Assessing the Individual and Interactive Associations of Pre- and Postnatal Air Pollution Exposures and Maternal Nutrition During Pregnancy with Child Blood Pressure: A Prospective Study in a Community-based Birth Cohort

Yu Ni

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Reading Committee:
Catherine J. Karr, Chair
Adam Szpiro
Mario Kratz
Annette L. Fitzpatrick

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University of Washington

Abstract

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Yu Ni

Chair of the Supervisory Committee:
Catherine Karr, Professor
Department of Environmental and Occupational Health Sciences
Department of Epidemiology
Department of Pediatrics

Background:
Limited data suggest that the intrauterine environment and early life experience may contribute to increased child blood pressure, with effects possibly extending into adulthood. The individual and interactive effects of air pollution exposures and maternal nutrition during pregnancy upon child blood pressure are unclear.

Methods:
In this dissertation, we conducted two separate prospective studies with three aims to investigate these associations in the CANDLE study, a socioeconomically diverse birth cohort in the Memphis, Tennessee. Mother-child dyads were included if they had available residential address histories, pregnancy nutrition data (either the Healthy Eating Index 2010 [HEI], determined by a second trimester Food Frequency Questionnaires, or plasma folate measurement in mid- to late pregnancy), and a valid child blood pressure measurement at age 4-6 years. We calculated the systolic (SBP) and diastolic blood pressure (DBP) percentile incorporating sex, age and height, and categorized children as high blood pressure (HBP) ($\geq 90^{th}$ percentile) or normal.

In the first study, we assessed the associations between air pollution exposures and child blood pressure (Aim 1) and determined whether the associations would be modified by maternal nutrition (Aim 3). Effect modifications by child sex and maternal race were also examined. NO_2 and $PM_{2.5}$ estimates in both pre- and postnatal windows were obtained from annual national models and spatio-temporal models respectively. We fit multivariate Poisson and linear regressions to estimate the exposure-outcome associations, and quantified multiplicative joint effects with maternal nutrition, child sex and maternal race using interaction terms.

In the second study, we tested the hypothesis that better pregnancy nutrition status characterized by higher HEI and plasma folate would be related to lower child blood pressure percentiles and reduced risk of HBP (Aim 2). Linear, Poisson and Generalized Additive Models

were used with adjustments for socio-demographics, anthropometric measurements, behavioral factors, maternal stress and child nutrition. Interactions of maternal nutritional measurements with child sex, maternal race, pre-pregnancy maternal overweight or obesity, maternal smoking and breastfeeding for the associations of interest were explored.

Results:
In the first study with 822 mother-child dyads, mean $PM_{2.5}$ and NO_2 in the prenatal period were 10.8 μg/m^3 and 10.0 ppb, respectively, and 9.9 μg/m^3 and 8.8 ppb from birth to age 4 birthday. 29.2% of the children were classified as HBP, largely driven by isolated diastolic HBP. There was an estimated 13.6 percentile increase in SBP (Beta: 13.6, 95%CI: 3.7, 23.5) and an 8.0 percentile increase in DBP (Beta: 8.0, 95%CI: 0.7, 15.3) for each 2 μg/m^3 increase of $PM_{2.5}$ in the 2nd trimester. $PM_{2.5}$ averaged over the full prenatal period was only associated with higher DBP percentiles (Beta: 11.6, 95%CI: 3.0, 20.2). The adverse effects were more pronounced in children whose mother had low-quality diet or possible folate deficiency during pregnancy, female children and children with a mother self-identified as Black. We detected no association of NO_2, road proximity and postnatal $PM_{2.5}$ with any outcomes.

In the second study with 846 mother-child dyads, mean HEI and folate were 60.0 (SD: 11.32) and 23.1 (SD: 11.1) nmol/L respectively. 29.6% of the children were defined as HBP based on measurements at one visit. Maternal HEI and plasma folate were not associated with child BP percentiles and HBP in the full cohort. The conclusion remained the same after adjustments for potential mediators and in sensitivity analyses. Among mothers self-identified as White, there was an inverse relationship between maternal HEI and child SBP percentile (Beta: -0.45, 95%CI: -0.80, -0.09). Maternal HEI 59 and above was associated with reduced HBP in girls (IRR: 0.57, 95% CI: 0.34, 0.96). A moderate non-linear relationship was suggested for maternal plasma folate and child SBP percentile in women with pre-pregnancy overweight or obesity. It was also indicated that child nutrition may confound the non-linear maternal folate-child SBP associations. We found no evidence of modified associations by maternal smoking and breastfeeding.

Conclusion:
The findings from this dissertation add to the limited evidence that higher prenatal $PM_{2.5}$ exposure, particularly in the 2nd trimester, is associated with elevated early childhood blood pressure. The results also suggest that healthy maternal nutrition may potentially ameliorate the adverse cardiovascular effects of air pollutants. However, no independent association between maternal nutrition during pregnancy and childhood blood pressure was detected. This dissertation contributes to the evolving scientific inquiry regarding developmental origins of disease. The evidence generated from this dissertation can be used to inform regulatory policy on acceptable air pollution levels, and to raise interest for further studies to understand the role of pregnancy dietary interventions in child health.

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INTRODUCTION

Background and Rationale

High blood pressure (HBP) is a major risk factor for heart disease and stroke, two leading causes of death in adults.[1] HBP in early life may persist over time and progress to clinical hypertension in adulthood.[2,3] Large cohorts and national representative data have shown the prevalence of HBP in U.S. children and adolescents to be 15-20% based on a single blood pressure measurement, with the highest rates in Hispanic and African American youth.[4–6] Untreated pediatric hypertension is also directly associated with target organ damage, such as left ventricular hypertrophy and decreased arterial compliance.[7] Child HBP is multifactorial. Primary HBP has been chiefly attributed to heredity, obesity and diet, while major determinants of secondary HBP include renal abnormalities, medications and neoplasm.[8] However, currently recognized risk factors do not sufficiently explain disease occurrence. A growing body of literature suggests that environmental risk factors may play a role, and it is also increasingly recognized that in-utero exposure to poor maternal nutrition may predispose to future cardiometabolic abnormalities.[9–14]

Air pollution exposures have been linked with several chronic health conditions in children, such as asthma and neurodevelopmental disorders, even in regions where the level of exposure is in compliance with regulations.[15–17] Lab science literatures suggest that prenatal air pollution exposures may induce placental and fetal systematic inflammation, oxidative stress or endocrine disruption, change placental vascularization, and subsequently restrict fetal programming. [18,19] There is also mounting evidence from animal studies and epidemiologic studies of adult cohorts implicating air pollution as a driver of cardiovascular outcomes, with a particular focus on ambient particulate matter less than 2.5 micrometers in diameter ($PM_{2.5}$) and nitrogen dioxide (NO_2). [20,21] However, population studies of prenatal or early life air pollution exposure and child cardiovascular health are sparse.

The evidence for developmental origins of HBP from nutrition studies are circumstantial: they represent a diversity of study designs and varying features of early life nutrition. Earlier population cohorts have focused on maternal famine or malnutrition during pregnancy, and reported long lasting effects on offspring cardiovascular impairments even in mid and old age. [22–26] Due to the obesity epidemic, overnutrition and suboptimal nutrition during pregnancy have been of greater concern in the past decades. Observational studies examining the influence of single foods or nutrients have reported inconsistent associations with child blood pressure.[27–33] Clinical trials and their follow-up studies, primarily of supplement intakes, have uniformly yielded null results except for fish oil supplementation.[34–41] There is a growing interest in the utilization of dietary pattern analysis and micronutrient biomarkers to assess the effects of maternal nutrition on several offspring outcomes, but little is known about child blood pressure.

Both living environment and nutritional status are strongly related to socioeconomic status (SES). Several studies in North America have shown that lower SES communities face a greater burden of environmental toxicants such as air pollution and lead exposures.[42,43] Lower residential

property values, education and income have also been associated with poorer diet quality measured by Healthy Eating Index (HEI).[44] Suboptimal maternal food patterns are more commonly observed among African American and Hispanic populations, and among pregnant women with relatively low health literacy.[45] Although daily folic acid supplementation is universally advised for women who are planning pregnancy or currently pregnant in the U.S., around one-quarter of this population do not follow the recommendation.[46] A recent study in the urban northeastern U.S. reported a disparity in sufficient dietary folate intake between African American pregnant women and their counterparts in the other racial groups.[47] These findings suggest that populations disproportionately exposed to ambient air pollution may also be more likely to have poorer nutrition status. Moreover, there are shared biological mechanisms linking air pollution exposures and poor maternal nutrition with child cardiovascular health: both of them may relate to child metabolic and developmental disorders through intra-uterine growth restriction (Figure 1). [9,10,48] Epigenetic studies have indicated that adequate maternal folate levels may protect against elevated blood pressure by increasing nitric oxide synthesis in endothelial cells, and subsequently counteracting oxidative stress caused by prenatal air pollution exposure and promoting resilience of vessels.[49,50] We are aware of no previous publication exploring the potential interactive effect between air pollution exposures and maternal nutrition status during pregnancy on offspring blood pressure.

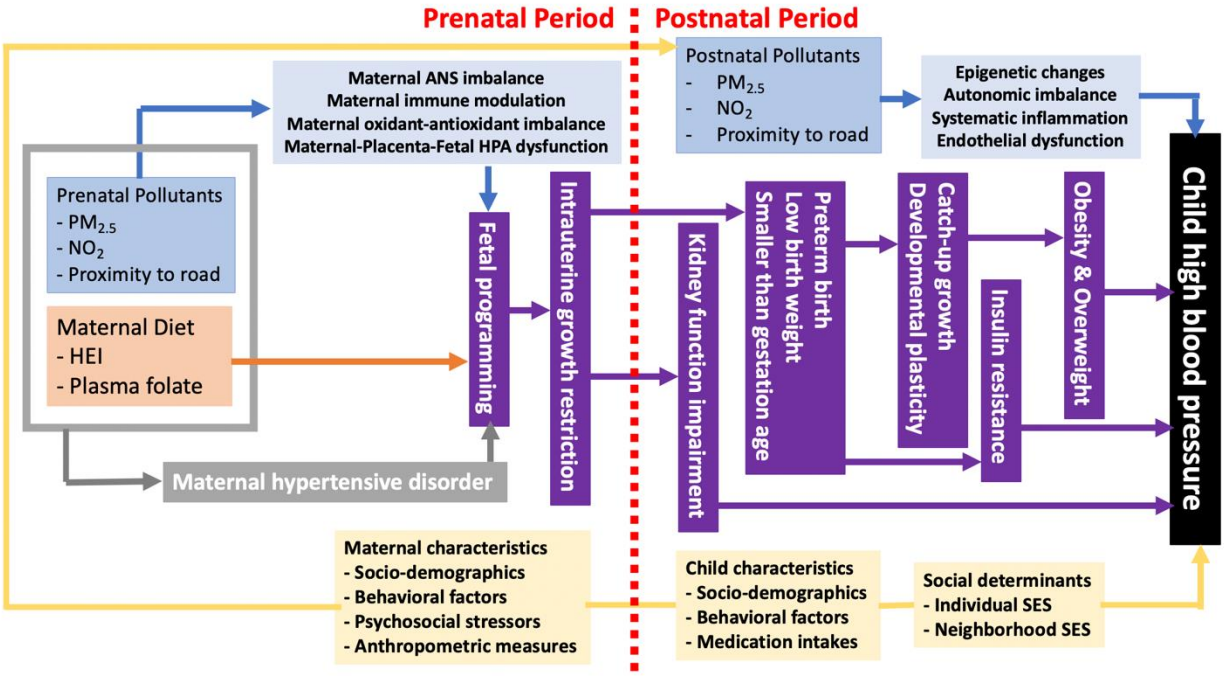

Figure 1. Conceptual model of the associations of air pollution exposures and maternal nutrition with child blood pressure

Objectives and Specific Aims

The purpose of this dissertation is to investigate the individual and interactive associations of pre- and postnatal air pollution exposures, including $PM_{2.5}$, NO_2 and proximity to main roadway, and maternal nutrition status during pregnancy characterized by the Healthy Eating Index 2010 (HEI) and plasma folate with child blood pressure at age 4-6 years visit. It seeks to build on the following three emerging hypotheses: First, both pre- and postnatal air pollution exposures may increase child systolic and diastolic blood pressure percentiles and risks of HBP at age 4-6 years. Second, it was hypothesized that children would have lower blood pressure percentiles and reduced risks of high blood pressure if their mothers had better adherence to the 2010 Dietary Guidelines for Americans, and/or had higher levels of plasma folate during pregnancy. Finally, we hypothesized that optimal maternal nutrition during pregnancy may ameliorate the adverse effects of air pollution on child cardiovascular health.

To conduct this research, we used data from the Conditions Affecting Neurocognitive Development and Learning in Early Childhood (CANDLE) Study, a large socio-demographically diverse birth cohort in the urban South of the U.S. The CANDLE study was originally established to identify risk factors that impact child neurodevelopment and learning, and became part of the ECHO PATHWAYS Consortium in 2016. The enrolled CANDLE population was representative of the source population in Shelby County, Tennessee with regard to sociodemographic characteristics. From the prenatal period to child age 3, families completed eight in-person visits (two prenatal clinic visits, one hospital visit at delivery, three postnatal clinic visits and two postnatal home visits) and nine phone-based assessments. At the ages 4-6 clinic visit, 1,143 mother-child dyads completed staff administered questionnaires and anthropometric measures, achieving a retention rate of 76% (Figure 2).

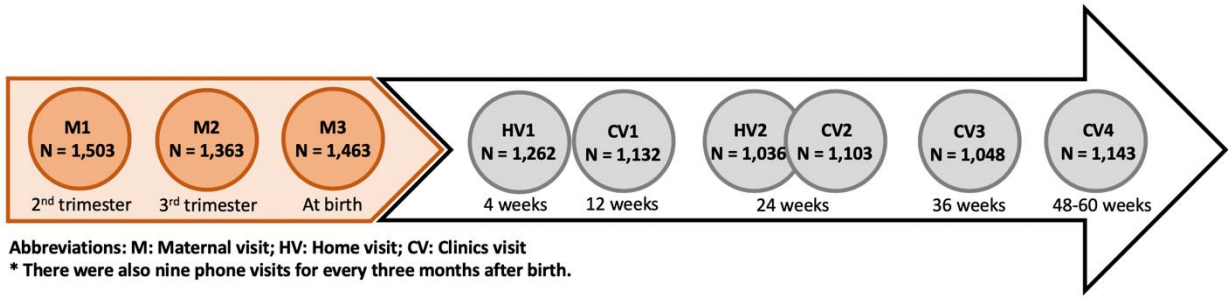

Figure 2. Timelines of study visits in CANDLE

Two distinct projects were implemented to answer the aforementioned research questions in the context of a single large birth cohort. We presented the study process and analytic results for both Aim 1 and Aim 3 in Chapter 1. We characterized the associations of air pollution exposures in different pre- and postnatal windows with child blood pressure, and determined whether these associations would be modified by maternal nutrition during pregnancy. In Chapter 2, we described the relationships of maternal HEI and plasma folate during pregnancy and child blood pressure, in both linear and non-linear forms, estimated the potential confounding by child nutrition, and explored the potential effect modifiers of child sex, maternal race, pre-pregnancy overweight or obesity, maternal smoking and breastfeeding practice (Aim 2). The conclusions of

these studies, their major strengths and limitations, the public health implications and potential future research directions were summarized in Chapter 3. Please refer to each chapter for more details. This work contributes to the evolving science regarding the developmental origins of disease. The ultimate objectives of this research are to provide evidence to inform regulatory policy on acceptable air pollution levels, support dietary interventions in pregnancy, and raise interest in the development of pregnancy-specific dietary guidelines.

CHAPTER 1: Associations of Pre- and Postnatal Air Pollution Exposures and Child Blood Pressure and Potential Modification by Maternal Nutrition: Results from the Conditions Affecting Neurocognitive Development and Learning in Early Childhood (CANDLE) Study

Authors:
Yu Ni[1], Adam Szpiro[2], Michael Young[3], Christine Loftus[3], Nicole R. Bush[4,5], Kaja LeWinn[4], Sheela Sathyanarayana[3,6,7], Daniel A. Enquobahrie[1,8], Robert Davis[9,10], Mario Kratz[1,11], Annette L. Fitzpatrick[1,12,13], Jennifer Sonney[14], Frances Tylavsky[15], Catherine J. Karr[1,3,6]

Institutions:
1. Department of Epidemiology, School of Public Health, University of Washington, Seattle, WA, the U.S.
2. Department of Biostatistics, School of Public Health, University of Washington, Seattle, WA, the U.S.
3. Department of Environmental and Occupational Health Sciences, School of Public Health, University of Washington, Seattle, WA, the U.S.
4. Department of Psychiatry, School of Medicine, University of California, San Francisco, San Francisco, CA, the U.S.
5. Department of Pediatrics, School of Medicine, University of California, San Francisco, San Francisco, CA, the U.S.
6. Department of Pediatrics, School of Medicine, University of Washington, Seattle, WA, the U.S.
7. Seattle Children's Research Institute, Seattle, WA, the U.S.
8. Department of Health Services, School of Public Health, University of Washington, Seattle, WA, the U.S.
9. Center for Biomedical Informatics, University of Tennessee Health Science Center, Memphis, TN, the U.S.
10. Department of Pediatrics, University of Tennessee Health Science Center, Memphis, TN, the U.S.
11. Public Health Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, WA, the U.S.
12. Department of Family Medicine, School of Medicine, University of Washington, Seattle, WA, the U.S.
13. Department of Global Health, School of Public Health, University of Washington, Seattle, WA, the U.S.
14. Department of Child, Family, and Population Health Nursing, School of Nursing, University of Washington, Seattle, WA, the U.S.
15. Department of Preventive Medicine, University of Tennessee Health Science Center, Memphis, TN, the U.S.

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The data from the Conditions Affecting Neurocognitive Development and Learning in Early Childhood (CANDLE) Study can be requested from the study website (https://candlestudy.uthsc.edu/research/guidelines-collaboration).

Introduction

High blood pressure (HBP) is a major contributor to cardiovascular disease (CVD) in adults.[51] In U.S. children and adolescents, the HBP prevalence has been shown to be 15-20% based on a single BP measurement[4,6], with the highest rates in Hispanic and African American youth[5]. Early identification of children with HBP is crucial, as untreated hypertension is associated with target organ damage in childhood, such as left ventricular hypertrophy and decreased arterial compliance.[7] In addition, HBP in early life may persist over time and progress to clinical hypertension in adulthood.[2] Child HBP is multifactorial. Primary HBP has been chiefly attributed to heredity, obesity and diet, while major determinants of secondary HBP include renal abnormalities, medications and neoplasm.[8] However, currently recognized risk factors do not sufficiently explain disease occurrence, and a growing body of literature suggests that environmental risk factors may play a role.[11–13]

Air pollution exposures have been linked with several chronic health conditions in children, such as asthma and neurodevelopmental disorders, even in regions where the level of exposure is in compliance with regulations.[15–17] There is mounting evidence from animal studies and epidemiologic studies of adult cohorts implicating air pollution as a driver of cardiovascular outcomes, with a particular focus on ambient particulate matter less than 2.5 micrometers in diameter ($PM_{2.5}$) and nitrogen dioxide (NO_2).[20,21] However, studies of prenatal or early life air pollution exposure and child cardiovascular health are sparse. To the best of our knowledge, only one population study in Boston[52] has estimated the associations of child blood pressure with air pollution exposures in both pre- and postnatal windows, while three other studies – two in the U.S. and one in Europe[53–55] – have assessed the associations with prenatal exposures only. All of them provided evidence of positive relationships. More studies, including many conducted in China and Europe, investigated adverse cardiovascular effects of postnatal air pollution exposures, but the conclusions varied by population characteristics, study design, pollutant types and exposure windows.[56–69]

This study seeks to build on the emerging hypothesis that both pre- and postnatal air pollution exposures may increase child blood pressure. Using data from the Conditions Affecting Neurocognitive Development and Learning in Early Childhood (CANDLE) Study, a large socio-demographically diverse birth cohort in the urban South, we investigated associations of child blood pressure with air pollution exposures in different windows, and assessed whether the associations would be modified by child sex and maternal race. Furthermore, we examined pregnancy nutritional factors as potential modifiers, a topic that has not been previously explored, despite their known critical roles in fetal programming.

Methods

Study Population

The CANDLE study is an ongoing longitudinal study established to identify risk factors that impact child neurodevelopment and learning. From 2006 to 2011, 1,503 pregnant women were recruited in their 2nd trimester from prenatal care clinics and the community at large via media campaigns. Details of the sampling, recruitment and data collection have been described

elsewhere.[70] In brief, women were considered eligible if they were Shelby County, Tennessee residents between 16-40 years of age, had singleton pregnancies without complications, and planned to deliver at a participating study hospital. All women provided informed consent upon enrollment. The enrolled CANDLE population was representative of the source population in terms of sociodemographic characteristics. For this analysis, conducted as part of the ECHO PATHWAYS Consortium, we included 822 mother-child dyads who completed the age 4-6 years visit with blood pressure assessment and had valid residential address history. All CANDLE research activities were approved by the Institutional Review Board of the University of Tennessee Health Sciences Center, and the analyses were approved by the University of Washington Human Subjects Division.

Blood pressure assessment

At the age 4-6 years visit, child systolic (SBP) and diastolic blood pressure (DBP) were measured by clinical researchers using BP Tru Medical Devices, Model BPM-100 according to a standardized protocol, which included assessment of the participant's arm circumference to estimate correct cuff size (https://www.cdc.gov/nchs/data/nhanes/nhanes_07_08/manual_pe.pdf). Following at least two minutes of rest, child BP was measured twice in the right arm at heart level. Up to four measurements were taken if there was a discrepancy greater than 5 mmHg. Final BP values were calculated by averaging the measurements within a 5-mmHg difference. Using the American Academy of Pediatrics 2017 Clinical Practice Guideline[71], we calculated sex-, age- and height specific BP percentile based on the U.S. pediatric population with normal weight. HBP was defined as systolic and/or diastolic BP at 90th percentile and above.

Air pollution assessments

Residential addresses were collected at enrollment and updated at each subsequent visit. NO_2 exposures were estimated using regionalized annual average national models – land use regressions with universal Kriging components.[72] Briefly, the models utilized monitoring data from regulatory networks, further enhanced with satellite data. We used a geographic information system to identify covariates representing land-use characteristics that could reflect spatial variability in air pollution distributions. Final dimension-reduced regression covariates were obtained using Partial Least Squares (PLS) from more than 400 geographic variables. We calculated average NO_2 exposures corresponding to both pre- and postnatal windows (from birth to age 4 birthday) by applying the varied-year models from 2006 to 2014 to participants' residential addresses.

$PM_{2.5}$ exposures were obtained from spatio-temporal models predicting point-based estimates on a two-week time scale.[73,74] The models utilized monitoring data from regulatory networks supplemented with $PM_{2.5}$ measurements from intensive research cohort-specific monitors. The model decomposed the space-time field of concentrations into spatially varying long-term averages, spatially varying seasonal and long-term trends, and spatially correlated but temporally independent residuals. Time trends were estimated from observed time series, and spatial smoothing spline methods were used to borrow strength between observations. We estimated $PM_{2.5}$ by averaging biweekly predictions over the following windows: 1st, 2nd and 3rd trimester, full prenatal period, and postnatal period from birth to age 4 birthday. For families who moved

during an exposure window, we calculated the time-weighted averages of NO_2 and $PM_{2.5}$ across all addresses. Finally, we estimated residential distance to the nearest roadways as a proxy of exposure to traffic-related air pollutants. Specifically, major roads were classified as A1, A2 or A3 according to the census feature class, and "near road" was defined as a distance of <150 m from the nearest major roadway. If a family moved during one of the pre- or postnatal time windows, road proximity was assigned based on the single address with the longest residential history in the window.

Covariates

CANDLE conducted extensive data collection on mother, child and family characteristics, including multi-level social determinants of health. Maternal characteristics included age at delivery, race, education, insurance status, income adjusted by household size[75], pregnancy smoking, breastfeeding, Body Mass Index (BMI) class before pregnancy, maternal psychopathology measured by the Global Severity Index (GSI) [76], maternal hypertensive disorder and gestational age. Child characteristics included sex, age, height and BMI at visit, gestational age at birth, birthweight, physical activity level, sleep quality and use of medication that potentially increased blood pressure. Neighborhood conditions were estimated using the Childhood Opportunity Index (COI), a spatial measurement of relative childhood neighborhood opportunity.[77] We used two of the three "opportunity domains" comprising the COI: educational and economic opportunity. Other potential confounders were also incorporated, including recruitment sites (safety net clinics vs. private clinics) and time splines of conception date and visit date.

Maternal Healthy Eating Index (HEI-2010) was calculated from the Block (2005) Food Frequency Questionnaire during the 2nd trimester.[78] In addition, maternal folate concentration was derived from maternal 2nd and 3rd trimester blood samples. Plasma folate was assessed using the Lactobacillus casei microbiological assay, with a minimum detection limit of 3 ng/mL[79], and measurements from both trimesters were averaged. We dichotomized HEI at the median and folate at the 1st quartile.

Statistical analysis

We conducted descriptive analyses to summarize the characteristics of the participants. Associations between each outcome measurement and $PM_{2.5}$ and NO_2 in each window were examined independently. Linear and Poisson regression with robust standard error were the primary modeling approaches used for continuous BP percentiles and binary HBP, respectively. Effect estimates were rescaled to two-unit increments of $PM_{2.5}$ and NO_2. Families who moved out of Shelby County between enrollment and the age 4-6 years visit were excluded from analyses with postnatal exposures. No families moved out of the study area before childbirth.

We developed a staged approach of four models for the main analysis. Model 1 was adjusted for child sex, age and height at age 4-6 years visit, and recruitment site. Model 2 (for $PM_{2.5}$ and NO_2 only) additionally controlled for time splines of both visit date and date of conception. Visit date was used to capture the secular trends of child BP, while date of conception was included as it was correlated with air pollution concentrations and might be associated with child BP due to

time-varying enrollment patterns. Time splines for visit date were universally modeled with 1 degree of freedom (df)/year in all models. Date of conception was modeled with 1 df/year for analyses with NO_2. For $PM_{2.5}$, the df of splines for date of conception varied by exposure windows due to differences in temporal variabilities in the exposures: 8 df/year for trimester specific $PM_{2.5}$, 4 df/year for prenatal $PM_{2.5}$ and 1 df/year for postnatal $PM_{2.5}$. Model 3 was considered the full model, and included other potential confounders: maternal race, maternal age at childbirth, maternal education levels, income adjusted by household size, breastfeeding, smoking during pregnancy, BMI class before pregnancy, insurance status, maternal GSI, child sleep quality scores, child physical activity levels, child use of medication that potentially increased blood pressure, and COI. Model 4 was further adjusted for potential mediators including maternal hypertensive disorder, gestational age, birthweight and child BMI at the age 4-6 years visit. To assess effect modifications, we included cross product terms of maternal HEI, plasma folate, child sex or maternal race with exposures in each window in separate analyses using the full models, and estimated interaction p-values as well as strata specific associations.

Four sensitivity analyses were performed. 1) To disentangle the effects of spatial contrast from temporal variability, we replaced time-varying NO_2 concentrations from the annual national models and the $PM_{2.5}$ concentrations from the spatio-temporal models in the pre- and postnatal windows with predictions from 2006 (the 1st year of recruitment) and 2011 (the 1st year of age 4-6 years visit) national models respectively, based on residential history during the relevant exposure periods. 2) We varied the df for date of conception from zero (no date of conception adjustment) to twelve in full adjustment models, to evaluate the robustness of results when progressively controlling for temporal trends. 3) We simultaneously included $PM_{2.5}$ estimates in all three trimesters in the full model, then further adjusted for postnatal $PM_{2.5}$ exposure. 4) Child age and height at the age 4-6 years visit were modeled flexibly using two-dimensional unpenalized thin-plate regression splines (TPRS) for adjustment in the analyses of two sets of continuous outcomes -- BP percentiles and BP raw measurements. TPRS were generated from the MGCV package with varied df from five to twelve.

All analyses were conducted in STATA 15 (StataCorp, 2017, Texas, the U.S.) and R 3.6.2 (R Cord Team, 2019, Vienna, Austria).

Results

Characteristics of the study population

Figure 3 illustrates CANDLE cohort retention between enrollment and the age 4-6 years visit as well as sample sizes for primary analysis of each exposure. Mothers included in the analysis were racially diverse, with 67% identifying as Black and 26% identifying as White (Table 1). 60% of them had a high school education or less, and about 40% reported never being married. One third of the participating families had a household income less than $20,000 per year, and more than half were covered by Medicaid or Medicare only. Few mothers reported smoking (9.5%) during pregnancy, and 60% breastfed their newborn. The median HEI was 60.1 HEI below the median indicates poor adherence to the Dietary Guidelines for Americans according to a grading system recently proposed by Krebs-Smith et al.[80] The 1st quarter of plasma folate ranged from 2.6 to 15.0 ng/mL, suggesting possible folate deficiency. (Appendix Table 1)

Children were on average 4.4 years old (SD 0.6) at the time of BP measurement, with an approximately equal sex distribution. Seventeen percent met the definition of obesity. According to parent reports, 7.8% were taking medications that potentially increased blood pressure, such as albuterol or methylphenidate. Compared to the 1503 who enrolled in CANDLE, children in our analysis were more likely to come from low income families, participate in vigorous physical activities and use medication that potentially increased blood pressure (Appendix Table 2). The two groups were similar with respect to other characteristics. Mean SBP was 92.3 mmHg (SD 10.0) for raw measurement and 48.6 (SD 25.7) for percentile, and mean DBP was 61.1 mmHg (SD 9.2) for raw measurement and 75.6 (SD 19.3) for percentile. Both BP raw measurements were normally distributed (data not shown), while the DBP percentile was left skewed. 29.2% of the children were classified as HBP, largely driven by isolated diastolic HBP.

Air pollution exposures

A summary of air pollution exposures is shown in Table 2. Trimester specific $PM_{2.5}$ ranged from 6.15 to 17.01 μg/m^3. Mean $PM_{2.5}$ levels were 10.8 μg/m^3 (SD 0.9) during the prenatal period, and 9.9 μg/m^3 (SD 0.6) averaged between birth and age 4 birthday. There was little correlation among the $PM_{2.5}$ in three trimesters (corr: -0.09 to 0.15), but a moderate correlation between prenatal and postnatal measurements (corr: 0.34). Prenatal and postnatal NO_2 had average levels of 10.0 ppb (SD 2.4) and 8.8 ppb (SD 1.9) respectively and were highly correlated (corr: 0.82). More than one fourth of the families lived <150 meters from an A1, A2 or A3 main road at some point during the study period. We inspected distributions of exposure between strata of effect modifiers. Mothers who self-identified as Black, had HEI lower than median or plasma folate in the 1st quartile were more likely to live near major roadways and had higher NO_2 exposures in both pre- and postnatal windows (data not shown). There were no meaningful differences in $PM_{2.5}$ by any effect modifiers.

Associations between air pollution exposures and child BP

Associations between air pollution exposures in each window and child BP are shown in Table 3. When BP was analyzed as continuous percentiles, we found evidence supporting adverse effects of $PM_{2.5}$ in the 2nd trimester on both SBP and DBP. The fully adjusted model estimated a 13.6 percentile increase in SBP (Beta: 13.6, 95% CI: 3.7-23.5) and an 8.0 percentile increase in DBP (Beta: 8.0, 95% CI: 0.7, 15.3) for each 2 μg/m^3 increase of $PM_{2.5}$ in the 2nd trimester. Prenatal average $PM_{2.5}$ was associated with DBP only, with each 2 μg/m^3 increase of $PM_{2.5}$ associated with an 11.6 percentile elevation (Beta: 11.6, 95% CI: 3.0, 20.2). The conclusions remained the same after additionally adjusting for potential mediators. Postnatal $PM_{2.5}$ was not associated with any outcome. There was also no evidence that either NO_2 or road proximity was associated with BP, regardless of exposure timing. When BP was analyzed as a binary outcome, we observed increased risks of HBP with both pre- and postnatal $PM_{2.5}$, although associations were imprecise ($IRR_{prenatal}$: 1.5, 95% CI: 0.7, 3.2; $IRR_{postnatal}$: 2.1, 95% CI: 0.7, 6.0).

We investigated four potential effect modifiers of associations between air pollution and child BP: maternal HEI, maternal plasma folate, child sex and maternal race (Figure 4). The notable associations between $PM_{2.5}$ in the 2nd trimester and SBP percentile were observed in all the

strata. For DBP percentile, the effect of $PM_{2.5}$ in the 2nd trimester was limited to strata of children whose mother had HEI below median or folate in the lowest quartile during pregnancy, female children, and children whose mother self-identified as Black. However, these differences achieved significance only for interactions of maternal folate levels (P=0.036) and child sex (P=0.035). Associations between prenatal $PM_{2.5}$ and DBP percentile were apparent in all strata except for children with a non-Black mother, but none of the interactions were significant.

Sensitivity analyses

Replacing NO_2 and $PM_{2.5}$ assessments with predictions from the fixed year 2006 and 2011 national models did not produce meaningful changes in the effect estimates obtained from the models that incorporated time spline adjustment (Models 2-4) (Appendix Table 3 and 4). When varying the df/year from 0 to 5 for temporal adjustment of conception date, the estimated effects of $PM_{2.5}$ in the 2nd trimester became stronger, and the result gained significance (Appendix Figure 1). The associations remained stable with 5 df/year and above. For prenatal $PM_{2.5}$, similar patterns were observed in the associations with DBP percentile, with 3 df/year as the turning point. Varying the df/year had little impact on the precision of the associations between prenatal $PM_{2.5}$ and SBP percentile. Including all three trimester-specific $PM_{2.5}$ exposures in the full models, the effect estimates for $PM_{2.5}$ in the 2nd trimester and SBP percentile became even stronger (Beta: 16.4, 95% CI: 5.6, 27.2), but the association was attenuated for DBP percentile (Beta: 7.0, 95% CI: -0.9, 14.9). Additionally, adjusting for postnatal $PM_{2.5}$ in the models of prenatal exposures in each window produced no appreciable changes (data not shown). When controlling for child age and height using TPRS with varied degrees of freedom, we obtained similar effect estimates with the primary analysis of BP percentile, and equivalent estimates of raw BP (1 percentile $\approx$ 0.35 mmHg) (Appendix Figure 2).

Discussion

In this community-based birth cohort in the Southern U.S., we found that children exposed to higher $PM_{2.5}$ in utero had elevated BP percentiles at age 4-6 years. Specifically, $PM_{2.5}$ in the 2nd trimester was associated with increased SBP and DBP percentiles, while $PM_{2.5}$ averaged over the full prenatal period was only associated with higher DBP percentiles. These findings were robust to adjustments for potential mediators, exposure assessments in the other pre- and postnatal periods, as well as numerous sensitivity analyses. The adverse effects, particularly on DBP, were more pronounced in children whose mother had low-quality diet or possible folate deficiency during pregnancy, female children and children whose mother self-identified as Black. Notably, our observed associations occurred in a setting with $PM_{2.5}$ levels that fall within current regulatory guidelines. No association was detected between NO_2, proximity to road or postnatal $PM_{2.5}$ with any outcome.

This study has several important strengths. The cohort was well-characterized, allowing control for key confounders of concern. Missing data was rare, and the large sample size enabled us to conduct well-powered analyses for effect modification. In addition, we estimated spatiotemporally resolved $PM_{2.5}$ and spatially resolved NO_2 using well-validated advanced modeling approaches that predict exposures at individually geocoded subject home locations, allowing us to exploit small-scale spatial variability in the pollutant surfaces. Moving is common

in families with young parents and low socioeconomic status (SES)[81], and we were able to leverage detailed address history data collected by CANDLE and further improve the accuracy of exposure assessment. The biweekly resolution of the PM2.5 model also enabled evaluations of critical window(s) of exposure among multiple pre- and post-natal periods. Lastly, two nutritional measurements – the HEI (a validated and standardized tool) and plasma folate (a biomarker), were utilized to assess potential effect modification by maternal diet during pregnancy.

The effect estimates measured from our study were in general larger than in similar previous studies. We detected convincing positive associations between prenatal $PM_{2.5}$ and DBP, in common with the study of Madhloum et al, in Belgium[55], but not with SBP. Similar to the two studies conducted in Massachusetts[52,53], we observed adverse effects on both BP percentiles from trimester-specific $PM_{2.5}$, but with the 2nd not 3rd trimester. However, no association of NO_2 with child BP was found in the CANDLE cohort, whereas the Child Health Study in California [54] detected effects of NO_2 exposure in the 3rd trimester on SBP. We also did not observe any associations between postnatal exposures and the outcomes of interest, contrary to most recent studies conducted in medium to highly polluted areas. Five studies in China have reported positive relationships between air pollution exposures and both SBP and DBP[56,57,63,65,66], while five other studies [58,60,62,64,69], mainly in Europe, have detected positive associations with either measurement but not both. Several factors could potentially explain the different findings between our study and the others: First, the blood pressure was assessed earlier in life in our participants than in most of the other studies. In particular, the studies conducted outside of the U.S. primarily targeted schoolchildren or adolescents. Our population is diverse, with a high proportion of low SES families and an elevated child obesity rate, likely contributing to the high incidence of HBP. Second, the outcomes of interest were standardized using the 2017 guidelines with the normal weight pediatric population as the reference, which performed better than the 2004 guidelines in identifying children with adverse cardiometabolic profiles.[82] Third, all air pollution exposure prediction models, including ours, may induce complex forms of exposure measurement error that can introduce bias in either direction, and lead to excess variability in estimating effect sizes.[83,84] And finally, we were able to adjust rigorously for confounders, particularly the individual and neighborhood level SES variables and time trends.

In hypertensive youth, elevated peripheral DBP is superior to SBP in predicting future CVD, but with advancing age, SBP gradually overtakes DBP as a more powerful predictor[85]. We elected to examine both SBP and DBP and found effects for both. The existing science suggests that potential mechanisms for associations of prenatal air pollution exposures with child BP may involve induction of placenta and fetal systematic inflammation, oxidative stress or endocrine disruption, subsequent changes of placental vascularization, and restricted fetal programming.[18,19] Three major pathways linking postnatal air pollution exposures and cardiovascular health have been substantiated from previous studies, including “spillover” of pulmonary inflammation which further induces systemic inflammation, modulation of autonomic influences, and direct target organ effects of pollutants or their products.[21,86–88] The differences in routes of exposure and biological mechanism of disease progression between the two windows may partially explain why we detected notable associations only in the prenatal period. In addition, although the cardiovascular and renal systems develop throughout pregnancy, there

is an exponential increase in nephrons between 18 and 32 weeks, which may explain the trimester-specific associations detected in this study.[89–91] Animal and human data also support the theory that maternal nutritional deficiencies may cause intrauterine growth restriction.[9,10,48] When exposed to an adequate nutrient supply after birth, an undernourished infant might experience catch-up growth, which has been associated with amplified risks of hypertension, diabetes and CVD later in life.[92–94] Folate may protect against elevated blood pressure by increasing nitric oxide synthesis in endothelial cells, and subsequently counteracting oxidative stress caused by prenatal air pollution exposure and promoting resilience.[49,50]

Our study has some limitations. One is the potential misclassification of HBP. Although BP was measured repeatedly during assessment, the examination was performed on a single occasion. As such, the definition of HBP in our analysis does not meet the clinical definition.[82,95,96] In addition, address was first ascertained at enrollment, and we assumed each mother had the same address since the start of pregnancy, which could reduce the accuracy of exposure assessments. Also, we excluded approximately half of the original study population from this analysis. Although there was no meaningful difference in baseline characteristics between the families at enrollment and the analytic sample, selection bias remains a concern. Further, as plasma folate is an indicator for recent folate exposure, it is an imperfect measure of chronic deficiency in the prenatal period.[97] Another limitation is the lack of data regarding $PM_{2.5}$ composition as well as information on indoor air pollution exposures.

Despite the limitations, the primary contribution of this study is twofold. It highlights the potential harmful effect of prenatal $PM_{2.5}$, even at low levels, on an important feature of child cardiovascular health. Such considerations can inform regulatory policy on acceptable air pollution levels and appropriate controls. Unfortunately, gathering sufficient scientific evidence takes time, and regulatory action does not always follow closely behind. To partially address this problem, we also identified maternal nutritional factors that may potentially ameliorate the adverse effects of air pollution on child health, to inform timely intervention.

Tables and Figures

Table 1: Characteristics of CANDLE participants included in analysis (n=822)

	Analytic sample (N=822)	
Variables	**n**	**Mean (SD) /Percentage**
Child characteristics		
Child age at age 4-6 years visit (yrs)	822	4.4 (0.6)
Child height at age 4-6 years visit (cm)	822	106.5 (6.1)
Child sex		
Male	410	49.9%
Female	412	50.1%
Birth weight (kg)	817	3.2 (0.5)
Gestational age at childbirth (wks)	818	38.8 (1.8)
BMI at age 4-6 years visit (kg/m^2)	821	16.5 (2.3)
Medication use potentially leading to HBP*		
No	758	92.2%
Yes	64	7.8%
Child sleep score at age 4-6 years visit	816	46.9 (7.2)
Vigorous activity frequency		
Never or occasionally	119	14.5%
Once or twice per week	87	10.6%
Three or more times per week	604	73.5%
Missing	12	1.5%
Maternal characteristics		
Maternal age at birth (years)	822	26 (5.5)
Maternal race		
Black	552	67.2%
White	217	26.4%
Asian	8	1.0%
Other	1	0.1%
Multiple race	44	5.4%
Maternal education at enrollment		
< High School	113	13.8%
High School/GED/Technical School	482	58.6%
College Degree	142	17.3%
Grad/Professional Degree	84	10.2%
missing	1	0.1%
Maternal marital status at enrollment		
Married/Living with partner	430	52.3%
Widowed/Divorced/Separated	22	2.7%
Never Married	369	44.9%
missing	1	0.1%

Insurance status at enrollment		
No insurance	2	0.2%
Medicaid or Medicare only	503	61.2%
Medicaid/Medicare and private insurance	28	3.4%
Private insurance only	289	35.2%
Household income at enrollment		
$0-$19,999	303	36.9%
$20,000-$44,999	197	24.0%
$45,000-$74,999	138	16.8%
$75,000 or over	110	13.4%
missing	74	9.0%
Income adjusted by household size	817	16629.1 (16387.5)
Prenatal smoking		
No	743	90.4%
Yes	78	9.5%
missing	1	0.1%
BMI class		
Underweight	38	4.6%
Normal	329	40.0%
Overweight	180	21.9%
Obese	272	33.1%
missing	3	0.4%
Breastfeeding		
No	312	38.0%
Yes (6 months or less)	295	35.9%
Yes (Above 6 months)	205	24.9%
Missing	10	1.2%
Pregnancy hypertensive disorder		
No	773	94.0%
Yes	49	6.0%
The BSI Global Severity Index (GSI)	798	46.8 (10.9)
Prenatal plasma folate (ng/mL)	822	23 (11.1)
Prenatal Healthy eating index	725	59.9 (11.3)
Other characteristics		
Childhood Opportunity Index		
Prenatal Educational index	818	-0.03 (0.5)
Prenatal Economics index	818	-0.1 (0.6)
Postnatal Educational index	812	-0.03 (0.5)
Postnatal Economics index	812	-0.1 (0.6)

Abbreviation: BMI: Body Mass Index

Table 2. Distribution of exposure measurements

Measurements	n	Mean (SD)	Median	Min	Max	IQR
Prenatal exposures						
$PM_{2.5}$ in 1st trimester ($\mu g/m^3$)	818	10.67 (1.47)	10.43	7.69	16.81	1.34
$PM_{2.5}$ in 2nd trimester ($\mu g/m^3$)	818	10.71 (1.38)	10.48	7.70	16.56	1.53
$PM_{2.5}$ in 3rd trimester ($\mu g/m^3$)	815	11.01 (1.71)	10.75	6.15	17.01	1.77
Prenatal $PM_{2.5}$ ($\mu g/m^3$)	805	10.78 (0.90)	10.83	8.61	13.80	1.14
Prenatal NO_2 (ppb)	818	10.02 (2.39)	10.04	3.95	16.37	3.43
Distance to A1 roadway (m)	818	2489.53 (1840.57)	2017	35	11400	2313
Distance to A2 roadway (m)	818	1919.55 (1630.68)	1385	9	8658	2320
Distance to A3 roadway (m)	818	449 (542.34)	307	9	6220	404
Postnatal exposures						
Postnatal $PM_{2.5}$ ($\mu g/m^3$)	822	9.88 (0.55)	9.85	8.52	11.65	0.75
Postnatal NO_2 (ppb)	822	8.80 (1.88)	8.79	3.38	13.46	2.65
Distance to A1 roadway (m)	817	2630.20 (2354.96)	2010	38	25000	2433
Distance to A2 roadway (m)	817	1994.03 (1678.44)	1538	9	11833	2426
Distance to A3 roadway (m)	817	447.60 (485.54)	313	9	3747	406

Abbreviation: IQR: Interquartile range

Table 3. Estimated effects of air pollution exposures on BP percentiles and HBP from the fully adjusted model (Model 3)

	SBP percentile		DBP percentile		HBP	
Measurements[a]	**Beta[b]**	**95% CI**	**Beta[b]**	**95% CI**	**IRR[b]**	**95% CI**
Prenatal exposures						
$PM_{2.5}$ in 1st trimester	0.96	(-8.17, 10.09)	3.90	(-2.89, 10.68)	0.89	(0.50, 1.57)
$PM_{2.5}$ in 2nd trimester	13.60	(3.74, 23.46)	7.97	(0.68, 15.26)	0.97	(0.52, 1.81)
$PM_{2.5}$ in 3rd trimester	-2.17	(-9.45, 5.10)	3.47	(-2.25, 9.18)	1.09	(0.72, 1.67)
Prenatal $PM_{2.5}$	7.74	(-3.48, 18.96)	11.58	(2.96, 20.21)	1.52	(0.73, 3.20)
Prenatal NO_2	1.33	(-0.67, 3.33)	0.59	(-0.91, 2.10)	1.04	(0.91, 1.19)
Proximity to road	0.09	(-4.05, 4.22)	-0.04	(-3.16, 3.08)	1.03	(0.81, 1.31)
Postnatal exposures						
Postnatal $PM_{2.5}$	9.26	(-9.07, 27.59)	10.05	(-3.00, 23.10)	2.06	(0.71, 5.97)
Postnatal NO_2	0.16	(-2.61, 2.94)	1.04	(-1.03, 3.11)	1.15	(0.96, 1.37)
Proximity to road	-0.48	(-4.75, 3.79)	-0.84	(-4.11, 2.43)	0.95	(0.74, 1.23)

Abbreviations: SBP: systolic blood pressure, DBP: diastolic blood pressure, HBP: high blood pressure, IRR: incidence rate ratio.

a. $PM_{2.5}$ and NO_2 in each window were rescaled to two-unit increment.

b. The full models (linear regressions for BP percentiles and Poisson regressions for HBP) were adjusted for child sex, child age and height at age 4-6 years visit, times splines for visit date and date of conception ($PM_{2.5}$ and NO_2 only), maternal race, maternal age at childbirth, maternal education, income adjusted by household size, breastfeeding, smoking during pregnancy, BMI class before pregnancy, insurance status, maternal GSI, child sleeping scores, child physical activity levels, child use of medication that potentially increased blood pressure and COI.

Figure 3: Inclusion Flowchart

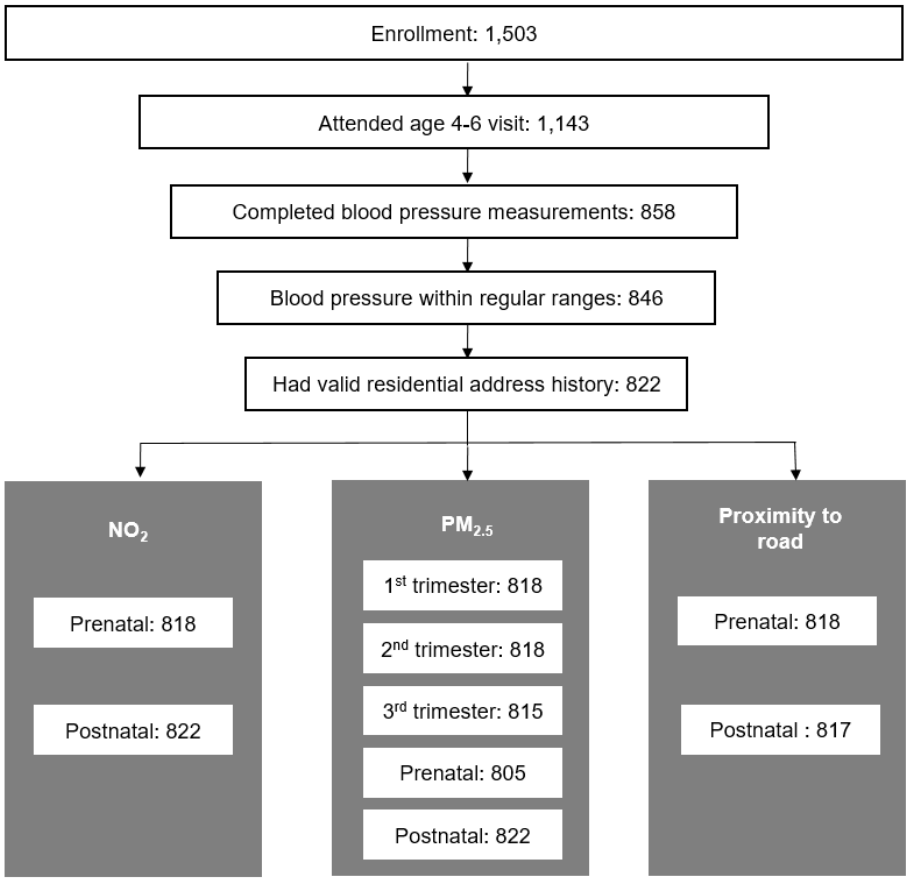
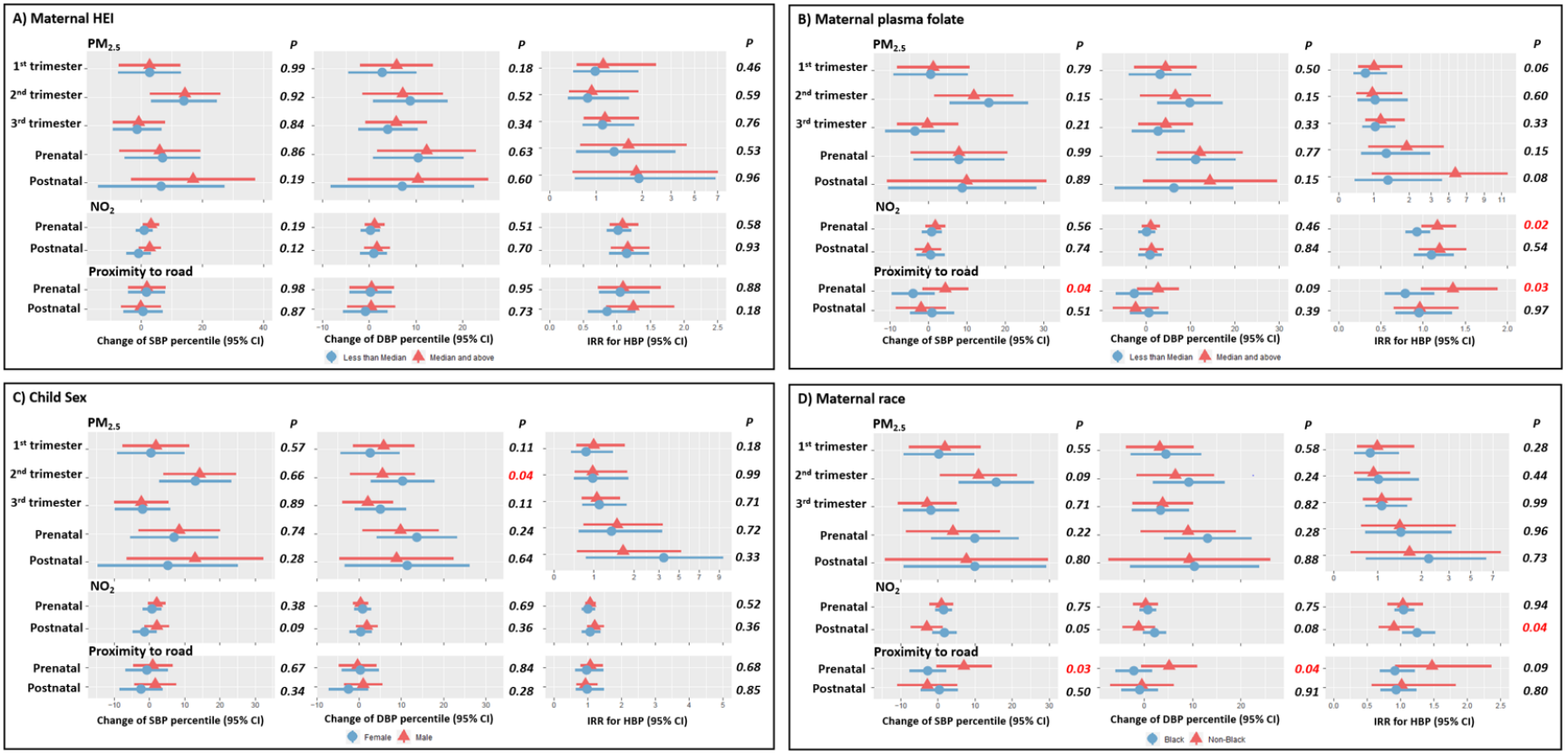


Figure 4. Estimated effects of air pollution exposures on BP percentiles and HBP by maternal HEI levels (Median and above vs. Below Median), maternal plasma folate (1st quartile vs. > 1st quartile), child sex (female vs. male) and maternal race (Black vs. non-Black) from the interaction models.



Appendix Tables and Figures

Appendix Table 1: Distribution of nutritional measurements by categories

	Healthy Eating Index				
	N	Mean	Std.	Min	Max
Overall	725	59.94	11.31	26.16	88.42
Below median	363	50.64	6.69	26.16	60.07
Median and above	362	69.26	6.13	60.10	88.42
	Plasma folate (ng/mL)				
	N	Mean	Std.	Min	Max
Overall	822	23.00	11.10	2.56	80.48
1st Quartile	206	10.44	3.23	2.56	15.01
2nd Quartile	205	18.60	2.01	15.02	22.01
3rd Quartile	206	25.24	2.05	22.01	28.81
4th Quartile	205	37.78	8.60	28.86	80.48

Appendix Table 2. Comparisons of baseline characteristics between the analytic sample and the full CANDLE cohort

	Analytic sample (N=822)		CANDLE mothers ever enrolled (N = 1503)	
Variables	**N**	**Mean (SD) /Percentage**	**N**	**Mean (SD) /Percentage**
Child characteristics				
Child age at age 4-6 years visit (yrs)	822	4.4 (0.6)	1,157	4.4 (0.6)
Child height at age 4-6 years visit (cm)	822	106.5 (6.1)	1,069	106.2 (6.0)
Child sex				
Male	410	49.9%	736	49.0%
Female	412	50.1%	726	48.3%
Missing	0	0.0%	41	2.7%
Birth weight (kg)	817	3.2 (0.5)	1,454	3.2 (0.6)
Gestational age at childbirth (wks)	818	38.8 (1.8)	1,456	38.8 (1.9)
BMI at age 4-6 years visit (kg/m2)	821	16.5 (2.3)	1,064	16.5 (2.2)
Medication use potentially leading to hypertension				
No	758	92.2%	1,416	94.2%
Yes	64	7.8%	87	5.8%
Child sleep score at age 4-6 years visit	816	46.9 (7.2)	1,078	46.6 (7.2)
Vigorous activity frequency				
Never or occasionally	119	14.5%	144	9.6%
Once or twice per week	87	10.6%	111	7.4%
Three or more times per week	604	73.5%	888	59.1%
Missing	12	1.5%	360	24.0%
Maternal characteristics				
Maternal age at birth (years)	822	26 (5.5)	1,503	26 (5.4)
Maternal race				
Black	552	67.2%	936	62.3%
White	217	26.4%	467	31.1%
Asian	8	1.0%	13	0.9%
American Indian	0	0.0%	1	0.1%

Native Hawaiian/Pacific Islander	0	0.0%	1	0.1%
Other	1	0.1%	6	0.4%
Multiple race	44	5.4%	77	5.1%
Missing	0	0.0%	2	0.1%
Maternal education at enrollment				
< High School	113	13.8%	184	12.2%
High School/GED/Technical School	482	58.6%	847	56.4%
College Degree	142	17.3%	299	19.9%
Grad/Professional Degree	84	10.2%	171	11.4%
missing	1	0.1%	2	0.1%
Maternal marital status at enrollment				
Married/Living with partner	430	52.3%	848	56.4%
Widowed/Divorced/Separated	22	2.7%	40	2.7%
Never Married	369	44.9%	614	40.9%
missing	1	0.1%	1	0.1%
Insurance status at enrollment				
No insurance	2	0.2%	2	0.1%
Medicaid or Medicare only	503	61.2%	859	57.2%
Medicaid/Medicare and private insurance	28	3.4%	42	2.8%
Private insurance only	289	35.2%	600	39.9%
Household income at enrollment				
$0-$19,999	303	36.9%	493	32.8%
$20,000-$44,999	197	24.0%	370	24.6%
$45,000-$74,999	138	16.8%	271	18.0%
$75,000 or over	110	13.4%	234	15.6%
missing	74	9.0%	135	9.0%
Income adjusted by household size	817	16629.1 (16387.5)	1356	18864.5 (17229.4)
Prenatal smoking				
No	743	90.4%	1,351	89.9%
Yes	78	9.5%	151	10.1%

missing	1	0.1%	1	0.1%
Maternal supplement intake of Vitamins				
No	48	5.8%	78	5.2%
Yes	758	92.2%	1,392	92.6%
missing	16	2.0%	33	2.2%
BMI class				
Underweight	38	4.6%	66	4.4%
Normal	329	40.0%	633	42.1%
Overweight	180	21.9%	354	23.6%
Obese	272	33.1%	445	29.6%
missing	3	0.4%	5	0.3%
Breastfeeding				
No	312	38.0%	401	26.7%
Yes (6 months or less)	295	35.9%	434	28.9%
Yes (Above 6 months)	205	24.9%	309	20.6%
Missing	10	1.2%	359	23.9%
Pregnancy hypertensive disorder				
No	773	94.0%	1,408	93.7%
Yes	49	6.0%	95	6.3%
The BSI Global Severity Index (GSI)	798	46.8 (10.9)	1,055	46.6 (10.6)
Prenatal plasma folate (ng/mL)	822	23 (11.1)	1,502	23.5 (11)
Prenatal healthy eating index	725	59.9 (11.3)	1,322	60.2 (11.4)
Other characteristics				
Childhood Opportunity Index				
Prenatal Educational index	818	-0.03 (0.5)	1,415	0.02 (0.5)
Prenatal Economics index	818	-0.1 (0.6)	1,415	-0.04 (0.6)
Postnatal Educational index	812	-0.03 (0.5)	1,080	0.01 (0.5)
Postnatal Economics index	812	-0.1 (0.6)	1,080	-0.04 (0.6)

Abbreviation: BMI: Body Mass Index

Appendix Table 3. Comparisons of estimates and 95% CI for $PM_{2.5}$ from spatio-temporal models and fixed year national models

	Prenatal $PM_{2.5}$[a]		Postnatal $PM_{2.5}$[a]	
	Spatio-temporal models	2006 national models	Spatio-temporal models	2011 national models
Models[b]	Beta/IRR (95% CI)	Beta/IRR (95% CI)	Beta/IRR (95% CI)	Beta/IRR (95% CI)
Systolic BP percentile				
Model 1	-2.34 (-7.19, 2.51)	9.11 (-0.34, 18.56)	16.99 (7.46, 26.51)	12.85 (-0.51, 26.21)
Model 2	9.35 (0.10, 18.60)	10.33 (0.82, 19.84)	15.09 (1.45, 28.73)	14.02 (0.57, 27.46)
Model 3	7.74 (-3.48, 18.96)	8.48 (-2.95, 19.920)	9.26 (-9.07, 27.59)	8.84 (-9.34, 27.03)
Model 4	6.61 (-4.74, 17.96)	7.83 (-3.61, 19.28)	7.42 (-11.09, 25.93)	7.72 (-10.71, 26.15)
Diastolic BP percentile				
Model 1	-0.09 (-3.55, 3.37)	8.24 (1.23, 15.25)	6.70 (-0.25, 13.65)	8.97 (-1.00, 18.94)
Model 2	9.05 (1.99, 16.11)	9.73 (2.80, 16.67)	9.13 (-0.63, 18.89)	9.64 (-0.04, 19.31)
Model 3	11.58 (2.96, 20.21)	12.02 (3.85, 20.19)	10.05 (-3.00, 23.10)	10.99 (-2.06, 24.05)
Model 4	10.64 (2.09, 19.18)	11.78 (3.68, 19.87)	9.55 (-3.45, 22.56)	10.74 (-2.3, 23.79)
HBP				
Model 1	0.78 (0.59, 1.03)	0.99 (0.55, 1.78)	1.74 (0.98, 3.10)	1.65 (0.70, 3.89)
Model 2	1.28 (0.70, 2.32)	1.03 (0.57, 1.86)	1.79 (0.77, 4.20)	1.82 (0.75, 4.40)
Model 3	1.52 (0.73, 3.20)	1.10 (0.56, 2.18)	2.06 (0.71, 5.97)	2.06 (0.69, 6.14)
Model 4	1.37 (0.65, 2.88)	1.05 (0.53, 2.08)	2.02 (0.70, 5.83)	2.04 (0.68, 6.10)

Abbreviations: SBP: systolic blood pressure, DBP: diastolic blood pressure, HBP: high blood pressure, IRR: incidence rate ratio.

a. $PM_{2.5}$ in each window were rescaled to two-unit increment.

b. Model 1 was adjusted for child sex, age and height at age 4 visit, and study site.

Model 2 was additionally controlled for time splines of both visit date and date of conception

Model 3 was further controlled for maternal race, maternal age at childbirth, maternal education, income adjusted by household size, breastfeeding, smoking during pregnancy, BMI class before pregnancy, insurance status, maternal GSI, child sleeping scores, child physical activity levels, child use of medication that potentially increased blood pressure and COI.

Model 4 was additionally adjusted for potential mediators of maternal hypertensive disorder, gestational age, birthweight and child BMI at age 4-6 years visit.

Appendix Table 4. Comparisons of estimates and 95% CI for NO_2 from annual national models and fixed year national models

	Prenatal NO_2[a]		Postnatal NO_2[a]	
	Annual national models	2006 national models	Annual national models	2011 national models
Models[b]	Beta/IRR (95% CI)	Beta/IRR (95% CI)	Beta/IRR (95% CI)	Beta/IRR (95% CI)
Systolic BP percentile				
Model 1	0.79 (-0.92, 2.49)	0.85 (-0.64, 2.34)	1.55 (-0.66, 3.76)	0.82 (-1.26, 2.90)
Model 2	1.13 (-0.56, 2.82)	0.95 (-0.53, 2.42)	1.09 (-1.15, 3.32)	1.01 (-1.08, 3.10)
Model 3	1.33 (-0.67, 3.33)	1.12 (-0.63, 2.87)	0.16 (-2.61, 2.94)	0.13 (-2.46, 2.72)
Model 4	0.92 (-1.06, 2.91)	0.80 (-0.95, 2.54)	-0.13 (-2.89, 2.62)	-0.19 (-2.77, 2.39)
Diastolic BP percentile				
Model 1	-0.10 (-1.39, 1.19)	0.12 (-1.01, 1.25)	1.13 (-0.52, 2.77)	0.86 (-0.7, 2.42)
Model 2	0.30 (-0.98, 1.58)	0.18 (-0.93, 1.30)	0.88 (-0.78, 2.53)	0.84 (-0.71, 2.40)
Model 3	0.59 (-0.91, 2.10)	0.43 (-0.88, 1.74)	1.04 (-1.03, 3.11)	1.01 (-0.93, 2.96)
Model 4	0.4 (-1.06, 1.86)	0.28 (-0.99, 1.56)	0.91 (-1.12, 2.94)	0.86 (-1.05, 2.78)
HBP				
Model 1	0.97 (0.87, 1.08)	0.99 (0.9, 1.09)	1.13 (0.98, 1.30)	1.10 (0.96, 1.25)
Model 2	1.00 (0.90, 1.12)	1.00 (0.91, 1.10)	1.11 (0.96, 1.28)	1.11 (0.97, 1.27)
Model 3	1.04 (0.91, 1.19)	1.03 (0.92, 1.16)	1.15 (0.96, 1.37)	1.15 (0.97, 1.36)
Model 4	1.03 (0.91, 1.18)	1.03 (0.92, 1.15)	1.13 (0.94, 1.35)	1.13 (0.95, 1.34)

Abbreviations: SBP: systolic blood pressure, DBP: diastolic blood pressure, HBP: high blood pressure, IRR: incidence rate ratio.

a. NO_2 in each window were rescaled to two-unit increment.

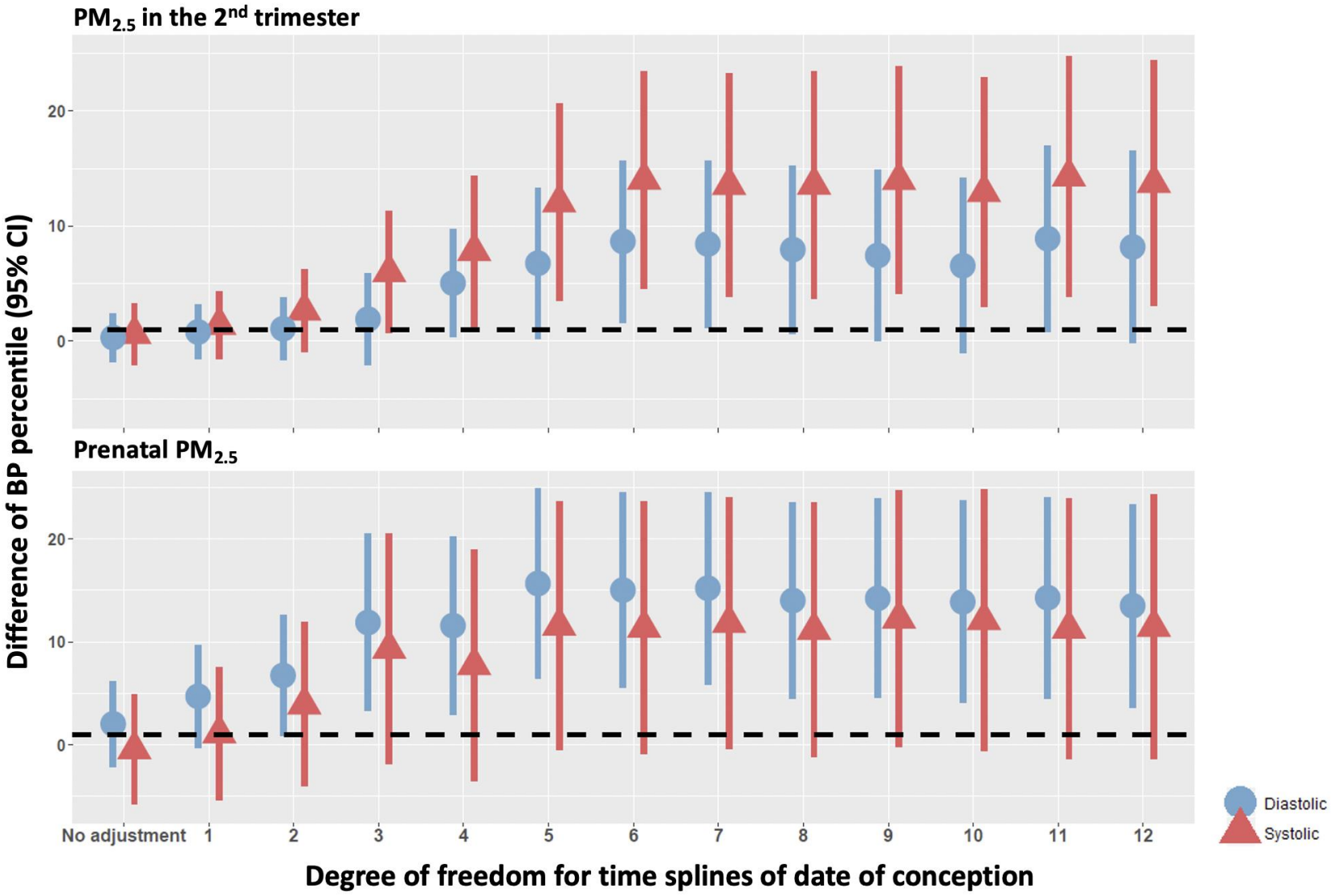
b. Model 1 was adjusted for child sex, age and height at age 4 visit, and study site.

Model 2 was additionally controlled for time splines of both visit date and date of conception

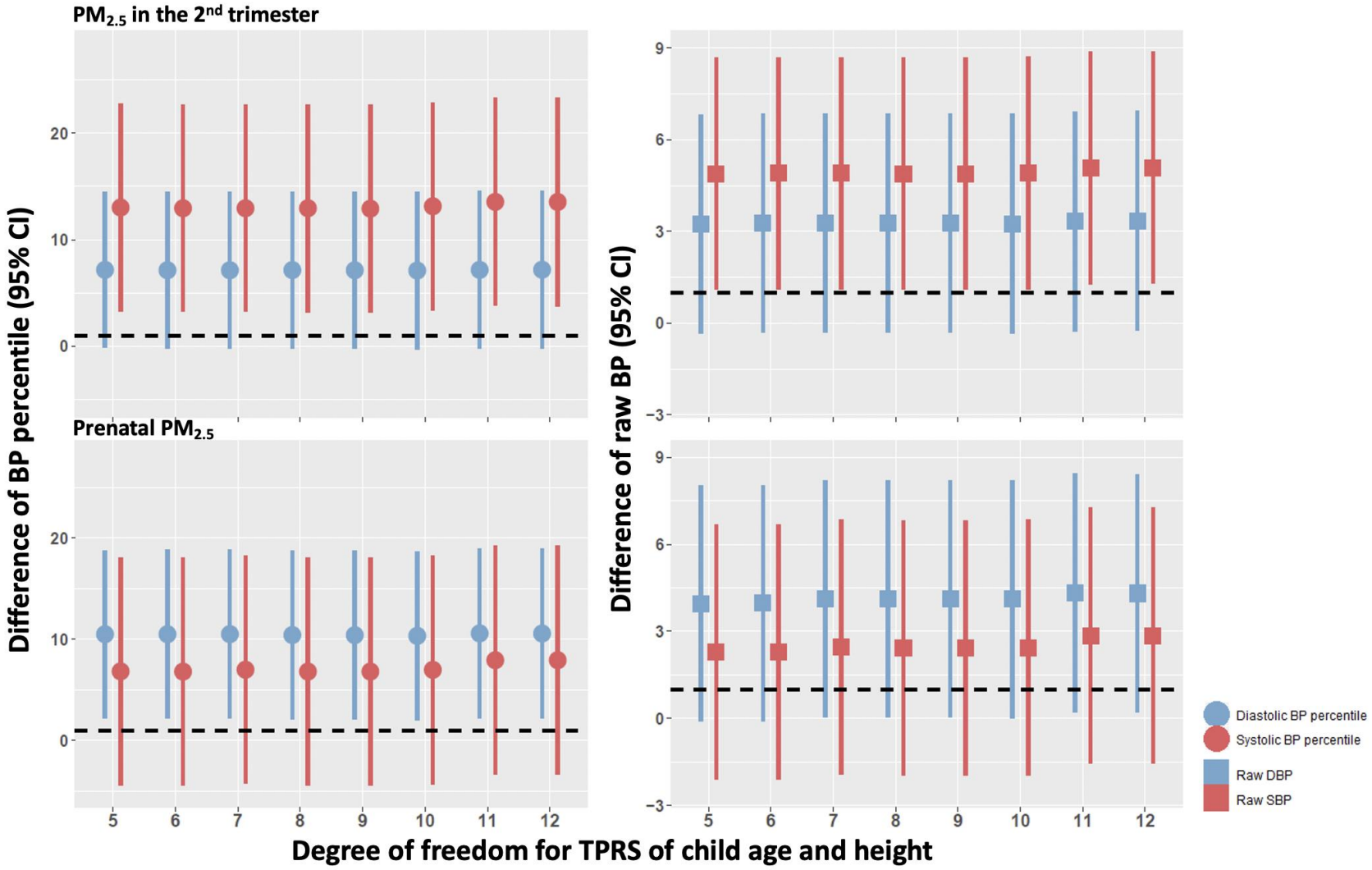
Model 3 was further controlled for maternal race, maternal age at childbirth, maternal education, income adjusted by household size, breastfeeding, smoking during pregnancy, BMI class before pregnancy, insurance status, maternal GSI, child sleeping scores, child physical activity levels, child use of medication that potentially increased blood pressure and COI.

Model 4 was additionally adjusted for potential mediators of maternal hypertensive disorder, gestational age, birthweight and child BMI at age 4-6 years visit.

Appendix Figure 1. Estimated effects of $PM_{2.5}$ in the 2nd trimester and during prenatal period on BP percentiles from the full model (Model 3) adjusted for time splines of date of conception with varied degree of freedom



Appendix Figure 2. Estimated effects of $PM_{2.5}$ in the 2nd trimester and during prenatal period on BP percentiles and raw BP from the full model (Model 3) adjusted for thin-plate regression splines (TPRS) of child height and age with varied degree of freedom.



CHAPTER 2: Associations between Maternal Nutrition in Pregnancy and Child Blood Pressure: A Prospective Study in a Community-based Birth Cohort

Authors:
Yu Ni[1], Adam Szpiro[2], Christine Loftus[3], Frances Tylavsky[4], Mario Kratz[1,5], Nicole R. Bush[6,7], Kaja LeWinn[6], Sheela Sathyanarayana[3,8,9], Daniel A. Enquobahrie[1], Robert Davis[10,11], Annette L. Fitzpatrick[1,12,13], Jennifer Sonney[14], Catherine J. Karr[1,3,8]

Institutes:
1. Department of Epidemiology, School of Public Health, University of Washington
2. Department of Biostatistics, School of Public Health, University of Washington
3. Department of Environmental and Occupational Health Sciences, School of Public Health, University of Washington
4. Department of Preventive Medicine, University of Tennessee Health Science Center
5. Public Health Sciences Division, Fred Hutch
6. Department of Psychiatry, School of Medicine, University of California, San Francisco
7. Department of Pediatrics, School of Medicine, University of California, San Francisco
8. Department of Pediatrics, School of Medicine, University of Washington
9. Seattle Children's Research Institute
10. Center for Biomedical Informatics, University of Tennessee Health Science Center
11. Department of Pediatrics, University of Tennessee Health Science Center
12. Department of Family Medicine, School of Medicine, University of Washington
13. Department of Global Health, School of Public Health, University of Washington
14. Department of Child, Family, and Population Health Nursing, School of Nursing, University of Washington

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Introduction

High blood pressure (HBP) is a major risk factor for heart disease and stroke, two leading causes of death in the U.S.[1] Large cohort studies show that blood pressure can track from childhood to adulthood.[2,3] Untreated pediatric hypertension is also directly associated with target organ damage.[7] It is increasingly recognized that adverse early-life experience, including in-utero exposure to poor maternal nutrition, may predispose to future cardiometabolic abnormalities.[9,14,98]

The evidence supporting this hypothesis is circumstantial, representing a diversity of study designs and features of early life nutrition. Animal experiments provide important mechanistic insights: studies from rodents and sheep have suggested associations between nutritional deprivation during pregnancy and vascular resistance, and studies from diet induced obese dams have shown endothelial dysfunction and elevated blood pressure in offspring.[99–102] Earlier cohort studies, mostly in Europe, have focused on maternal famine or malnutrition during pregnancy, and reported long lasting effects on offspring cardiovascular impairments even in mid and old age.[22–26] Due to the obesity epidemic, overnutrition and suboptimal nutrition during pregnancy have been of greater concerns in the past decades. Observational studies of single foods or nutrients, including fish intake, total energy intake, carbohydrate and protein, have reported inconsistent associations with child blood pressure.[27–33] Clinical trials and their follow-up studies, primarily of supplement intakes, have uniformly yielded null results except for fish oil supplementation.[34–41] As nutrients are not consumed in isolation, dietary pattern analysis has been used increasingly to assess the effects of maternal overall food intake on several offspring outcomes, but little is known about child blood pressure.[103–105] We are aware of only three studies, two using Mediterranean Diet scores and one using a data driven approach to define dietary patterns, have evaluated this exposure-outcome association.[106–108] Among these, only one study with Mediterranean Diet scores has detected weak protective effects on both systolic (SBP) and diastolic blood pressure (DBP) in the two pooled cohorts from Greece and the U.S.[108]

In addition to dietary patterns, there is a growing interest in the utilization of biomarkers in nutrition studies, particularly for micronutrients. Biomarkers directly reflect physiological responses to certain food components or supplement intakes after absorptive and metabolic processes.[109] Folate is an essential B vitamin involved in nucleic acid synthesis, DNA methylation and cellular division. The preventative effect of sufficient folate intake on fetal neural tube defects is well-established, and its benefits on reproductive health and cardiovascular health have been shown by previous research.[110–113] Although daily folic acid supplementation is universally advised for women who are planning pregnancy or having pregnancy in the U.S., around one-quarter of this population do not follow the recommendation.[46] A mechanism by which maternal sufficient folate intake may protect against elevated blood pressure in children has been proposed.[114] Yet to the best of our knowledge, only three studies have estimated the effects of maternal folate using biomarkers in pregnancy on child blood pressure.[49,50,115] Of these, only the study by Wang et al. (2017) in Boston has found notably reduced odds of child elevated blood pressure with higher folate, restricted to the subset of mothers with cardiometabolic conditions.[116] The need for more evidence is acknowledged.

We investigated the associations of maternal dietary pattern measured by the Healthy Eating Index 2010 (HEI) and plasma folate with blood pressure in children aged 4-6, using data from the Conditions Affecting Neurocognitive Development and Learning in Early Childhood (CANDLE) Study. It was hypothesized that children would have lower blood pressure percentiles and reduced risks of high blood pressure if their mothers had better adherence to the 2010 Dietary Guideline for Americans, and/or had higher levels of plasma folate during pregnancy. This well characterized cohort has a large sample size adequate for examining important potential effect modifiers. Nutrition status is an indicator of socioeconomic status (SES) and health literacy, and obstacles to improving diet may co-occur with other behaviors that may impact child health, such as smoking or formula feeding.[45,117–119] Over- or suboptimal nutrition is directly linked with being overweight and obese.[120] In addition, there is evidence for sexual and racial difference in nutrient status and cardiovascular disease programming.[121,122] As such, we also examined whether the factors aforementioned, including child sex, maternal race, pre-pregnancy overweight or obesity, maternal smoking and breastfeeding practice, would modify the associations of interests in the current study.

Methods

Study population

The CANDLE study is a socio-demographically diverse birth cohort in Memphis, Tennessee, originally established to identify risk factors that impact child neurodevelopment and learning. It included pregnant women residing in Shelby County aged 16-40 who were 16-27 weeks of gestation with a singleton and low risk pregnancy, planned to deliver at a participating study hospital, and were able to speak and understand English. From 2006 to 2011, 1,503 participants were recruited from prenatal care clinics and the community. Women provided informed consent upon enrollment. More details of the sampling, recruitment and data collection have been described elsewhere.[123] We included 846 mother-child dyads with a primary exposure metric (either the Food Frequency Questionnaires (FFQ) in the 2nd trimester or plasma folate), and a valid measure of child blood pressure at age 4-6 years. All CANDLE research activities were approved by the Institutional Review Board of the University of Tennessee Health Sciences Center, and this secondary analysis was approved by the University of Washington Human Subjects Division.

Blood pressure assessment

Child blood pressure assessments were conducted by clinical researchers according to a standardized protocol at the age 4-6 years visit.[124] We first conducted an assessment of the child's arm circumference to estimate correct cuff size. After a rest period of at least two minutes, blood pressure measurements were taken twice in the right arm at heart level using BP Tru Medical Devices, Model BPM-100. Up to four measurements were taken if there was a discrepancy greater than 5 mmHg. Final BP values were calculated by averaging the measurements within a 5-mmHg difference. We calculated sex-, age- and height specific BP percentile using the American Academy of Pediatrics 2017 Clinical Practice Guideline, based on the U.S. pediatric population with normal weight.[71] HBP was defined as systolic and/or diastolic blood pressure at 90th percentile and above.

Maternal nutrition assessment

Maternal dietary patterns were assessed using the Block (2005) FFQ in the 2nd trimester, in which mothers reported intake of 111 food and beverage groups over the past 3 months.[125] The HEI-2010 and total energy intake for each woman were calculated based on her response. The HEI uses a scoring system to evaluate a set of foods aligning with key dietary recommendations from the 2010 U.S. Dietary Guidelines for Americans. [78] It comprises 12 components that sum to a total score ranging from 0 to 100, nine of which underline intake adequacy including fruits, vegetables and protein, and three underline moderation including refined grains, sodium and empty calories. The HEI components are nutrient density measurements expressed as either a percent of calories or per 1,000 calories.

Folate concentration was determined from maternal blood samples collected in the 2nd and 3rd trimester. Plasma was separated by centrifuging at 3000 pm for 10 minutes and stored at -70℃ until analysis. Folate levels were assessed using the 96-well plate adaptation of the Lactobacillus casei microbiological assay, with a minimum detection limit of 3 ng/mL.[79] All measurements were performed within 3 months of sample collection by one research associate throughout the study period using samples that were never previously thawed. Measurements from both trimesters were averaged. We further defined HEI below 59 as poor adherence to the dietary Guidelines according to grading system recently proposed by Krebs-Smith et al[80], and grouped maternal folate into two categories (1st quartile vs. 2nd – 4th quartiles).

Effect modifier assessments

We evaluated several potential effect modifiers. Child sex (female vs. male) was obtained from birth record. Maternal race (Black vs. White vs. others) and breastfeeding (ever vs. none) were self-reported. We identified prenatal smokers as those who reported smoking at enrollment and/or had a positive cotinine test with a minimum detective level of 10 ng/mL based on third trimester urine. [126] Pre-pregnancy overweight or obesity was defined as body mass index (BMI) 25.0 kg/m^2 and above based on clinical measure.[127]

Covariates

We included a number of maternal, child and other characteristics in this study. Maternal characteristics included age at delivery, race, education levels, marital status, insurance coverage, income adjusted by household size [75], parity, maternal psychopathology measured by the Global Severity Index (GSI) [76], hypertensive disorders of pregnancy and weight gained during pregnancy. Child characteristics included height, age and BMI z scores at age 4-6 years visit, small for gestational age[128], sleep quality, physical activity levels, medication use that potentially increases blood pressure (e.g. albuterol or methylphenidate). We also calculated the average child HEI, total energy intake and nutrition density adjusted folate from 24-hour food recall by parental reports at age 2 and age 3 visits.[129] Neighborhood level SES was measured using two domains of the Childhood Opportunity Index (COI) -- educational and economic opportunity.[77] We also included study recruitment site (safety net clinics vs. private clinics).

Statistical analysis

We conducted descriptive analyses to summarize the characteristics of the analytic sample overall and by maternal nutrition levels, and to estimate the distributions of exposures and outcomes. For continuous SBP and DBP percentiles, linear regressions with robust standard error and Generalized Additive Models (GAM) with a fitted smooth curve were used to assess the linear and non-linear associations with maternal HEI and plasma folate. As GAM can be sensitive to the presence of extreme predictors, the curves for plasma folate were truncated at 60 ng/mL. Poisson regressions with robust standard error were performed to estimate the incidence rate ratio (IRR) of binary HBP. Based on existing literature regarding risk factors for pediatric HBP as well as Directed Acyclic Graphs (DAG), we identified confounders, precision variables and potential mediators, and developed a hierarchical adjustment approach of four models. Model 1 was minimally adjusted for child sex, child height and age at age 4-6 years visit and study site. Model 2 was considered as the full model and was additionally controlled for maternal age at delivery, maternal race, education, marital status, insurance coverage, income adjusted by household size, pre-pregnancy BMI, breastfeeding practices, smoking during pregnancy, parity, GSI, child sleep quality, child physical activity levels, child medication use that potentially increase blood pressure, and COI. We further included total energy intake truncated at 750 and 5000 kcal in the models with maternal HEI to address potential residual confounding. In Model 3, we additionally controlled for child HEI and total energy intake in analyses of maternal HEI and child nutrient density adjusted folate in analyses of maternal plasma folate. Model 4 was an extended model with extra adjustments of four potential mediators: hypertensive disorders of pregnancy, weight gained during pregnancy, small for gestational age and child BMI z scores.

In the secondary analysis of effect modification, we included cross product terms of each exposure and effect modifier of interest (child sex, etc) and estimated interaction p-values and strata-specific associations using fully adjusted linear or Poisson models. In GAMs, we created factor-by-curve interactions, and obtained individual smoothing curves implying the exposure-outcome associations and p-values in each stratum. In an additional secondary analysis, we replaced the continuous maternal HEI and plasma folate with their binary forms and repeated the primary analyses and interaction models.

Three sensitivity analyses were performed. To explore whether confounding by child nutrition would be non-linear, we categorized child HEI and folate into quartiles and substituted the continuous measurements in Model 3. To estimate the magnitude of potential selection bias induced by availability of HEI data, we performed inverse-probability weighting (IPW) in the linear and Poisson regressions using the R package "ipw". Stabilized weights were computed using the ratio of predicted densities with truncation at 0.01 (weights in 1^{st} – 99^{th} percentiles were kept). As such, the pseudosample may be viewed as a random sample from the target population so that the estimates obtained are interpretable in terms of the scientific population of interest. To evaluate whether the percentiles accurately expressed a child's raw blood pressure in the reference population, child age and height at blood pressure assessment were modeled flexibly using two-dimensional unpenalized thin-plate regression splines (TPRS) in the full models. TPRS were generated from the MGCV package with varied df from five to twelve. All analyses were conducted in R 3.6.2 (R Cord Team, Vienna, Austria).

Results

Characteristics of the study population

The retention of the CANDLE study from enrollment to the age 4-6 years visit as well as the sample sizes for primary analyses of each maternal nutrition are illustrated in Figure 5. Two third of mothers included in this analysis self-identified as Black and approximately one fourth self-identified as White (Table 1). 61% of them had a high school education or less, half reported being married or living with a partner, and 48% of the participating families lived with a household income less than $25,000 per year. More than half of the mothers were classified as overweight or obese before pregnancy. 14% were defined as smokers during pregnancy based on self-report and/or urinary cotinine analyses. The average weight gained during pregnancy was 14.5 kg (SD: 7.2), and 5.8% developed hypertensive disorder. About two third breastfed their newborn, but less than half of them breastfed more than six months.

Children had an equal sex distribution, with a mean age of 4.4 years old (SD 0.6) at the time of blood pressure measurement. Less than 10% were small for gestational age, according to an updated U.S.-based sex-specific birth weight for gestational age reference. Although 75% reported having vigorous physical activities three or more times per week, 15% were categorized as overweight at the age 4-6 years visit, and another 15% as obese. Based on parent report, 8% were taking medications that potentially increased blood pressure, such as albuterol or methylphenidate. The results from 24-hour food recall showed the average child HEI as 52.4 (SD: 10.4), total energy intake as 1481.1 kcal (SD: 851.8) and nutrient density adjusted folate as 209.1 μg/1000 kcal (SD: 92.2).

Compared with mothers with HEI greater than 59, mothers with HEI 59 or below were more likely to be younger at CANDLE child delivery, self-identify as Black, have a lower annual household income and education level, smoke during pregnancy and feed their newborn with formula (Table 1). Their children were more likely to be small for gestational age, take medication that potentially increases blood pressure, be less physically active, have poorer dietary quality and lower folate intake. Similarly, higher maternal plasma folate levels were related with higher SES and more health promoting behaviors. We did not observe meaningful difference in baseline characteristics comparing the CANDLE population at enrollment and the two analytic samples available for maternal HEI and plasma folate, except that a larger portion of mothers recruited from the private clinics had available FFQ data than their counterparts from the safety net hospitals (Appendix Table 1).

Child blood pressure

Average SBP was 92.3 mmHg (SD: 9.9) for raw measurement and 48.6 (SD: 25.5) for percentile, and average raw DBP and DBP percentile were 61.1 mmHg (SD: 9.1) and 75.7 (SD: 19.3), respectively. The data of both BP raw measurements (data not shown) and SBP percentile (Figure 6) was normally distributed, while DBP percentile was left skewed. 29.6% of the children with SBP or DBP percentile at 90th percentile and above were classified as HBP, largely driven by isolated diastolic HBP.

Maternal nutrition

Distributions of maternal HEI and plasma folate are shown in Figure 7. Maternal HEI ranged from 26.2 to 88.4 with a mean of 60.0 (SD: 11.32), and more than 75% were under 70. The average maternal total energy intake was 2396 kcal (SD: 965). Plasma folate ranged from 2.6 ng/mL to 80.5 ng/mL with a mean of 23.1 ng/mL (SD: 11.1), and the maximum of folate in the 1st quartile was 15.7 ng/mL. Although universally accepted cutoffs to define folate deficiency using plasma samples in pregnancy are uncertain, the folate concentrations within the 2nd - 4th quartiles were considered as adequate according to a report from the WHO Technical Consultation and a population-based randomized trial in China.[130,131] The pairwise correlations of maternal HEI and plasma folate (corr: 0.30), maternal and child HEI (corr: 0.27), and maternal plasma folate and child folate (corr: 0.11) were moderate.

Associations between maternal nutrition and child blood pressure

The DAG showing the exposure-outcome associations are presented in Appendix Figure 3. Overall, we found no evidence of the associations of maternal HEI and plasma folate with child BP percentiles and HBP from multivariate linear and Poisson regressions (Table 2). The smooth effect curves generated from GAMs (Figure 8) indicated no significant departures from linearity overall, except that we observed a "w" shaped visual pattern for the association between maternal plasma folate and child SBP percentile from Model 3 (p = 0.027). For maternal folate levels below 15 ng/mL and between 30-40 ng/mL, there was an inverse association with child SBP percentile independent of child nutrient density adjusted folate, however, the associations were positive for maternal folate levels between 20-30 ng/mL or above 40 ng/mL. Nonetheless, this finding might be driven by the right tail and could be spurious.

Five potential effect modifiers for the associations of maternal nutrition in pregnancy with offspring cardiometabolic traits were investigated – child sex, maternal race, maternal pre-pregnancy weight status, smoking during pregnancy and breastfeeding (Figure 9). In mothers self-identified as White, we observed an inverse association between maternal HEI and child SBP percentile. Each 1-unit increase of maternal HEI was associated with 0.45 lower SBP percentile in offspring (beta: -0.45, 95% CI: -0.80, -0.09). This association was null in mothers self-identified as Black (beta: 0.09, 95% CI: -0.19, 0.37) or other race (beta: -0.14, 95% CI: -0.79, 0.50), and the interaction term between maternal race and the HEI was insignificant (p = 0.07). In addition, a marginally reduced risk of child HBP for each 1-ng/mL increase of maternal plasma folate (IRR: 0.94, 95% CI: 0.88-0.998) was suggested in mothers self-identified as other race. In women who were overweight or obese before pregnancy, a moderate w-shaped non-linear relationship between plasma folate and child SBP percentile was observed (p = 0.026).

Results from the analyses with binary maternal HEI indicated that an index greater than 59 was associated with 4.09 lower child DBP percentile (beta: -4.09, 95% CI: -8.16, -0.03) when child HEI, total energy intake and other confounders were taken account (Model 3) (Table 3). In agreement with the results from the effect modification assessment with continuous form, in Whites, child SBP percentile was 10.87 lower (beta: -10.87, 95% CI: -20.00, -1.76) comparing mothers with HEI above 59 to those with HEI 59 and below (Figure 10). We also found a significant interaction between child sex and maternal HEI on child HBP (p interaction = 0.01): maternal HEI above 59 was associated with a 43% reduction in risk of HBP in girls but no reduction in boys (Girls: IRR:

0.57, 95% CI: 0.34, 0.96; Boys: IRR: 1.24, 95% CI: 0.84, 1.84). In women who were overweight or obese before pregnancy, a moderate w-shaped non-linear relationship between plasma folate and child SBP percentile was suggested (p = 0.026) (Figure 11). No interaction of smoking during pregnancy and breastfeeding with any outcome was detected. When dichotomized at the 1st quartile, plasma folate was not related to any outcome in overall sample or any stratum. In the sensitivity analyses, replacing continuous child HEI and folate with their quartiles did not produce meaningful changes in effect estimates and precision (Appendix Table 2). There was no meaningful difference in point estimates and precisions comparing the linear and Poisson regressions with or without IPW (Appendix Table 3). We also obtained similar results with the primary analysis of BP percentile when controlling for child age and height at visit using TPRS with degree of freedom varying from 5 to 12 (Appendix Figure 4).

Discussion

In this analysis of prospective data from a U.S. urban birth cohort with high socio-demographic diversity, we found no association in the overall sample of maternal nutrition in pregnancy, measured by HEI and plasma folate, with child blood pressure at age 4-6 years visit. The conclusion remained the same after adjustments for potential mediators and in sensitivity analyses. In White mothers, those with a better adherence to the 2010 Dietary Guideline for Americans in early pregnancy had offspring with lower SBP percentile. Relatively better maternal dietary quality (HEI>59) was associated with reduced HBP in girls. A moderate non-linear relationship was suggested for maternal plasma folate and child SBP percentile in women with pre-pregnancy overweight or obesity. However, these findings should be interpreted with caution owing to the multiple comparisons in the analysis of effect modifiers. The results also suggested that child nutrition may confound the non-linear maternal nutrition-child blood pressure relationships.

Our study has several strengths. To our knowledge, we are the first study to use HEI as a measurement for diet quality in pregnant women to predict offspring blood pressure. Although other diet quality scores, such as Mediterranean Diet Score or Dietary Approaches to Stop Hypertension (DASH), are also designed to evaluate adherence to specific dietary recommendations, the food compositions, weights assigned to each food group, and cutoffs of intake of nutrients vary widely for each score, suggesting that identification of suitable measurements for intended populations and health outcomes is influencial.[132,133] We elected to use HEI because the Dietary Guidelines aim to help the general American population to make healthy choices about food and beverages in their daily lives, and systematic reviews and large meta-analyses have convincingly demonstrated that higher diet quality, as described with the use of HEI, is associated with lower risks of CVD mobility and mortality.[134,135] Another strength of our study is the application of nutrient biomarker data. Folate levels determined from self-reported data could overestimate the intake from diet and supplemental folic acid, particularly when multiple folate sources are consumed together, as one may reduce the absorption and bioavailability of the others.[136] Utilization of plasma folate, which serves as an endpoint of folate intake, is more likely to reveal the true exposure-outcome associations. In addition, contributory evidence from observational nutrition studies is commonly subject to residual confounding. A predisposition to cardiometabolic disorder may be attributable to postnatal nutrition as much as

prenatal nutrition. We were able to adjust rigorously for individual and neighborhood level SES and extensively control for child diet. Furthermore, previous studies have suggested adverse health effects from both under- and overnutrition, and non-linear associations were frequently assessed by using tertiles or quartiles, the arbitrary cut-offs of which limit the external generalizability to other populations. Here we adopted two approaches to assess the non-linear associations -- curve fitting as well as analysis of cut-offs with public health meaning. Finally, child blood pressures were standardized using the 2017 guidelines with the normal weight pediatric population as the reference, which performed better than the 2004 guidelines in identifying children with adverse cardiometabolic profiles. [82]

In this analysis, we did not detect associations between maternal HEI and child blood pressure at age 4-6 years. The findings are in agreement with the two European studies with maternal dietary quality. The Infancia y Medio Ambiente (INMA) study in Spain reported null relationships between maternal Mediterranean diet scores and child cardiometabolic risk at age 4. The Generation R study in the Netherlands used a data-driven approach to define maternal posteriori-dietary patterns and found no association with child blood pressure at age 6.[106,107] However, a similar study based on pooled data from two birth cohorts, one in Greece and one in the U.S., observed lower offspring SBP and DBP in mid-childhood if mothers had a better adherence to the Mediterranean diet.[108] Other observational studies also have estimated the effects of single food or nutrient intakes in pregnancy on child blood pressure, such as carbohydrate, protein or fat, or the effects of maternal dietary patterns on health conditions that are closely relevant to child blood pressure, such as fetal growth or obesity, but the results are inconclusive.[27–33,137–139] Good-quality diets defined by different indexes share certain characteristics in common, such as being high in vegetables, fruit and fish, and low in processed meat and saturated fats. The mechanism linking maternal dietary patterns and child blood pressure is uncertain. Three major hypotheses have been suggested: the “thrifty phenotype” proposes that suboptimal maternal nutrition leads to adaptive responses in utero, subsequently resulting in intrauterine growth restriction. When exposed to an abundant food supply after birth, an undernourished infant might experience catch-up growth, which has been associated with amplified risks of hypertension, diabetes and CVD later in life. [10,92–94,98] The epigenetic alternations induced by poor maternal nutrition consist of upregulation of gene expression in adipogenesis, glucose homeostasis, inflammation, and/or insulin signaling, and DNA methylation repressing transcriptions of hormones such as leptin, nuclear receptors, gluconeogenic enzymes and transmembrane proteins.[140,141] An adverse in-utero environment also has direct impacts on placenta morphology, feto-maternal exchanges and endocrine function, further leading to impaired fetal tissue development and a higher susceptibility to cardiometabolic diseases later in life.[142]

There may be several explanations for the null results in our study with maternal HEI. First, as the previous studies have shown, the effects of prenatal nutrition exposure might not appear until age 6.[108] Second, we administrated the FFQ in the 2nd trimester, which was more likely to reflect the nutrition status in early pregnancy. Although the 1st trimester is critical for programming the lipid profile, the 2nd and 3rd trimesters are the windows when nephron development, adipogenesis and fat accumulation mostly occur. [89–91,143,144] Third, despite that the CANDLE

population has a high proportion of low SES families and more than 75% of mothers had a poor pregnancy diet, they were relatively young with a low medical risk pregnancy, and extreme under- or overnutrition was rarely observed. The associations might be more detectable when studying extreme exposures with greater contrasts. Some stratum specific associations between maternal HEI and child blood pressure were evident. National representative data has indicated sexual and racial differences in cardiovascular disease programming [122]. Mothers who self-identified as White in the CANDLE cohort tended to have a higher HEI, and there is a potential misclassification of exposures when some foods and nutrients consumed by the other race groups are underrepresented by the Dietary Guidelines. Sex-specific associations of child blood pressure with other maternal nutritional measurements have been previously reported [38,41,145]. Animal studies have also shown that the response to poor maternal diet triggers sex-specific epigenetic alternations in offspring, together with sexually dimorphic deregulation of certain genes, further resulting in sexual disparities in uptake and metabolism of amino acids, glucose and fatty and chronic disease development [146].

We detected little evidence of associations between maternal plasma folate and child blood pressure. The findings are consistent with the two micronutrient supplement trials in rural Nepal and Bangladesh, and the two observational studies addressing this research topic in the Netherlands.[35,49,115,147] The study based on the Boston Birth Cohort reported 40% reduced odds of child elevated blood pressure when comparing mothers with plasma folate levels above median to below median, in the subset of mothers with cardiometabolic conditions.[116] Another study by Wang et al. using data from the same cohort has reported an "L" shaped association between maternal plasma folate and child overweight or obesity.[148] In line with the Boston study, our results suggested a moderate non-linear relationship between maternal plasma folate and child SBP percentile in women with overweight or obesity before pregnancy, but the GAM curve indicated "w" shaped associations. Adequate maternal folate levels may protect against offspring elevated blood pressure by reducing plasma homocysteine concentrations and increasing NO synthesis in endothelial cells, scavenging superoxide anions, and subsequently counteracting oxidative stress and promoting resilience of vessels.[149–152] However, the plausible biological mechanism explaining this "w" shaped relationship needs future explorations. Even though our study had relatively higher maternal folate concentrations compared with the studies aforementioned and the national representative data of women of childbearing age[153], most of the studies including ours have obtained similar null results. It could be due to the fact that the associations between maternal folate and child cardiovascular health are likely to be non-linear, and the ability to detect potential effects may vary by the utilization of study specific plasma folate thresholds. Further investigations on potential universally accepted population cut-offs of pregnancy plasma folate to predict different child health outcomes are required.

Our study has some limitations. One is the self-reported nature of maternal HEI and child nutrition. Even though these measurements were nutrient density adjusted, potential measurement error may still exist. In addition, as plasma folate is an indicator for short-term exposure, it is an imperfect measure of chronic deficiency in pregnancy.[97] It is also difficult to pinpoint the food source of folate biomarker and therefore to inform dietary interventions. Furthermore, we cannot rule out the misclassification of HBP. Although BP was measured

repeatedly during assessment, the examination was performed on a single occasion. As such, the definition of HBP in our analysis does not meet the clinical definition. [82,95,96] Moreover, although we did not observe meaningful difference in baseline characteristics between the families at enrollment and the analytic samples for each nutritional measurement, and analyses with IPW indicated limited selection bias, selection bias remains a concern. Finally, due to the skewed socio-demographic features and other health characteristics in our study sample, the findings of this study may not be generalized to the other populations.

Despite the limitations, our study shows that lower maternal nutrient status characterized by the HEI and plasma folate in early pregnancy is not associated with child blood pressure in a community-based cohort in the U.S. It contributes to the evolving science regarding the developmental origins of disease, and informs the pregnancy nutrition interventions in subpopulations. Identification of unhealthy maternal dietary patterns and threshold effects of micronutrients is critical for the development of pregnancy-specific dietary guidelines, the implementation of which may largely improve both maternal and child health. Continuing investigations are needed to verify our hypotheses in other well characterized populations.

Tables and Figures

Figure 5. Inclusion Flowchart

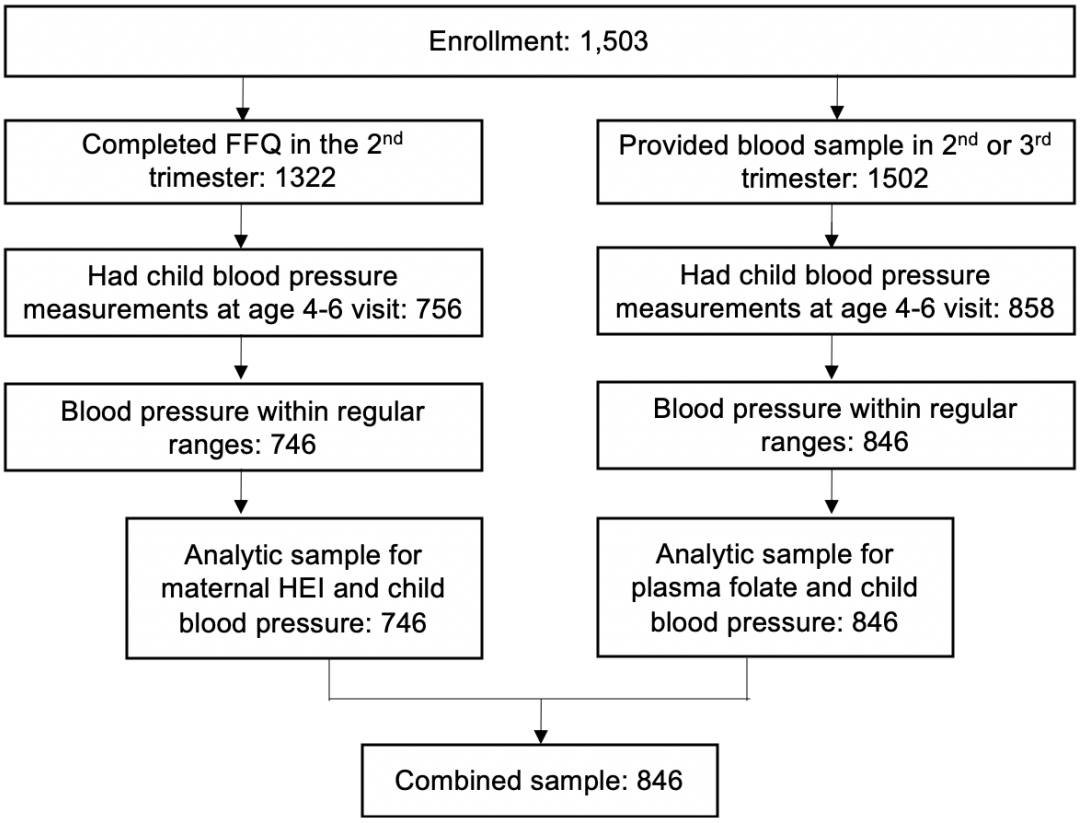


Table 4. Characteristics of CANDLE participants in the overall analytic sample and by maternal nutrition levels

	Analytic sample (N=846)		HEI≤59 (N=352)		HEI>59 (N=394)		1st folate quartile (N=225)		2nd-4th folate quartiles (N=621)	
	Mean/ Counts	Std/Percentage	Mean/ Counts	Std/Percentage	Mean/ Counts	Std/Percentage	Mean/ Counts	Std/Percentage	Mean/ Counts	Std/Percentage
Child characteristics										
Child age at CV4 (years)	4.4	0.6	4.5	0.7	4.3	0.5	4.5	0.7	4.4	0.6
Child height at CV4 (cm)	106.4	6.1	106.4	6.3	106.0	5.7	107.2	6.7	106.1	5.8
Sleeping scores at CV4	46.8	7.3	47.6	7.3	45.9	7.0	48.3	7.1	46.3	7.2
Birth weight	3.2	0.6	3.2	0.6	3.3	0.5	3.1	0.5	3.3	0.6
Gestational age (weeks)	38.8	1.8	38.6	2.0	38.9	1.7	38.8	2.0	38.8	1.7
Child diet										
Child HEI	52.4	10.4	50.2	10.7	54.8	9.9	50.8	9.6	52.9	10.6
Child total energy intake	1481.1	851.8	1517.2	596.2	1473.3	1067.6	1605.9	823.2	1440.2	857.8
Child nutrient density adjusted folate	209.1	92.2	198.8	86.1	215.4	91.0	199.1	91.2	212.4	92.4
Child sex										
Male	421	49.8%	177	50.3%	200	50.8%	116	51.6%	305	49.1%
Female	425	50.2%	175	49.7%	194	49.2%	109	48.4%	316	50.9%
Pre-term delivery (<37 weeks)	76	9.0%	40	11.4%	26	6.6%	24	10.7%	52	8.4%
Low birth weight	59	7.0%	37	10.6%	18	4.6%	24	10.7%	35	5.7%
Small for gestational age	77	9.2%	39	11.2%	27	6.9%	26	11.6%	51	8.3%
Medication use potentially leading to hypertension	67	7.9%	36	10.2%	26	6.6%	18	8.0%	49	7.9%
Vigorous activity frequency										
Never or occasionally	121	14.5%	60	17.3%	33	8.5%	42	19.1%	79	12.9%
Once or twice per week	90	10.8%	42	12.1%	36	9.2%	29	13.2%	61	9.9%
Three or more times per week	623	74.7%	244	70.5%	321	82.3%	149	67.7%	474	77.2%
BMI class at visit										

Underweight	21	2.5%	11	3.1%	10	2.5%	5	2.2%	16	2.6%
Normal weight	571	67.6%	239	68.1%	267	67.8%	150	66.7%	421	67.9%
Overweight	125	14.8%	48	13.7%	58	14.7%	32	14.2%	93	15.0%
Obesity	128	15.2%	53	15.1%	59	15.0%	38	16.9%	90	14.5%
Maternal characteristics										
Maternal age at birth (years)	26.1	5.5	24.4	4.9	28.0	5.4	24.5	4.7	26.6	5.7
Adjusted household income	17019.8	16638.2	10966.1	12436.6	24097.8	17549.3	8827.2	10041.6	19958.0	17534.5
Total weight gained during pregnancy	14.5	7.2	14.2	7.5	14.7	6.8	12.9	7.9	15.0	6.8
GSI scores	50.3	9.5	50.8	9.9	49.7	9.3	51.6	10.1	49.8	9.2
Maternal race										
Black	563	66.6%	274	77.8%	211	53.6%	202	89.8%	361	58.1%
White	229	27.1%	56	15.9%	159	40.4%	12	5.3%	217	34.9%
Asian	8	1.0%	0	0.0%	8	2.0%	1	0.4%	7	1.1%
Other	1	0.1%	1	0.3%	0	0.0%	0	0.0%	1	0.2%
Multiple race	45	5.3%	21	6.0%	16	4.1%	10	4.4%	35	5.6%
Maternal education										
< High School	114	13.5%	66	18.8%	26	6.6%	55	24.4%	59	9.5%
High School/GED	403	47.7%	207	58.8%	147	37.3%	131	58.2%	272	43.9%
Technical School	85	10.1%	30	8.5%	42	10.7%	18	8.0%	67	10.8%
College Degree	150	17.8%	32	9.1%	108	27.4%	16	7.1%	134	21.6%
Grad/Professional Degree	93	11.0%	17	4.8%	71	18.0%	5	2.2%	88	14.2%
Maternal marital status at enrollment										
Married	296	35.0%	78	22.2%	199	50.5%	30	13.3%	266	42.9%
Widowed/Divorced/Separated/Never married	398	47.1%	191	54.3%	140	35.5%	136	60.4%	262	42.3%
Living with partner	151	17.9%	83	23.6%	55	14.0%	59	26.2%	92	14.8%
Insurance status										

No insurance	2	0.2%	0	0.0%	2	0.5%	0	0.0%	2	0.3%
Medicaid or Medicare only	510	60.3%	259	73.6%	170	43.2%	192	85.3%	318	51.2%
Medicaid/Medicare and private insurance	28	3.3%	17	4.8%	10	2.5%	8	3.6%	20	3.2%
Private insurance only	306	36.2%	76	21.6%	212	53.8%	25	11.1%	281	45.3%
Baseline household income										
$0-$24,999	372	48.3%	198	64.7%	114	29.8%	133	70.0%	239	41.2%
$25,000-$54,999	196	25.5%	70	22.9%	116	30.4%	42	22.1%	154	26.6%
$55,000-$74,999	83	10.8%	19	6.2%	61	16.0%	7	3.7%	76	13.1%
$75,000 or over	119	15.5%	19	6.2%	91	23.8%	8	4.2%	111	19.1%
Maternal smoking (Urinary Cotinine + Self report)	115	13.6%	58	16.5%	38	9.7%	45	20.1%	70	11.3%
Maternal alcohol consumption	76	9.0%	33	9.4%	40	10.2%	14	6.2%	62	10.0%
Maternal supplement intake of Vitamins	780	94.0%	316	92.9%	378	96.7%	190	87.6%	590	96.3%
BMI class										
Underweight	41	4.9%	18	5.1%	17	4.3%	12	5.4%	29	4.7%
Normal	340	40.3%	142	40.6%	165	42.0%	72	32.1%	268	43.3%
Overweight	186	22.1%	83	23.7%	75	19.1%	46	20.5%	140	22.6%
Obese	276	32.7%	107	30.6%	136	34.6%	94	42.0%	182	29.4%
Breastfeeding										
No	275	34.6%	157	48.9%	83	21.5%	96	48.2%	179	30.1%
Yes (6 months or less)	305	38.4%	116	36.1%	157	40.7%	63	31.7%	242	40.7%
Yes (Above 6 months)	214	27.0%	48	15.0%	146	37.8%	40	20.1%	174	29.2%
Pregnancy hypertensive disorder	49	5.8%	17	4.8%	27	6.9%	10	4.4%	39	6.3%
Parity										
No prior births	512	60.5%	232	65.9%	221	56.1%	177	78.7%	335	54.0%
At least one prior birth	334	39.5%	120	34.1%	173	43.9%	48	21.3%	286	46.1%

Other characteristics										
Childhood Opportunity Index										
Prenatal Educational index	-0.03	0.5	-0.2	0.5	0.1	0.6	-0.3	0.3	0.1	0.6
Prenatal Economics index	-0.1	0.6	-0.2	0.6	0.02	0.6	-0.4	0.6	-0.03	0.6
Postnatal Educational index	-0.02	0.5	-0.1	0.5	0.1	0.6	-0.3	0.3	0.1	0.6
Postnatal Economics index	-0.07	0.6	-0.2	0.6	0.1	0.6	-0.3	0.5	0.03	0.6
Site										
Private clinics	650	76.8%	252	71.6%	371	94.2%	137	60.9%	513	82.6%
Safety net hospitals	196	23.2%	100	28.4%	23	5.8%	88	39.1%	108	17.4%

Abbreviations: Body Mass Index, BMI; Global Severity Index, GSI

Figure 6. Distributions of child SBP and DBP percentiles

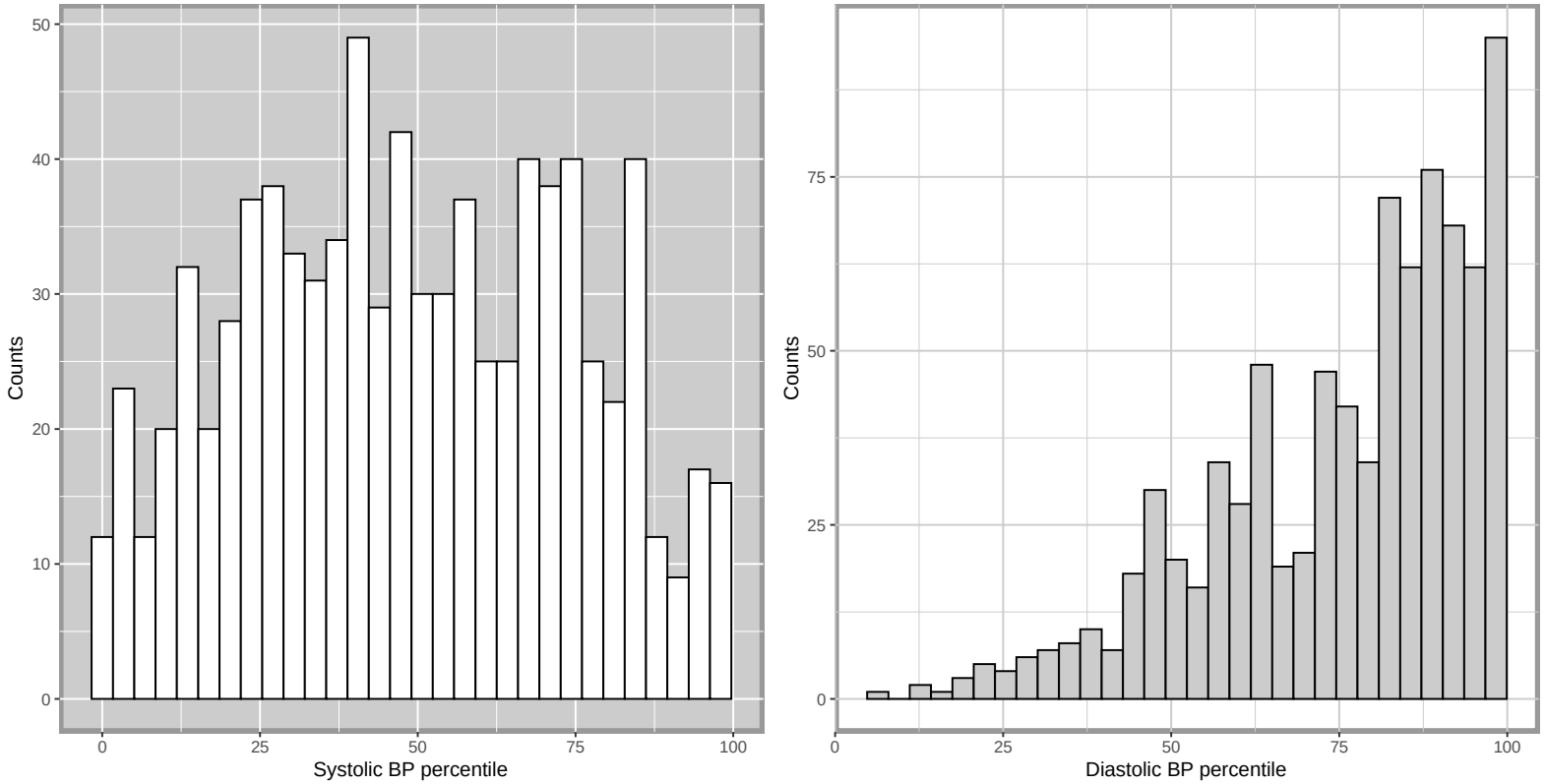


Figure 7. Distributions of maternal HEI and plasma folate during pregnancy

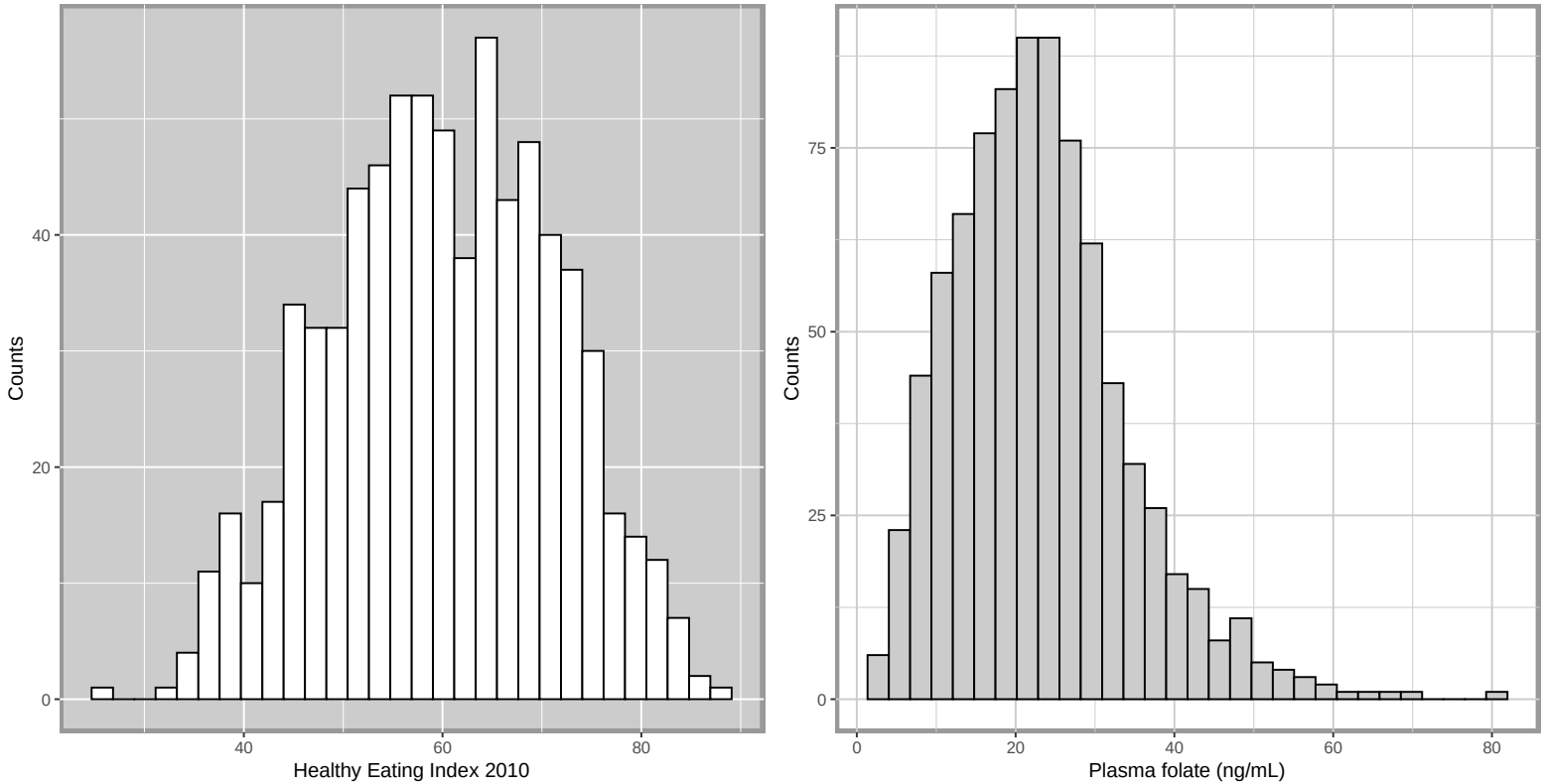


Table 5. Estimated effects of maternal HEI and plasma folate on BP percentiles and HBP

	Maternal HEI		Plasma folate	
Model	Beta/IRR	95% CI	Beta/IRR	95% CI
Systolic BP percentile				
Model 1	-0.14	(-0.31, 0.03)	-0.02	(-0.19, 0.14)
Model 2	-0.10	(-0.32, 0.13)	0.03	(-0.17, 0.24)
Model 3	-0.12	(-0.37, 0.12)	0.05	(-0.17, 0.27)
Model 4	-0.10	(-0.33, 0.12)	0.06	(-0.15, 0.27)
Diastolic BP percentile				
Model 1	-0.09	(-0.21, 0.04)	-0.01	(-0.12, 0.11)
Model 2	-0.05	(-0.23, 0.13)	0.03	(-0.12, 0.18)
Model 3	-0.06	(-0.26, 0.13)	0.01	(-0.15, 0.17)
Model 4	-0.05	(-0.24, 0.14)	0.02	(-0.13, 0.17)
HBP				
Model 1	0.995	(0.98, 1.01)	0.998	(0.99, 1.01)
Model 2	1.002	(0.99, 1.02)	0.998	(0.99, 1.01)
Model 3	0.997	(0.98, 1.01)	0.999	(0.99, 1.01)
Model 4	1.003	(0.99, 1.02)	0.998	(0.99, 1.01)

Abbreviations: HEI: Healthy Eating Index, BP: blood pressure, HBP: high blood pressure, IRR: incidence rate ratio

Model 1: adjusted for child sex, child height and age at age 4-6 years visit and study sites

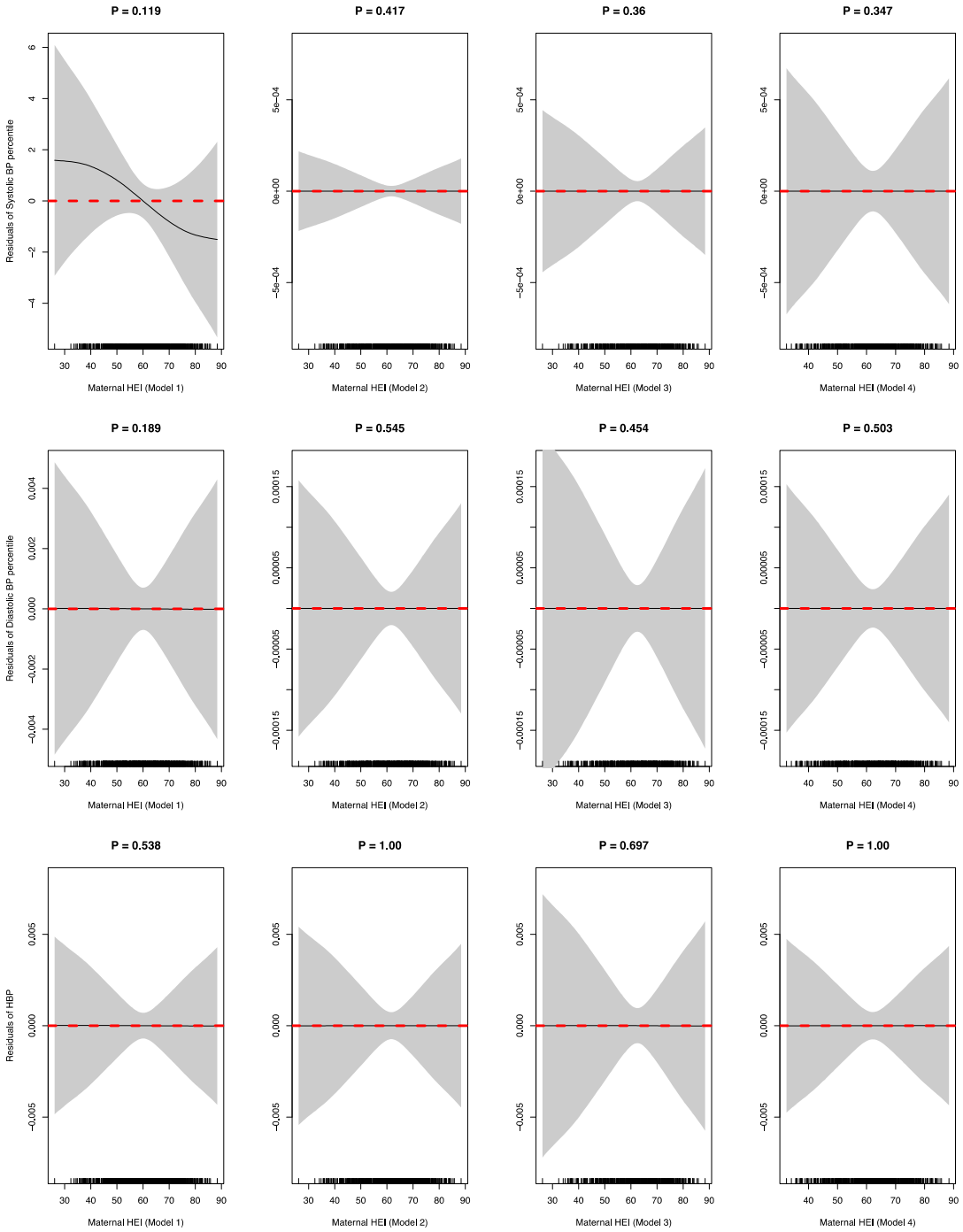
Model 2 (full model): Model 1 additionally controlled for maternal age at delivery, maternal race, education levels, marital status, insurance coverage, income adjusted by household size, pre-pregnancy BMI, breastfeeding practices, smoking during pregnancy, parity, GSI, child sleep quality, child physical activity levels, child medication use that potentially increase blood pressure, and COI. Maternal total energy intake was further controlled in the model with maternal HEI

Model 3: Model 2 with maternal HEI additionally controlled for child HEI and total energy intake, or Model 2 with maternal plasma folate additionally controlled for child nutrient density adjusted folate

Model 4: Model 2 additionally controlled for hypertensive disorders of pregnancy, weight gained during pregnancy, small for gestational age and BMI z scores

Figure 8. GAM curves for the associations of maternal HEI and plasma folate in pregnancy and child blood pressure

A) Maternal HEI



B) Maternal plasma folate

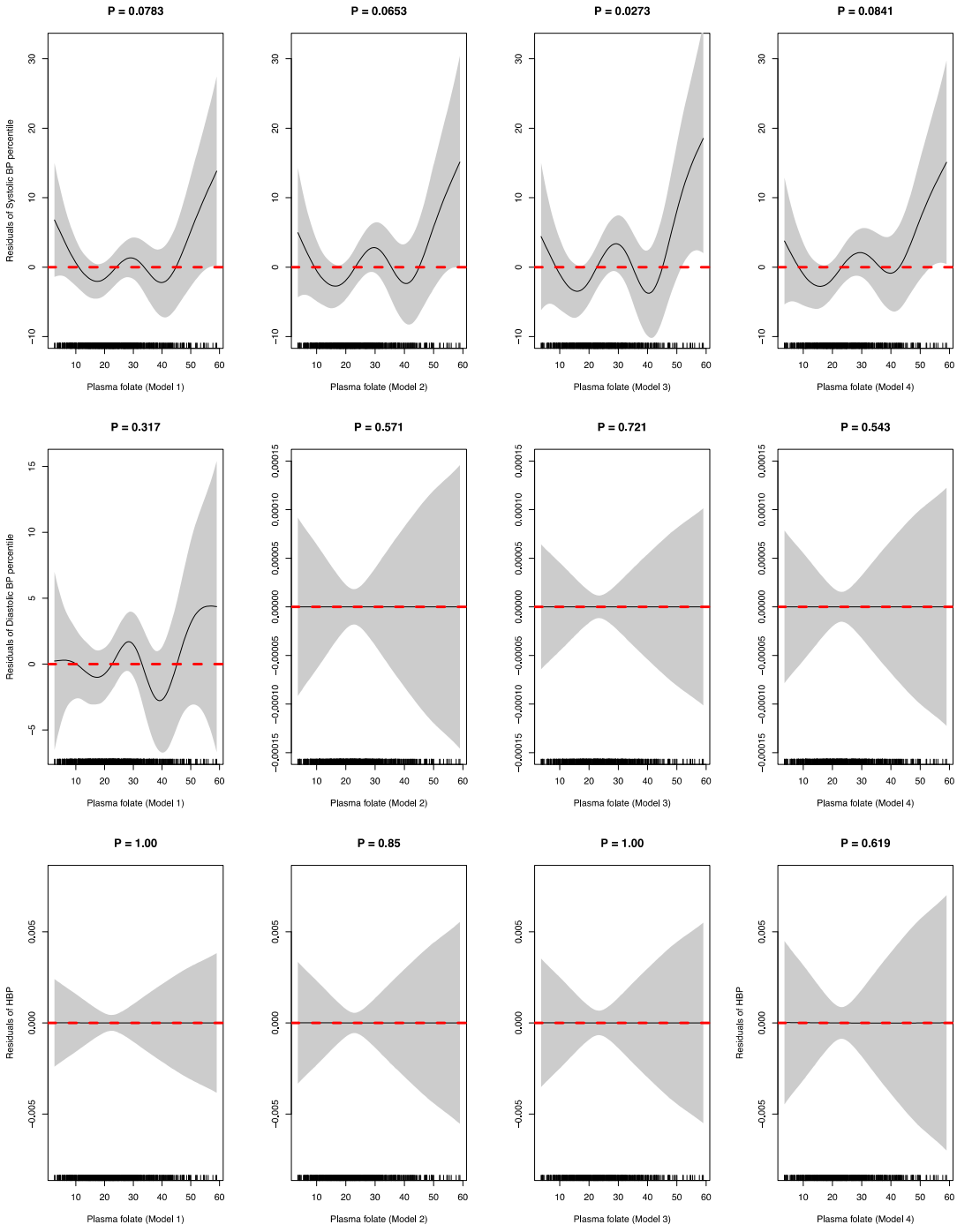


Figure 9. Estimated effects of maternal HEI and plasma folate on BP percentiles and HBP by child sex (female vs. male), maternal race (Black vs. White vs. Other), maternal overweight/obesity before pregnancy (Overweight/obesity vs. Non overweight/obesity), maternal smoking during pregnancy (Smoker vs. Nonsmoker) and breastfeeding practice (Ever vs. No breastfeeding) from linear and Poisson interaction models.

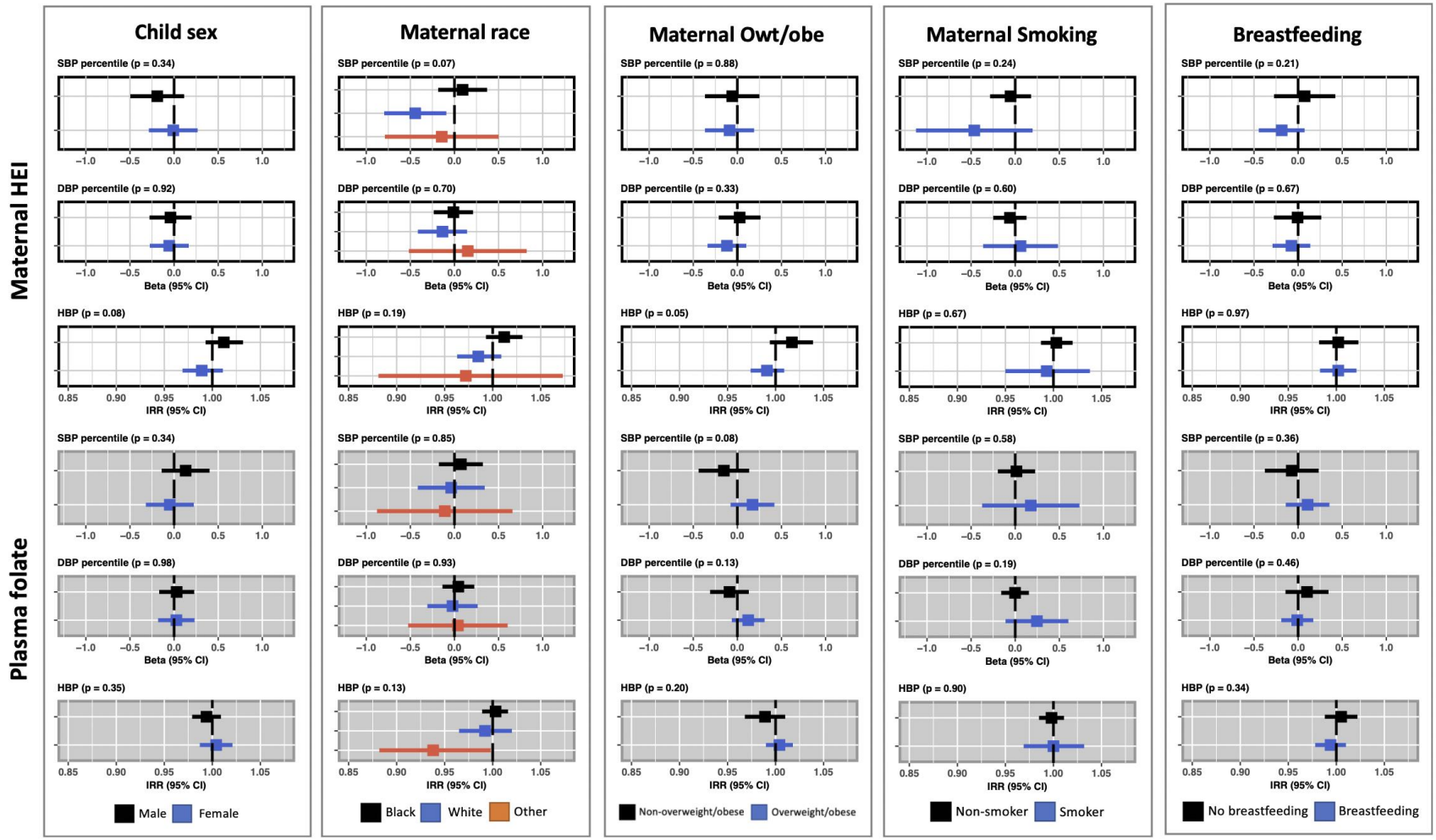
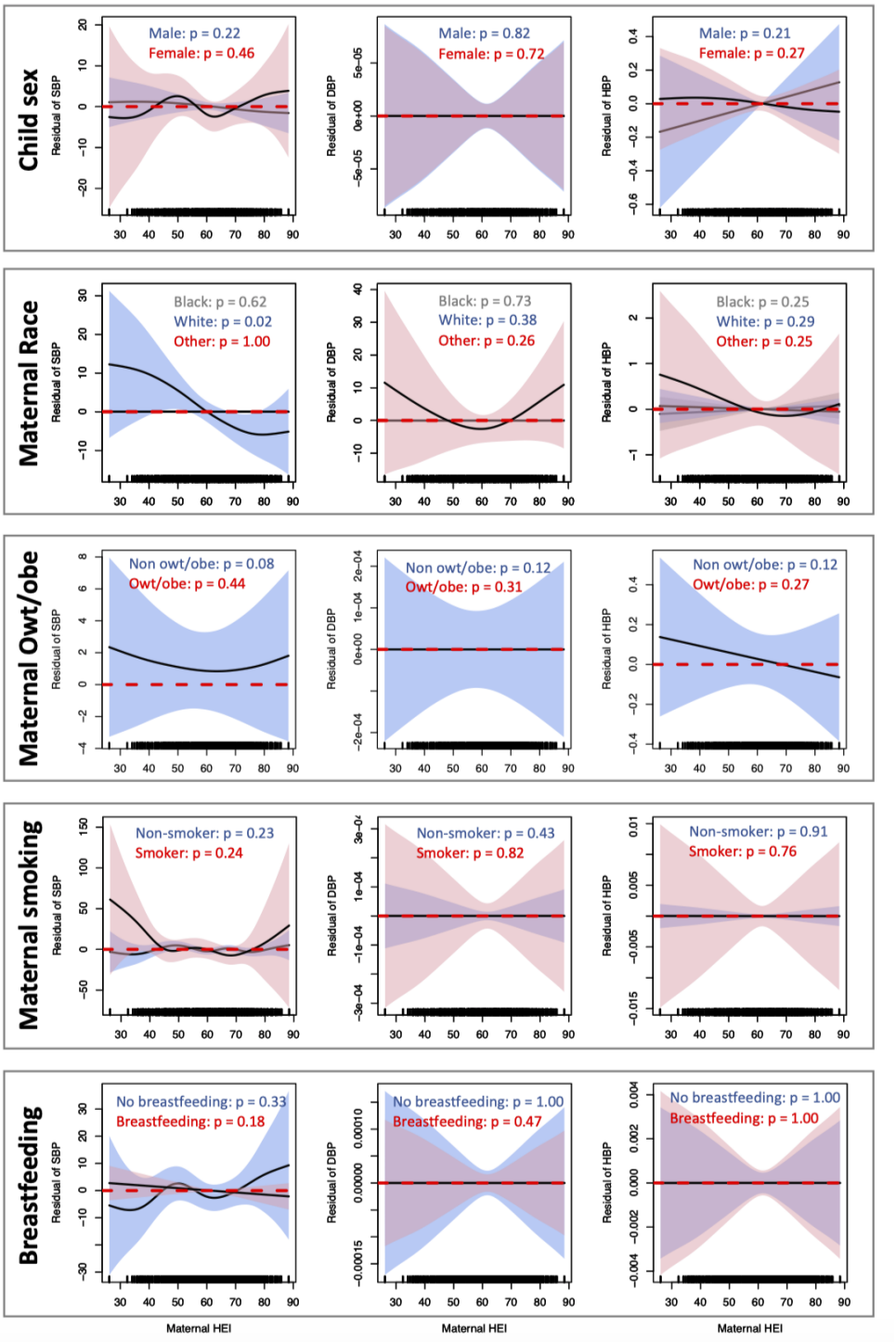


Figure 10. Estimated effects of maternal HEI and plasma folate on BP percentiles and HBP by child sex (female vs. male), maternal race (Black vs. White vs. Other), maternal overweight/obesity before pregnancy (Overweight/obesity vs. Non overweight/obesity), maternal smoking during pregnancy (Smoker vs. Nonsmoker) and breastfeeding practice (Breastfeeding vs. No breastfeeding) from GAM interaction models.

A) Maternal HEI



B) Plasma folate

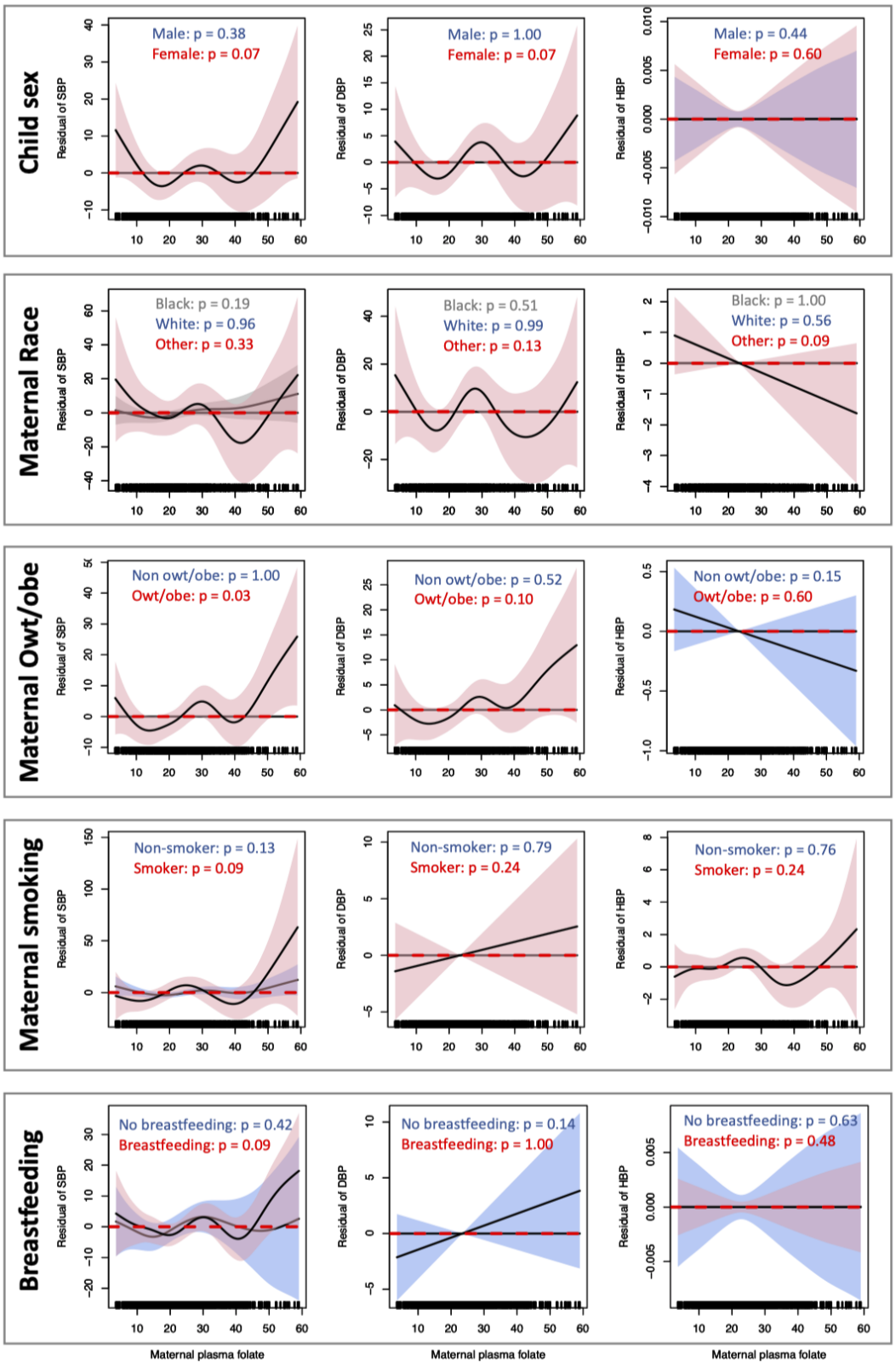


Table 6. Estimated effects of binary maternal HEI and plasma folate levels on BP percentiles and HBP

	Maternal HEI (<= 59 vs. > 59)		Plasma folate (1st quartile vs. 2-4 quartiles)	
Model	Beta/IRR	95% CI	Beta/IRR	95% CI
Systolic BP percentile				
Model 1	-3.22	(-6.93, 0.50)	-1.41	(-5.38, 2.55)
Model 2	-2.83	(-7.74, 2.09)	0.02	(-5.00, 5.04)
Model 3	-4.33	(-9.83, 1.17)	-0.20	(-5.92, 5.51)
Model 4	-3.56	(-8.50, 1.38)	0.21	(-4.99, 5.40)
Diastolic BP percentile				
Model 1	-2.57	(-5.29, 0.16)	0.26	(-2.55, 3.07)
Model 2	-2.20	(-5.83, 1.44)	1.10	(-2.66, 4.86)
Model 3	-4.09	(-8.16, -0.02)	1.05	(-3.22, 5.31)
Model 4	-2.75	(-6.50, 1.00)	0.67	(-3.24, 4.58)
HBP				
Model 1	0.83	(0.65, 1.05)	0.96	(0.76, 1.21)
Model 2	0.91	(0.66, 1.27)	0.96	(0.71, 1.29)
Model 3	0.77	(0.55, 1.09)	1.00	(0.72, 1.38)
Model 4	0.89	(0.64, 1.24)	0.87	(0.64, 1.17)

Abbreviations: HEI: Healthy Eating Index, BP: blood pressure, HBP: high blood pressure, IRR: incidence rate ratio

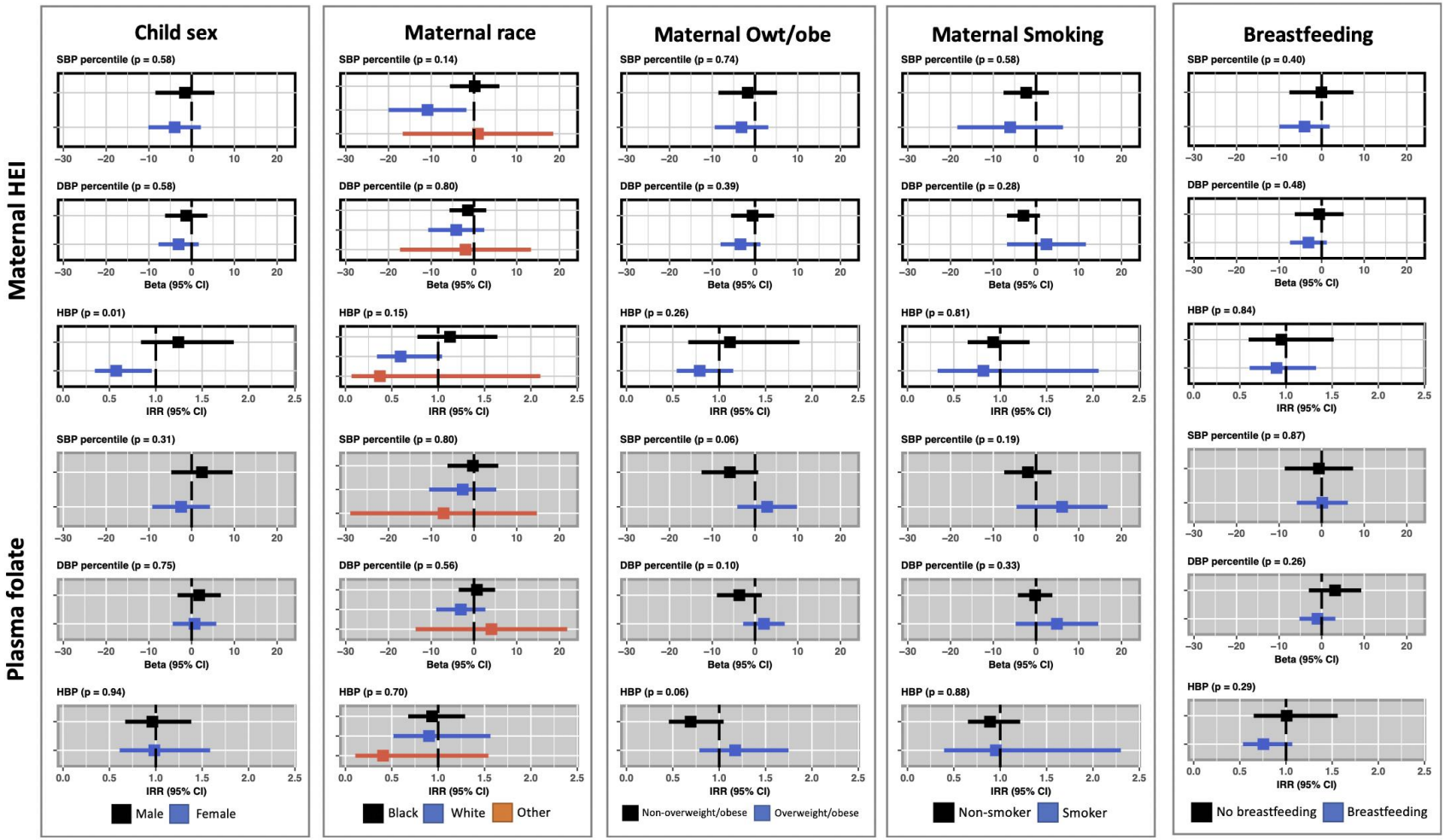
Model 1: adjusted for child sex, child height and age at age 4-6 years visit and study sites

Model 2 (full model): Model 1 additionally controlled for maternal age at delivery, maternal race, education levels, marital status, insurance coverage, income adjusted by household size, pre-pregnancy BMI, breastfeeding practices, smoking during pregnancy, parity, GSI, child sleep quality, child physical activity levels, child medication use that potentially increase blood pressure, and COI. Maternal total energy intake was further controlled in the model with maternal HEI.

Model 3: Model 2 with maternal HEI additionally controlled for child HEI and total energy intake, or Model 2 with maternal plasma folate additionally controlled for child nutrient density adjusted folate

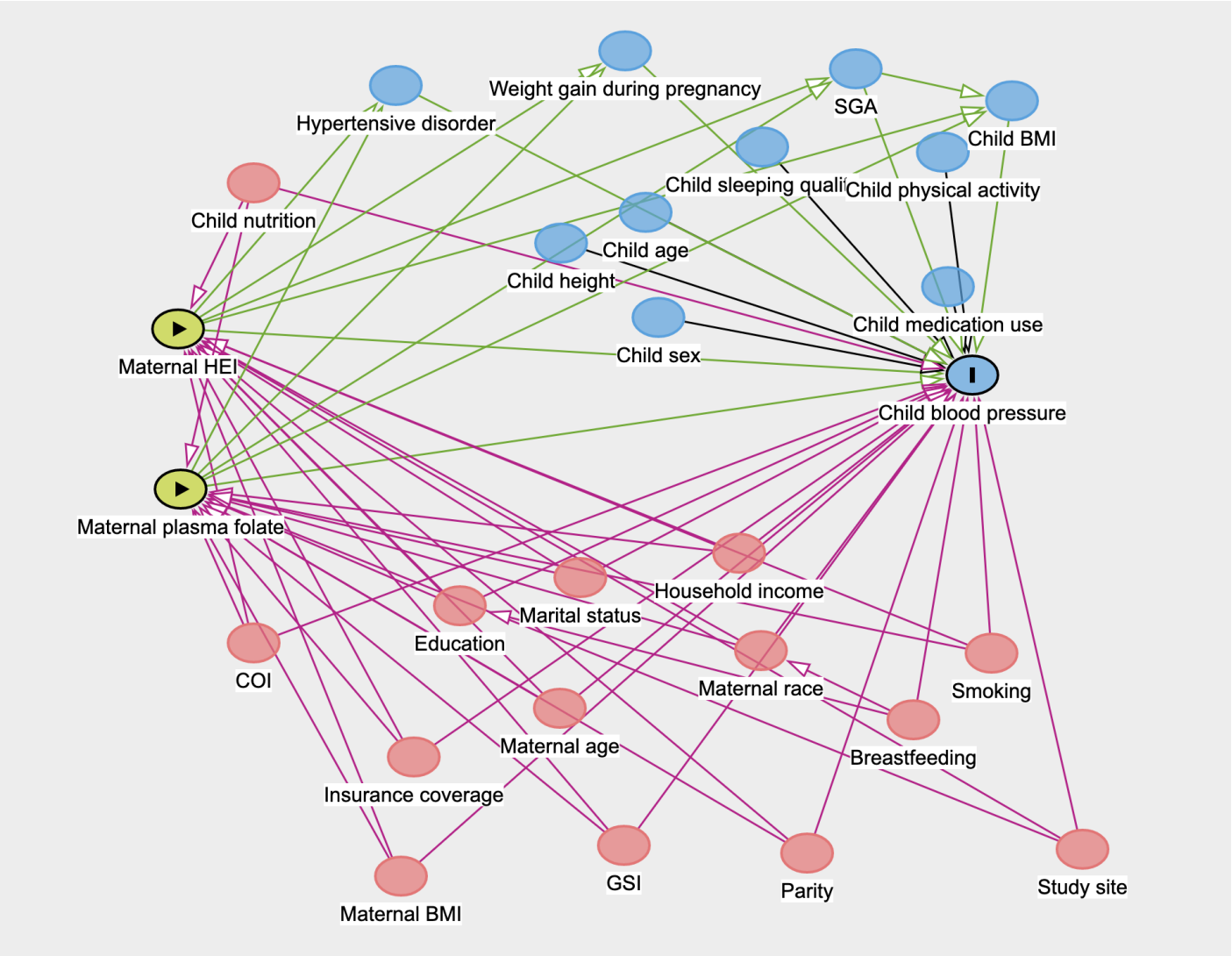
Model 4: Model 2 additionally controlled for hypertensive disorders of pregnancy, weight gained during pregnancy, small for gestational age and BMI z scores

Figure 11. Estimated effects of binary maternal HEI and plasma folate levels on BP percentiles and HBP by child sex (female vs. male), maternal race (Black vs. White vs. Other), maternal overweight/obesity before pregnancy (Overweight/obesity vs. Non overweight/obesity), maternal smoking during pregnancy (Smoker vs. Nonsmoker) and breastfeeding practice (Ever vs. No breastfeeding) from linear and Poisson interaction models.



Appendix Tables and Figures

Appendix Figure 3. Directed Acyclic Graphs for the maternal nutrition-child blood pressure associations



Appendix Table 5. Comparisons of baseline characteristics between the full CANDLE cohort and the analytic samples for each maternal nutritional measurement

	Analytic sample with folate (N=846)		Analytic sample with HEI (N=746)		Participants at enrollment (N=1503)	
	Mean/ Counts	Std/Percentage	Mean/ Counts	Std/Percentage	Mean/ Counts	Std/Percentage
Child characteristics						
Child age at CV4 (years)	4.4	0.6	4.4	0.6	4.4	0.6
Child height at CV4 (cm)	106.4	6.1	106.2	6.0	106.2	6.0
Sleeping scores at CV4	46.8	7.3	46.7	7.2	46.6	7.2
Birth weight	3.2	0.6	3.2	0.6	3.2	0.6
Gestational age (weeks)	38.8	1.8	38.8	1.8	38.8	1.9
Child diet						
Child HEI	52.4	10.4	52.8	10.5	53.0	10.2
Child total energy intake	1481.1	851.8	1492.4	893.5	1441.7	734.6
Child nutrient density adjusted folate	209.1	92.2	208.2	89.2	207.2	94.5
Child sex						
Male	421	49.8%	377	50.5%	736	50.3%
Female	425	50.2%	369	49.5%	726	49.7%
Pre-term delivery (<37 weeks)	76	9.0%	66	8.9%	133	9.1%
Low birth weight	59	7.0%	55	7.4%	107	7.4%
Smaller than gestational age	77	9.2%	66	8.9%	161	11.1%
Medication use potentially leading to hypertension	67	7.9%	62	8.3%	87	5.8%
Vigorous activity frequency						
Never or occasionally	121	14.5%	93	12.6%	144	12.6%
Once or twice per week	90	10.8%	78	10.6%	111	9.7%
Three or more times per week	623	74.7%	565	76.8%	888	77.7%
BMI class at visit						
Underweight	21	2.5%	21	2.8%	25	2.4%
Normal weight	571	67.6%	506	67.9%	719	67.6%
Overweight	125	14.8%	106	14.2%	158	14.9%
Obesity	128	15.2%	112	15.0%	162	15.2%
Maternal characteristics						
Maternal age at birth (years)	26.1	5.5	26.3	5.5	26.0	5.4

Adjusted household income	17019.8	16638.2	17929.6	16693.5	18864.5	17229.4
Total weight gained during pregnancy	14.5	7.2	14.5	7.1	14.6	7.4
GSI scores	50.3	9.5	50.2	9.5	50.5	9.3
Maternal race						
Black	563	66.6%	485	65.0%	936	62.4%
White	229	27.1%	215	28.8%	467	31.1%
Asian	8	1.0%	8	1.1%	13	0.9%
American Indian	0	0.0%	0	0.0%	1	0.1%
Native Hawaiian/Pacific Islander	0	0.0%	0	0.0%	1	0.1%
Other	1	0.1%	1	0.1%	6	0.4%
Multiple race	45	5.3%	37	5.0%	77	5.1%
Maternal education						
< High School	114	13.5%	92	12.3%	184	12.3%
High School/GED	403	47.7%	354	47.5%	709	47.2%
Technical School	85	10.1%	72	9.7%	138	9.2%
College Degree	150	17.8%	140	18.8%	299	19.9%
Grad/Professional Degree	93	11.0%	88	11.8%	171	11.4%
Maternal marital status at enrollment						
Married	296	35.0%	277	37.1%	563	37.5%
Widowed/Divorced/Separated/Never married	398	47.1%	331	44.4%	654	43.6%
Living with partner	151	17.9%	138	18.5%	285	19.0%
Insurance status						
No insurance	2	0.2%	2	0.3%	2	0.1%
Medicaid or Medicare only	510	60.3%	429	57.5%	859	57.2%
Medicaid/Medicare and private insurance	28	3.3%	27	3.6%	42	2.8%
Private insurance only	306	36.2%	288	38.6%	600	39.9%
Baseline household income						
$0-$24,999	372	48.3%	312	45.4%	599	43.8%
$25,000-$54,999	196	25.5%	186	27.0%	370	27.1%
$55,000-$74,999	83	10.8%	80	11.6%	165	12.1%
$75,000 or over	119	15.5%	110	16.0%	234	17.1%
Maternal smoking (Urinary Cotinine + Self report)	115	13.6%	96	12.9%	207	14.2%
Maternal alcohol consumption	76	9.0%	73	9.8%	121	8.1%

Maternal supplement intake of Vitamins	780	94.0%	694	94.9%	1392	94.7%
BMI class						
Underweight	41	4.9%	35	4.7%	66	4.4%
Normal	340	40.3%	307	41.3%	633	42.3%
Overweight	186	22.1%	158	21.3%	354	23.6%
Obese	276	32.7%	243	32.7%	445	29.7%
Breastfeeding						
No	275	34.6%	240	34.0%	347	31.8%
Yes (6 months or less)	305	38.4%	273	38.6%	434	39.8%
Yes (Above 6 months)	214	27.0%	194	27.4%	309	28.4%
Pregnancy hypertensive disorder	49	5.8%	44	5.9%	95	6.3%
Parity						
No prior births	512	60.5%	453	60.7%	882	58.7%
At least one prior birth	334	39.5%	293	39.3%	621	41.3%
Other characteristics						
Childhood Opportunity Index						
Prenatal Educational index	-0.03	0.5	-0.01	0.5	0.02	0.5
Prenatal Economics index	-0.1	0.6	-0.1	0.6	-0.04	0.6
Postnatal Educational index	-0.02	0.5	0.01	0.5	0.02	0.6
Postnatal Economics index	-0.1	0.6	-0.1	0.6	-0.03	0.6
Site						
East	650	76.8%	623	83.5%	1160	77.2%
Med	196	23.2%	123	16.5%	343	22.8%

Abbreviations: Body Mass Index, BMI; Global Severity Index, GSI

Appendix Table 6. Comparisons of effect estimates between Model 3 adjusted for continuous child nutrition and the same model structure adjusted for child nutrition quartiles

	Model 3 wht continuous child nutrition		Model 3 wht child nutrition quartiles	
	Estimate	95%CI	Estimate	95%CI
Systolic BP percentile				
HEI	-0.12	(-0.37, 0.12)	-0.12	(-0.37, 0.13)
Folate	0.05	(-0.17, 0.27)	0.05	(-0.17, 0.26)
Diastolic BP percentile				
HEI	-0.06	(-0.26, 0.13)	-0.07	(-0.26, 0.13)
Folate	0.01	(-0.15, 0.17)	0.01	(-0.15, 0.17)
HBP				
HEI	1.00	(0.98, 1.01)	1.00	(0.98, 1.01)
Folate	1.00	(0.99, 1.01)	1.00	(0.99, 1.01)

Abbreviations: HEI: Healthy Eating Index, BP: blood pressure, HBP: high blood pressure, IRR: incidence rate ratio

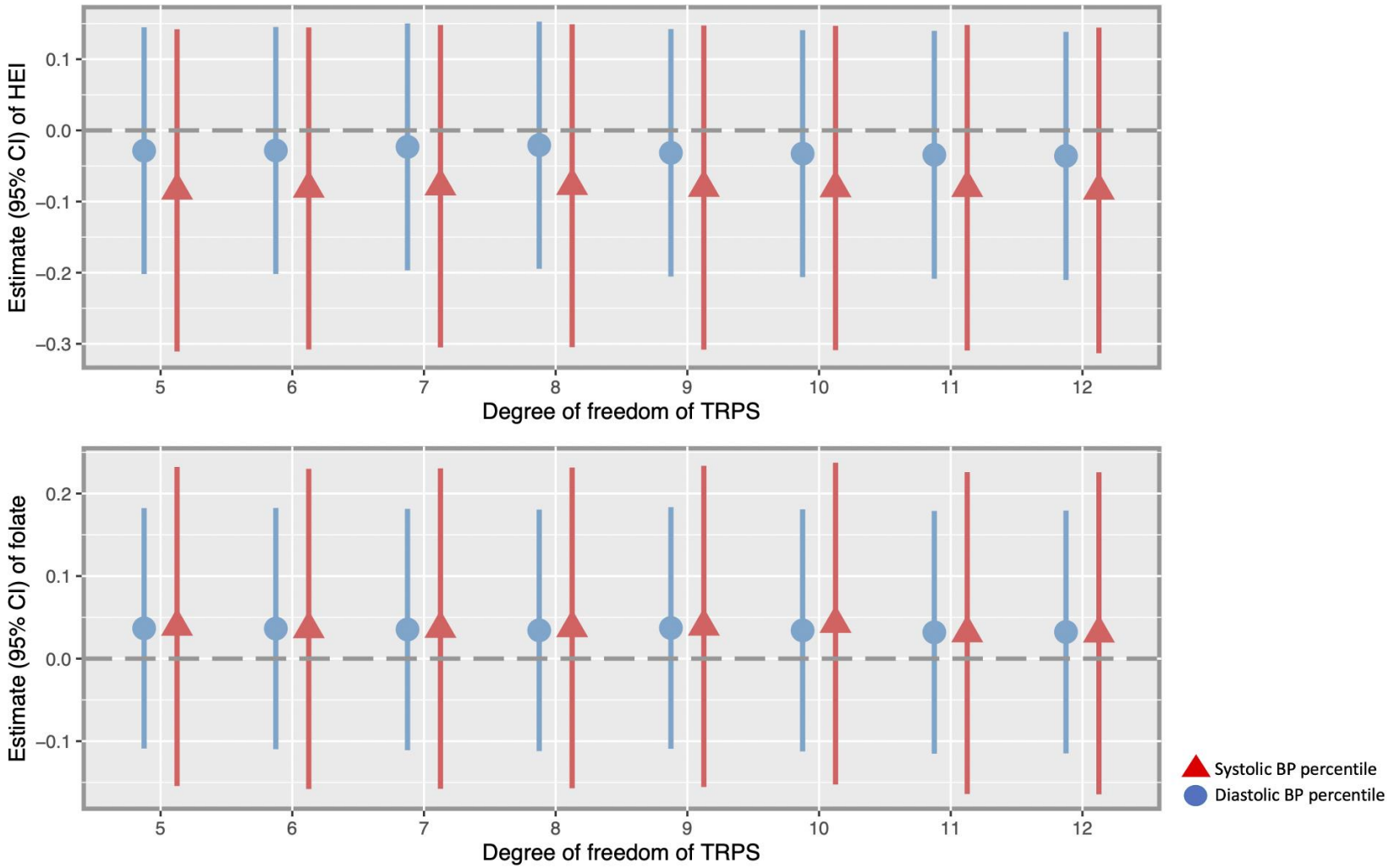
Model 3: adjusted for child sex, child height and age at age 4-6 years visit, study sites, maternal age at delivery, maternal race, education levels, marital status, insurance coverage, income adjusted by household size, pre-pregnancy BMI, breastfeeding practices, smoking during pregnancy, parity, GSI, child sleep quality, child physical activity levels, child medication use that potentially increase blood pressure, COI, and maternal total energy intake, child HEI and total energy intake in the model with maternal HEI, or child nutrient density adjusted folate in the model with maternal plasma folate

Appendix Table 7. Comparisons of effect estimates of maternal HEI between models with IPW and models without IPW

	Primary analysis		Analysis with IPW	
Model	Estimate	95%CI	Estimate	95%CI
Outcome of systolic BP percentile				
Model 1	-0.14	(-0.31, 0.03)	-0.14	(-0.32, 0.03)
Model 2	-0.10	(-0.32, 0.13)	-0.23	(-0.46, 0.00)
Model 3	-0.12	(-0.37, 0.12)	-0.21	(-0.44, 0.03)
Model 4	-0.10	(-0.33, 0.12)	-0.22	(-0.45, 0.01)
Outcome of diastolic BP percentile				
Model 1	-0.09	(-0.21, 0.04)	-0.08	(-0.21, 0.05)
Model 2	-0.05	(-0.23, 0.13)	-0.03	(-0.24, 0.17)
Model 3	-0.06	(-0.26, 0.13)	-0.08	(-0.29, 0.13)
Model 4	-0.05	(-0.24, 0.14)	-0.02	(-0.23, 0.19)
Outcome of HBP (IRR)				
Model 1	1.00	(0.98, 1.01)	1.00	(0.99, 1.01)
Model 2	1.00	(0.99, 1.02)	1.00	(0.99, 1.02)
Model 3	1.00	(0.98, 1.01)	1.00	(0.98, 1.01)
Model 4	1.00	(0.99, 1.02)	1.00	(0.99, 1.02)

Abbreviation: IPW: inverse-probability weighting

Appendix Figure 4. Estimated effects of maternal HEI and plasma folate on BP percentiles from the fully adjusted model (Model 2) adjusted for TPRS of child height and age with varied degree of freedom.



CHAPTER 3: CONCLUSIONS

In this dissertation, we conducted two prospective studies in a large socio-demographically diverse birth cohort in the urban South of the U.S. to estimate the individual and interactive associations of pre- and postnatal air pollution exposures and maternal nutrition during pregnancy with child blood pressure. In Aim 1, we found that children exposed to higher $PM_{2.5}$ in utero had elevated BP percentiles at age 4-6 years. Specifically, $PM_{2.5}$ in the 2nd trimester was associated with increased SBP and DBP percentiles, while $PM_{2.5}$ averaged over the full prenatal period was only associated with higher DBP percentiles. No association was detected of NO_2, proximity to road and postnatal $PM_{2.5}$ with any outcome. These findings were robust to adjustments for potential mediators, exposure assessments in the other pre- and postnatal periods, as well as numerous sensitivity analyses. However, in Aim 2, maternal nutrition in pregnancy, characterized by HEI and plasma folate, was not associated with child blood pressure in the overall CANDLE data. In White mothers, those with a better adherence to the 2010 Dietary Guidelines for Americans in early pregnancy had offspring with lower SBP percentile. Maternal HEI 59 and above was associated with reduced HBP in girls. It also suggested that child nutrition may confound the non-linear maternal folate-child SBP associations. In Aim 3, we observed more pronounced adverse effects of prenatal $PM_{2.5}$, particularly on DBP in children whose mother had low-quality diet or possible folate deficiency during pregnancy, female children and children whose mother self-identified as Black.

This dissertation has several important strengths: 1. The CANDLE cohort was well-characterized, allowing control for key confounders of concern, and the large sample size enabled us to conduct well-powered analyses for effect modification. 2. We estimated spatiotemporally resolved $PM_{2.5}$ and spatially resolved NO_2 using well-validated advanced modeling approaches that predict exposures at individually geocoded subject home locations, allowing us to exploit small-scale spatial variability in the pollutant surfaces. 3. It is the first study to use HEI as a measurement for diet quality in pregnant women to predict offspring blood pressure. HEI is a valid tool to reflect the adherence to the Dietary Guidelines in the general American population, and higher HEI has been associated with lower risks of CVD mobility and mortality in large meta-analyses. 4. Folate levels determined from self-reported data are likely to be overestimated when multiple folate sources are consumed together. Utilization of plasma folate, which serves as an endpoint of folate intake, is more likely to reveal the true exposure-outcome associations. 5. We calculated the sex, age and height specific child blood pressures using the 2017 guidelines with the normal weight pediatric population as the reference, which performed better than the 2004 guidelines in identifying children with adverse cardiometabolic profiles. However, the dissertation also has some limitations. One is the potential misclassification of HBP. As the examination was performed on a single occasion, the definition of HBP in our analysis does not meet the clinical definition, which requires measurements on three different occasions. Another drawback is the self-reported nature of maternal HEI and child nutrition. Even though these measurements were nutrient density adjusted, measurement error remains a concern. Moreover, plasma folate is an imperfect measure of chronic deficiency in pregnancy.[97] It is also difficult to pinpoint the food source of folate biomarker and therefore to inform dietary interventions. In addition, residential

address was first ascertained at enrollment, and we assumed each mother had the same address since the start of pregnancy, which could reduce the accuracy of air pollution exposure assessments. Also, we excluded approximately half of the original study population from this analysis. Although no meaningful difference in baseline characteristics between the families at enrollment and analytic samples was observed, we cannot rule out potential selection bias due to loss to follow-up. Finally, due to the skewed socio-demographic features and other health characteristics in our study sample, the findings of this study may not be generalized to the other populations.

This dissertation has several implications for public health. First, the findings extend the current knowledge of early life experience and health outcomes, and contribute to the evolving science regarding the developmental origins of disease. Second, it highlights the potential harmful effect of prenatal $PM_{2.5}$, even at levels within current regulatory guidelines, on an important feature of child cardiovascular health. Such considerations can inform regulatory policy on acceptable air pollution levels and appropriate controls. Third, this dissertation provides insight into the unhealthy maternal dietary patterns and threshold effects of micronutrients during pregnancy, the recognition of which are critical for the development of pregnancy-specific dietary guidelines. Fourth, because time is required to gather sufficient scientific evidence to support policy changes in air pollution control, the regulatory action does not always follow closely behind. To partially address this problem, our studies were able to identify vulnerable populations and their nutritional risk factors which may potentially synergize the adverse effects of air pollution on child health, to inform timely and tailored interventions.

Based on the major findings from this dissertation, the following research directions could be pursued in the future studies: we could extend the scope of the existing exposures of interest (air pollution exposures and maternal nutrition) with other environmental exposures and behavioral risk factors in early life, such as phthalate exposures, pregnancy smoking or child sleep quality, and estimate the overall effects of the exposome and interactive effects among individual exposures on child cardiovascular health using advanced statistical methods. In addition, continuous investigations are needed to quantify the effects of in-utero and early life experience on blood pressure trajectory from early childhood, adolescence to adulthood, and to detect the critical windows of exposures. Moreover, the findings of this dissertation need to be verified in other well-characterized populations. The ECHO consortium, with about 50,000 children and expectant women enrolled in 84 separate cohort studies across the U.S., provides a unique opportunity to re-evaluate our research questions in meta populations with racially, socioeconomically and geographically diverse backgrounds. Furthermore, as obesity and kidney function impairment are two major drivers for child primary and secondary HBP, respectively, and HBP also co-occurs with the other diseases in metabolic syndrome, future studies are required to elucidate the mechanisms linking air pollution and/or nutritional status with these health conditions. There is also an increasing interest in gene-environment interactions on child health outcome, and a candidate research topic would be the interactive effects of air pollution exposures and risk variants of the apolipoprotein L1 gene (*APOL1*) on child blood pressure and kidney function. Finally, interventional studies quantifying the protective impacts of better air

quality and optimal nutritional status on child cardiovascular health would be encouraged, based on the findings from existing observational studies including ours.

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