exploration of these associations among more geographically and racially diverse populations of children, and with a greater range of exposures, is warranted. Lastly, there were a number of children with levels of Hg below the detection limit for the biomarkers measured. Because this population had relatively low levels of Hg, over half of the samples were below the level of detection and imputation of these samples may have affected the results of our analyses. However, similar results were obtained when using only detectable levels in our analyses and also when evaluating quantiles of Hg. Further exploration of the associations observed in our study is warranted, particularly among populations of children with greater variation in levels of exposure, as well as longitudinal follow up of children to determine whether Hg-related changes in BP may persist over time.

In conclusion, we observed positive associations between biomarkers of Hg exposure measured at two time points in childhood, and measures of BP in children ages 5–6. Our results suggest that early childhood, rather than the prenatal period, may be a critical window of exposure for Hg to influence BP. Our results also suggest this relationship may be modified by sex and birth weight, but further investigation of these potential effect modifiers are needed. Although the long-term effects of Hg exposure-related BP changes in childhood are unclear, it is possible that small changes in childhood may become amplified in adulthood and limiting childhood exposures may be an important strategy for improving long-term cardiovascular health.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

5. Funding sources and ethical considerations

Funding for this study was provided by a NIEHS K99/R00 Pathway to Independence Grant (PI: Farzan, R00ES024144). Additional funding for this study was provided by the NIEHS Superfund Research Program (P42ES007373), the Children’s Center for Environmental Health and Disease Prevention Research (NIEHS and USEPA grants ES022832 and RD83459901), and the NYU Environmental Health Sciences Core Center. Dr. Howe is supported by an NIEHS K99/R00 Pathway to Independence Award (K99ES030400). The funding agencies that supported this work had no role in the planning, design, or execution of this study, nor any role in data analysis or manuscript preparation.

Environ Int. Author manuscript; available in PMC 2021 January 02.
The authors have no competing personal or financial interests. All participants provided written, informed consent upon enrollment. All protocols were approved by the Dartmouth College Institutional Review Board (Dartmouth Committee for the Protection of Human Subjects Approval Reference #20844).

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**Table 1**

Selected demographic characteristics for 395 NHBCS children, with 5–6 year blood pressure assessment and childhood Hg exposure assessment (toenails at 3 year visit and/or urine at 5–6 year visit).

| Child Variables          | N (%) or Mean (SD) |
|--------------------------|--------------------|
| Age at time of assessment, years | 5.5 (0.4)          |
| Child Sex                |                    |
| Boys                     | 192 (48.6)         |
| Girls                    | 203 (51.4)         |
| Birth Weight, grams *    | 3440.7 (550.3)     |
| Gestational age, weeks   | 38.9 (1.7)         |
| Height, cm *             | 112.6 (5.0)        |
| Weight, kg *             | 20.8 (3.6)         |
| Systolic Blood Pressure, mmHg | 98.0 (7.2)        |
| Diastolic Blood Pressure, mmHg | 56.9 (4.1)       |

**Maternal Variables**

| Maternal age at enrollment, years | 30.9 (4.6) |
| Educational attainment *          |            |
| Less than college                 | 117 (31.0) |
| College graduate                  | 148 (39.1) |
| Any post-graduate schooling       | 113 (29.9) |
| Pre-pregnancy BMI, kg/m² *        | 26.3 (5.9)  |
| Smoking status during pregnancy * |            |
| Ever smoked                       | 24 (6.4)   |
| Never smoked                      | 354 (93.6) |

**Exposure Variables**

| Child 3-year toenail Hg, µg/g * | 0.055 (0.087) |
| Range                            | 0.0001–0.9232 |
| Child 5–6 year urine Hg, µg/L *  | 0.071 (0.119) |
| Range                            | 0.0003–0.986  |
| Maternal gestational week 24–28 toenail Hg, µg/g * | 0.129 (0.139) |
| Range                            | 0.0013–0.9742 |
| Maternal postnatal week 6 toenail Hg, µg/g * | 0.128 (0.157) |
| Range                            | 0.0001–1.444  |

* N = 378 for maternal smoking and education, N = 377 for gestational age, N = 388 for birth weight, N = 389 for maternal pre-pregnancy BMI, N = 393 for child weight, N = 392 for child height, N = 290 for those with 3-year visit toenail Hg, N = 363 for those with 5–6 year visit urine Hg, N = 301 with maternal gestational week 24–28 toenail Hg, N = 322 maternal postnatal week 6 toenail Hg.
Table 2

Linear regression models\(^a\) associating biomarkers of maternal and child Hg exposure (Hg levels in maternal prenatal and postpartum toenails, child toenails at age 3 years and child urine at age 5–6 years) with child blood pressure at age 5–6 years.

| Exposure                          | N   | SBP, mmHg β (95% CI) | DBP, mmHg β (95% CI) | MAP, mmHg β (95% CI) |
|----------------------------------|-----|----------------------|----------------------|----------------------|
| Prenatal toenail Hg, GW 24\(^b\) | 281 | −0.20 (−0.83, 0.42)  | 0.03 (−0.34, 0.40)  | −0.03 (−0.47, 0.41)  |
| Postpartum toenail Hg, 6W\(^b\)  | 305 | −0.21 (−0.72, 0.31)  | −0.07 (−0.36, 0.23)  | −0.09 (−0.45, 0.27)  |
| Child 3-year toenail Hg\(^b\)    | 274 | 0.81 (−0.16, 1.78)   | 0.53 (−0.02, 1.07)   | 0.67 (0.00, 1.33) *  |
| Child 5–6 year urine Hg\(^c\)   | 317 | 0.51 (−0.13, 1.16)   | 0.48 (0.10, 0.86) ** | 0.55 (0.10, 1.01) *  |

\(^a\)Adjusted for child age, height and weight at time of BP measurement; child sex, birth weight, gestational age; maternal education and smoking during pregnancy; urine specific gravity (for urine biomarkers only).

\(^b\)Estimates scaled to a 0.1 µg/g change in toenail Hg.

\(^c\)Estimates scaled to a 0.1 µg/L change in urine Hg.

* P < 0.05.

** P ≤ 0.01.
Table 3

Linear regression models \(^a\) associating biomarkers of child Hg exposure at two time points (Hg levels in toenails at age 3 years and urine at age 5–6 years) with blood pressure at age 5–6 years (N = 233).

|               | β Toenail Hg (95% CI) \(^b\) | β Urine Hg (95% CI) \(^c\) |
|---------------|-------------------------------|----------------------------|
| SBP, mmHg     | 0.51 (−0.56, 1.58)            | 0.35 (−0.46, 1.15)         |
| DBP, mmHg     | 0.33 (−0.26, 0.92)            | 0.56 (0.11, 1.00) \(^**\) |
| MAP, mmHg     | 0.42 (−0.31, 1.15)            | 0.56 (0.01, 1.11) \(^*\)  |

\(^{a}\) Models include both toenail Hg and urine Hg measures, adjusted for child age, height and weight at time of BP measurement; child sex, birth weight, gestational age; maternal education and smoking during pregnancy; and urine specific gravity.

\(^{b}\) Estimates scaled to a 0.1 µg/g change in toenail Hg.

\(^{c}\) Estimates scaled to a 0.1 µg/L change in urine Hg.

\(^*\) \(P < 0.05\).

\(^{**}\) \(P \leq 0.01\).