Relationships between Serum and Meconium Biomarkers of Prenatal Tobacco Smoke Exposure and their Association with Infant and Early Childhood Growth

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ABSTRACT

JOE BRAUN: Relationship between Serum and Meconium Biomarkers of Prenatal Tobacco Smoke Exposure and their Association with Infant and Child Growth (Under the direction of Julie Daniels)

Prenatal active and secondhand tobacco smoke exposures remain a prevalent and preventable risk factor for adverse infant and childhood health outcomes. I used a prospective birth cohort of 389 mothers and their infants who were followed from early pregnancy to three years of age to address two specific aims. First, I validated the utility of meconium as a biological matrix to quantify prenatal tobacco smoke exposure. Second, I examined the association between prenatal tobacco smoke exposure and early childhood body mass index (BMI).

I validated the utility of meconium tobacco smoke metabolites as biomarkers of prenatal tobacco smoke exposure against self-report and serum cotinine biomarkers of tobacco smoke exposure. I also estimated and compared associations between meconium and serum metabolite concentrations and infant birth weight. Nicotine, cotinine, and trans-3'-hydroxycotinine were detected in the majority of meconium samples (57-80%). Meconium tobacco smoke metabolite concentrations were positively associated with self-report and serum cotinine biomarkers of tobacco smoke exposure. The association between meconium metabolite concentrations and infant birth weight was similar to serum cotinine associations. Meconium is a promising biological matrix to quantify prenatal environmental toxicant exposure; however, meconium tobacco smoke metabolite concentrations did not provide additional information that could be obtained from a single serum cotinine measurement.

In the second aim, prenatal tobacco smoke exposures were quantified using maternal self-report and serum cotinine biomarkers. BMI was calculated from weight and height measurements taken at birth, 4 weeks, and 1, 2, and 3 years of age. During pregnancy, 51% of women had cotinine levels consistent with SHS exposure and 10% had cotinine concentrations indicative of active smoking. After adjustment for confounders, both self-report and serum biomarkers of active tobacco smoke exposures were associated with elevated BMI at 2 and 3 years of age. Estimates of association between self-reported SHS exposures and BMI were attenuated towards the null relative to serum cotinine concentration associations. These results suggest that prenatal tobacco smoke exposures may play a role in the development of overweight in early childhood and that self-reported prenatal SHS exposures are non-differentially misclassified, resulting in biased estimates of association with childhood BMI.

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LIST OF ABBREVIATIONS AND SYMBOLS

Symbols and Units of Measurement:

β: Beta

cm: Centimeter

gm: Grams

kg: Kilogram

μg: Microgram

mL: Milliliter

ng: Nanogram

Abbreviations:

3HC: Trans-3'-Hydroxycotinine

BDI: Beck Depression Inventory

BMI: Body Mass Index

CDC: Centers for Disease Control and Prevention

CI: Confidence Interval

COT: Cotinine

CV: Coefficient of Variation

DAG: Directed Acyclic Graph

GM: Geometric Mean

HOME Scale/Score: Home Observation for Measurement of the Environment

HOME Study: Health Outcomes and Measures of the Environment

HPLC-MS: High Performance Liquid Chromatography-Tandem Mass Spectroscopy

IRB: Institutional Review Board

LBW: Low Birth Weight

LOD: Limit of Detection

LOESS: Locally Weighted Scatter Plot Smoothing

MD: Mean Difference

MS: Mainstream

NCHS: National Center for Health Statistics

NCS: National Children's Study

NHANES: National Health and Nutrition Examination Survey

NIC: Nicotine

OR: Odds Ratio

QC: Quality Control

SHS: Secondhand Smoke

SS: Sidestream

US: United States

CHAPTER 1: INTRODUCTION AND SPECIFIC AIMS

Prenatal tobacco smoke exposure is a prevalent and preventable risk factor for low birth weight (LBW), preterm birth, spontaneous abortion, infant mortality, and behavioral problems in childhood.[1-6] Despite declines in the prevalence of smoking and exposure to second hand smoke (SHS) in the United States (US), 10% to 11% of mothers report smoking during pregnancy and a high proportion of women have exposure to tobacco smoke at home or work.[7-9] Even though the dangers of smoking are well known,[10, 11] prenatal tobacco smoke exposure is a growing problem in the developing world. [12-14]

Both active and second hand tobacco smoke exposure have been consistently associated with restricted fetal growth.[10, 11] Women who smoke during pregnancy give birth to infants who on average weigh 350 grams less than infants of non-smoking women,[15] while women exposed to SHS give birth to infants weighing 40-80 grams less than unexposed women.[6, 16, 17]

Reductions in birth weight related to prenatal tobacco smoke exposure may have lasting consequences on growth and development across the lifespan. A growing body of literature indicates that active prenatal tobacco smoke exposure increases the risk for overweight and obesity in childhood by approximately 50%.[18-20] Children born to active smokers are shorter and lighter at birth and go through a period of catch-up growth marked by increases in weight which results in increased body mass index (BMI).[19] Less is known about the association between prenatal SHS exposure and childhood BMI and growth.[21, 22] Prenatal SHS exposure may also increase the risk of overweight and obesity because of its association with low birth weight and decreased birth length.[5, 6, 11]

Sensitive and accurate biomarkers of prenatal tobacco smoke exposure are needed to study secondhand tobacco smoke exposures. Self-report of tobacco smoke is insensitive to many potential sources of tobacco exposure since many women are unaware of their presence.[9, 23] Meconium is a potentially promising biological matrix that is thought to represent cumulative exposure to environmental toxicants in the latter two-thirds of pregnancy.[24] Some investigators have used meconium as a biological matrix for examining prenatal tobacco smoke exposure.[25-29] To date, no studies have compared meconium metabolite concentrations to serial self-reported or biomarkers of prenatal tobacco smoke exposure. Prior studies have not elucidated if meconium tobacco smoke metabolites are indicative of cumulative gestational exposure to tobacco smoke.[25, 27, 29] In addition only two studies have examined the association between meconium tobacco smoke metabolite concentrations and infant health outcomes, but did not compare these associations to self-report or serum markers of exposure.[26, 28] The association between meconium metabolite concentrations and infant or child health outcomes needs to be compared to the associations with other validated biomarkers. The predictable association between prenatal tobacco smoke exposure and birth weight provides an ideal "model system" to compare meconium to other validated biomarkers of tobacco smoke exposure, as well as a measure of internal validity of the presented study.

I will use data from a prospective cohort of 389 women and their children to complete two aims.

Aim 1: Examine the utility of meconium nicotine, cotinine, and OH-cotinine as a biomarker of prenatal tobacco smoke exposure.

Sub Aim 1.1: Determine if three serial serum cotinine concentrations and self-reported tobacco smoke exposures are positively associated with meconium tobacco smoke metabolites.

Sub Aim 1.2: Determine if meconium tobacco smoke metabolites represent cumulative exposure to gestational tobacco smoke.

Sub Aim 1.3: Compare the associations between serum and meconium biomarkers of prenatal tobacco smoke exposure and infant birth weight.

Aim 2: Estimate the association between prenatal secondhand and active tobacco smoke exposure and childhood BMI in the first three years of life.

Sub Aim 2.1: Compare the association between self-report and serum biomarkers of prenatal tobacco smoke exposure and BMI over the first three years of life.

CHAPTER 2: LITERATURE REVIEW

A. Biomarkers of Tobacco Smoke

A.1. Measuring Exposure to Tobacco Smoke

Tobacco smoke is composed of over 4,000 compounds including nicotine, lead, cadmium, polycyclic aromatic hydrocarbons, and carbon monoxide.[30] Pregnant women can be exposed to tobacco smoke either through active or passive smoking. Active smokers are primarily exposed to inhaled mainstream (MS) smoke and to a lesser extent, sidestream (SS) smoke. SS smoke is the partially combusted material that is burnt off between inhalations. Secondhand smokers are exposed to both exhaled MS smoke and SS smoke. Both MS and SS smoke contain the same chemical constituents but in different concentrations due to the incomplete combustion of SS smoke.[31] MS smoke contains lower concentrations of carcinogenic and toxic substances than SS smoke due to a higher burning temperature and more complete combustion of the chemical constituents of the cigarette. Most studies of tobacco smoke exposure treat MS and SS smoke as the same exposures; however, it is important to recognize that the different concentrations of MS and SS smoke inhaled by active and secondhand smokers produces a continuum of exposure. Depending on the health outcome under study, there may be very distinct or overlapping effects of both secondhand and active exposure due to the different chemical composition of MS and SS smoke.

Measuring exposure to any environmental toxin is difficult. The difficulty is compounded by whether investigators measure exposure or effective dose. While both are of interest, they have the potential to produce different results. Historically, active smoking has been measured using questionnaires, the results of which are used as proxies for inhaled dose. Among active

smokers, the quantity, brand, and depth of inhalation are often assessed to determine inhaled dose. In addition, investigators often ask pregnant women the amount smoked during each trimester of pregnancy and whether she quit smoking during pregnancy. SHS exposure has typically been measured through self-report. Initial studies of the health effects of SHS exposure used spousal smoking as a proxy for SHS exposure.[32] More sophisticated questionnaires were developed that ask about the number of smokers, the quantity of cigarettes smoked by other smokers, the time spent in a smoky environment, and the smokiness of the environment.

Questionnaire based methods result in under-reporting and misclassification of active smoking among pregnant women due to social stigma and socioeconomic (SES) factors.[23, 33-35] In addition, women tend to underreport active smoking when questioned antenatally compared to their perinatal reports.[36, 37] Single measurements of active smoking status may lead to further misclassification if women quit smoking early in pregnancy, but resume later, or vice versa. Single measurements of active smoking may also miss critical periods of fetal susceptibility.

Questionnaires also cause substantial misclassification of SHS exposure among non-smokers for several reasons. First, different exposure metrics (hours, number of smokers, or smokiness) have been used in various studies, which reduces the comparability between studies.[5] Second, most studies dichotomize SHS exposure as exposed vs. non-exposed. While grouping all women with any exposure to SHS can increase power, it leads to increased exposure misclassification within the two groups. This method would classify a person exposed to one smoker four-feet away for eight hours a day the same as another person exposed to three smokers 100 feet away for 4 hours a day. Third, women (and men) are likely to underestimate their true exposure to SHS. It has been demonstrated that women who report no exposure to SHS at home or work have measurable levels of cotinine (a biomarker of nicotine exposure).[38, 39] Finally, questionnaire based methods measure the exposure of an individual, not their received dose. When studying infant outcomes, it can be preferable to have a

measure of the internal dose that a woman receives. Variations in ventilation, breathing rate, and genetics could cause variability in the received dose at the same levels of exposure.[31, 40]

A.2. Cotinine as a Biomarker of Tobacco Smoke Exposure

The National Research Council proposed the following criteria for biomarkers of tobacco smoke exposure: 1) should be unique or nearly unique for tobacco, 2) should be easily detectable, 3) should be detected with exposure to a variety of tobacco products or sources, and 4) should have a concentration proportional to other tobacco smoke constituents.[30] Based on these criteria, serum cotinine concentrations are considered the best biomarker of active and secondhand tobacco smoke exposure.[30, 31, 41] It is easily detectable in urine, saliva, serum, hair, and stool. Advances in analytical chemistry techniques have allowed cotinine to be detected in serum at levels two orders of magnitude lower (2 ng/mL vs. 0.015 ng/mL) than methods used 20 years ago.[42] Serum, saliva, urinary, and hair cotinine levels can accurately discriminate between smokers and non-smokers. There is little evidence to suggest that exogenous nicotine from fruits and vegetables (tomatoes, cauliflower, or eggplant) could result in a substantial increase in serum cotinine levels.[30] For example, a person would have to consume 130 grams of eggplant or 250 grams of pureed tomatoes to raise their serum cotinine levels by 1 ng/mL.

Numerous studies have documented elevated serum cotinine levels among women who report no active or secondhand tobacco smoke exposures.[9, 23, 43-45] The degree of misclassification of smoking based on self-report seems to vary considerably. It is likely influenced by cultural norms, social stigma, and SES status. Given the potential for misclassification, a biomarker of tobacco smoke exposure during pregnancy is preferable. .

A.3. Meconium Cotinine as a Marker of Tobacco Smoke Exposure

The use of serum cotinine levels as a biomarker of tobacco smoke exposure in pregnant women has several related shortcomings. First, women are often unaware of their pregnancy early in gestation. After women do become aware of their pregnancy, they often change their smoking habits as a result of pressure from friends and family, clinicians, and society.[34, 35]

Second, women may be intermittently exposed to secondhand tobacco smoke, resulting in exposure misspecification depending on the timing of exposure and serum measurement. Cumulative biomarkers of tobacco smoke exposure may be a potential solution to these two problems and would allow an investigator to determine whether the sum of exposure over gestation is an important predictor of health outcomes.

One novel biomarker of cumulative gestational exposure to environmental toxicants is meconium. Meconium is the first stools passed by an infant after birth. The majority of meconium remains in the intestine of the newborn at the time of birth, but occasionally fetal stress will cause the newborn to expel meconium *in utero*. Meconium is formed from swallowed amniotic fluid, shed epithelial cells, and intestinal secretions beginning in the 13th week of gestation.[24] Thus, meconium concentrations of environmental toxins reflect exposure in the latter two-thirds of pregnancy and may provide a better estimate of cumulative exposure than single or serial serum or urine cotinine measurements.

Several studies suggest that meconium is metabolically inert, making it an ideal matrix for analyzing chemicals with relatively short half-lives (nicotine, pesticides, cocaine, alcohol, and barbiturates).[46] This is substantiated by results showing that meconium has a greater sensitivity for illicit drugs compared to maternal urine, fetal urine, and cord blood samples.[47] Several studies have detected tobacco smoke metabolites in meconium and found that they are positively correlated with self-report or biomarkers of prenatal tobacco smoke exposure.[25, 27-29, 48] However, these studies have not had serial measurements of biomarkers or self-reports of prenatal tobacco smoke exposure throughout pregnancy to confirm that meconium metabolites represent cumulative gestational exposure. In addition, many of these studies have been conducted on relatively small samples of women (<50) and some have used outdated analytical chemistry techniques.

To date, only two studies have examined the association between meconium cotinine levels and infant or child health outcomes. Gray et al. found decrements in birth weight, length,

and head circumference in a sample of 51 women with detectable meconium metabolite tobacco smoke concentrations. They also detected similar decrements associated with self-reported prenatal tobacco smoke exposure, but assumed that detectable meconium metabolite concentrations were indicative of active smoking. Infants with detectable meconium metabolite concentrations could have been exposed to secondhand or active tobacco smoke exposure.

The second study conducted by Nuesslein et al. reported a 4.9-fold (95% CI: 1.2, 20.3) odds of lower respiratory tract infections in the first year of life among infants with greater than median meconium concentrations compared to infants with meconium concentrations below the median.[26] However, they were unable to validate their intriguing results against other markers of prenatal tobacco smoke exposure.

While meconium is reflective of total gestational exposure to environmental toxicants, it does not allow the identification of the timing of exposure. This may be detrimental when attempting to define critical windows of susceptibility. However, this pitfall may be offset by the difficulty in obtaining multiple serum cotinine measurements during pregnancy. In addition, transient exposures to environmental toxins have the potential to be associated with delays in growth and development which may not be captured even when collecting multiple serum measurements.

B. Fetal and Childhood Growth

B.1. Measurement of Birth Weight and Fetal Growth Restriction

LBW and decreased birth weight are used as indicators of fetal growth restriction because they are accurately measured and readily available on birth certificates and medical records.[49, 50] Among term infants, decreases in birth weight can provide an accurate indicator of fetal growth restriction.[51]

B.2. Etiology of Low Birth Weight

Low birth weight affects 7.5% of live-born infants in the US.[52] Infants are born low birth weight as a result of intrauterine growth restriction or premature birth. Preterm birth is one of

many predictors of low birth weight. Many of the same risk factors for preterm birth are associated with low birth weight. For instance, a nutritional factor that reduces fetal growth may also decrease gestational age. Disentangling the effects of an environmental factor on gestational age and low birth weight is difficult and requires many strong assumptions or advanced modeling techniques.[53-55]

Risk factors and predictors of low birth weight fall into maternal and fetal factors. Maternal factors include malnutrition, alcohol and cocaine use, certain medications, hypertension, autoimmune disorders, and lifestyle factors including active and secondhand smoking. Fetal factors include multiple gestation, infection, aneuploidy, and congenital malformations. Certain fetal disorders such as vascular, anatomical, and chromosomal abnormalities can also result in low birth weight. Some data suggest that air pollution may also be responsible for small declines in birth weight.[56, 57]

The use of birth weight as an indicator for fetal growth is not without critics.[51, 58] Differences in the estimated gestational age of infants may be responsible for reported decreases in birth weight, thus some infants who are less than 2500 grams may be misclassified as low birth weight, even though the infant is at a normal weight for their given gestational age. In order to correct for differences in fetal growth over time, some researchers use standardized measures of infant size based on their gestational age at birth.

B.3. Complications Related Low Birth Weight

LBW infants are at increased risk for death in the first year of life.[59, 60] Morbidity and mortality from respiratory problems and infection are also associated with LBW.[61-63] Treatment of complications related to LBW has substantial financial costs to society.[64] In 2001, admissions for LBW totaled $5.8 billion, representing almost half of the all the costs of infant hospitalizations. Beyond the quantitative costs, parents of LBW infants suffer unimaginable hardships.

The effects of LBW are not limited to the first year of life. Decreases in birth weight are associated with wheeze, cough, and respiratory infection in childhood among term infants.[65] Infants born LBW also have abnormal neurodevelopment in early childhood compared to their normal birth weight peers in.[66, 67] These deficits persist into later childhood where LBW children have decreased cognitive aptitude and academic skills.[68, 69] In addition, children born LBW are at increased risk for externalizing behaviors including attention-deficit/hyperactivity disorder and conduct disorder.[68, 70, 71] While there is some evidence that medical interventions may improve neurodevelopmental outcomes for children born LBW, it is imperative to prevent these outcomes given their substantial financial and societal cost.[72]

B.4. Birth Weight and Risk of Adult Mortality and Disease

Epidemiological studies that birth weight may play an important role in the development of circulatory disease or metabolic disorders in adulthood. In 1989, Barker observed that low weight in infancy was associated with mortality from ischemic heart disease.[73] Additional studies have confirmed that reduced birth weight is associated with diabetes and heart disease.[74-76] Baker et al. reported a 17% increased hazard (95% CI: 1.11, 1.22) for all-cause mortality among a cohort of Danish men and women who were between 2,000 and 2,750 grams at birth compared to those with a birth weight between 3,251 and 3,750 grams.[77]

The Barker Hypothesis posits that environmental factors act in early life to program the risk for the onset of adult metabolic and cardiovascular disease.[78, 79] Exposure to factors that decrease birth weight and alter postnatal growth trajectory may increase the risk for adult disease morbidity and mortality.[77, 80, 81] Prenatal growth restriction and alterations in early life growth trajectories may result in an overshoot of genetically programmed size and metabolism. Thus, factors like tobacco smoke that impair or restrict growth in early life may increase the risk for later morbidity and mortality. Some results suggest that this phenomenon is driven by early adiposity rebound, the nadir of early childhood BMI. Children with early adiposity rebound are more likely to be obese or overweight in childhood and have symptoms of metabolic

syndrome..[82-84] However, adiposity rebound may simply be a secondary phenomenon to children with higher BMIs having earlier rebounds.[85]

B.5. Impact of Small Shifts in Distribution of Birth Weight

Most documented associations between environmental toxicant exposure and continuous health outcomes, such as IQ or birth weight, are typically clinically insignificant. While these decrements would not produce tangible effects at the individual level, they can have profound impacts on the tails of the distribution of IQ at the population level. A well cited example comes from the literature on childhood lead exposure and IQ. A 4-point decrease in mean IQ was observed in children with elevated blood lead levels (10 μg/dL) compared to children with low blood lead levels (2.4 μg/dL).[86] Needleman and others have argued that a small shift in the mean of the distribution of IQ as a result of elevated blood lead levels can cause a 4-fold increase in the proportion of children classified as intellectually disabled (IQ<70).[87] Bellinger has also argued the importance of this phenomenon using the example of blood pressure from the cardiovascular disease literature.[88, 89] These studies show that exposure to low-level environmental toxins, like SHS, may cause small shifts in the mean of a continuous health outcome and lead to profound impacts on the tails of the distribution.

C. Association between Prenatal Tobacco Smoke Exposure and Infant Weight

C.1. Active Smoking and Infant Birth Weight

The US Surgeon General has reported that there is sufficient evidence to assume a causal relation between active maternal smoking during pregnancy and decreased birth weight.[10] Several compounds found in tobacco smoke are implicated in delayed fetal growth and development. Inhaled carbon monoxide is responsible for the production of carboxyhemoglobin which results in decreased rates of oxygen diffusion across the placenta.[90-92] Results from murine models suggest that nicotine exposure may result in delayed or altered embryonic implantation leading to growth deficits.[93] Accumulation of cadmium in the placenta

may cause morphological and functional impairment of the placenta.[94, 95] In addition, other tobacco smoke constituents may act through other mechanisms to produce decreased fetal growth and altered development.

Active smoking during pregnancy is associated with a 200-300 gram decrease in birth weight.[10] Data from randomized trials of smoking cessation interventions offered to pregnant women have provided the best evidence of active smoking induced fetal growth deficits.[15] Observational studies have documented results similar to those from randomized trials.

Results from the Generation R study revealed a 1.75-times (95% CI: 1.20, 2.56) odds of LBW among women who smoked throughout pregnancy compared to non-smokers.[96] The effect of smoking in late pregnancy was greatest among women who smoked $\geq$10 cigarettes per day (OR: 3.39; 95% CI: 1.45, 7.91). In addition, the authors found a dose-response relation between the number of cigarettes smoked and decreases in mean birth weight. Other studies have found similar decreases in mean birth weight and increased risk for LBW among women reporting active smoking.[97-99]

Several studies have used biomarkers of active smoking to examine and validate the relation between tobacco exposure and birth weight. England et al. found that both self-reported number of cigarettes smoked and urinary cotinine concentrations were associated with decreased birth weight.[100, 101] Haddow et al. found that serum cotinine levels were more strongly correlated with birth weight than self-reported tobacco smoke exposure.[102] Women who smoked $\geq$ 25 cigarettes per day, representing the top 2.7% of women, had infants that weighed 289 grams less than non-smokers (bottom 68%). Women with serum cotinine concentrations in the top 2.7% (>284 ng/mL) had infants that weighed 411 grams less than women in the bottom 68% of the serum cotinine distribution (<24 ng/mL). This comparison is somewhat biased because women in the bottom 68% of the cotinine distribution included actively smoking women. However, if serum cotinine levels in the lower range are associated

with decreases in birth weight, then the differences between women in the top 2.7% and non-smokers is likely greater.

Ellard et al. also found that urinary cotinine levels provided a better estimate of smoking related deficits on birth weight than self-reported tobacco use (cigarettes/day) among 3,038 pregnant women.[103] A dose-response relation between tobacco smoke exposure and birth weight was only apparent when they used urinary cotinine levels. In another cohort of 740 pregnant women in Boston, a clearer dose-response relation between active smoking and birth weight emerged when urinary cotinine levels were used instead of maternal self-report of tobacco use (cigs/day).[104] These results indicate that cotinine may provide a more accurate estimate of the dose of tobacco smoke constituents received by the mother and fetus, thus reducing exposure misclassification related to self-reported smoking.

C.2. SHS Exposure and Infant Birth Weight

Many studies reporting an association between SHS exposure and birth weight have relied on self-report of paternal smoking or number of hours exposed at home or work.[17, 99, 105-107] As noted earlier, self-report of SHS exposure is problematic, resulting in a substantial proportion of women being misclassified as unexposed.

There is a reasonably consistent inverse relation between cotinine levels and birth weight (**Table 1**). A meta-analysis of 11 studies with self-reported SHS exposure found a 28.5 gram (95% CI: 16.2, 40.8 gram) decrement in birth weight among women exposed to at least 1 hour of SHS per day compared to non-exposed women.[5] Among the three studies using cotinine as a marker of SHS exposure, an 81.6 gram (95% CI: 36.7, 126.4 gram) deficit in birth weight was found among exposed women. [16, 102, 108] The authors of this meta-analysis also reported a 40% (95% CI: 1.0, 1.9) increased odds of LBW among SHS exposed women compared to non-exposed women. If self-reported exposure does result in exposure misclassification, as this meta-analysis suggests, then using a biomarker is preferred since it will provide a more accurate estimate of the effect of SHS on fetal growth.

More recent studies have reported an inverse association between prenatal tobacco smoke exposure biomarkers and birth weight. Kharrazi et al. conducted an analysis examining the relationship between 2nd trimester serum cotinine levels and birth weight among 2,777 mother-infant dyads who were enrolled in the California alpha fetoprotein prenatal screening program in April of 1992.[6] A one $\log_{10}$-unit increase in serum cotinine levels (i.e., 1 to 10 ng/mL) was associated with a 52.5 gram (95% CI: 22.9, 82.2 gram) decrease in birth weight. A recent study in Washington DC collected two different salivary cotinine levels from 714 pregnant women during the 1st or 2nd trimester and before birth.[109] Reductions in birth weight ranged from 70 to 220 grams among women with higher salivary cotinine levels. Their results suggest that salivary cotinine levels collected at birth are more strongly associated with reductions in birth weight; however the confidence intervals from the two sets of estimates substantially overlapped.

C.3. Association between Prenatal Tobacco Smoke Exposure and Childhood Growth

A growing body of literature indicates that active prenatal tobacco smoke exposure increases the risk for overweight and obesity in childhood. A recent meta-analysis of 14 studies found that active tobacco smoke exposure during pregnancy increased the risk of childhood overweight and obesity by approximately 50%.[18] The results of this analysis did not suggest that confounding due to maternal anthropometry, sociodemographic factors, or child behaviors biased the estimated association between prenatal tobacco smoke exposure and the development of overweight or obese BMI.

Using data from the Collaborative Perinatal Project, Chen et al. reported that children born to smokers were lighter and shorter than non-exposed children at birth. Over the first 8 years of life, they reported that exposed children were born shorter and lighter and grew heavier and remained shorter until 8 years of age. Changes in these children's growth patterns resulted in a 20% increased risk of overweight BMI at 8 years of age.

Animal models show that prenatal nicotine administration results in increased adiposity at birth,[110] changes in postnatal growth trajectories,[111] and metabolic changes consistent with diabetes and obesity.[112] Some cross-sectional studies have observed an inverse association between active smoking and infant cord blood leptin levels,[113, 114] while others have not.[115, 116] Control over infant and childhood growth is a complicated enterprise and is controlled by numerous hormonal systems,[117] many of which could be altered by prenatal tobacco smoke exposure.

Less is known about the association between prenatal SHS exposure and childhood growth. There are six studies that have examined the effect of pre- and early postnatal tobacco smoke exposures on childhood growth (**Table 2).** There appears to be small decrements in height between 5 and 11 years of life associated with pre- and early postnatal SHS exposures. A study by von Kries et al. suggests that SHS exposures may be associated with increased odds for BMI later in life.[118] Two studies have examined the association between prenatal SHS exposure and childhood BMI. Leary et al. reported elevated BMI, total body fat, and truncal fat among 10-year old children whose mothers had a partner who smoked during pregnancy.[21] Oken et al. did not report any association between self-reported prenatal secondhand tobacco smoke exposure and BMI at 3 years of age.[22]

There are several limitations of the extant literature examining prenatal SHS exposure and childhood growth. First, all but one study has relied on self-reported tobacco smoke exposures.[119] Eskenazi and Bergmann's study relied on older laboratory methodology that had an increased limit of detection for serum cotinine. A valid biomarker of prenatal tobacco smoke exposures can reduce exposure misclassification of secondhand tobacco smoke exposures.[9, 23, 33] Third, prior studies have only had one postnatal measure of child size, thus there have been no studies examining the effect of SHS exposure on fetal growth trajectory. The trajectory of growth may be as or more important than differences in weight or length at any given time point. Finally, almost all of these studies were conducted on women who gave birth several decades

ago. Thus, the exposures experienced by these women are not relevant to contemporaneous women.

Table 1: Summary of studies examining the association between biomarkers of second hand smoke exposure and birth weigth

Author (year)	Location	Birth Year(s)	Sample Size	Biomarker Type	Timing of Exposure Measure	Exposure Definition	Birth Weight Decrement in Grams (95% CI)
Rebaglato et al. (1995)[108]	Spain	1989-1991	710	Saliva	3rd trimester	1.7-10 ng/mL vs. <0.5 ng/mL	87 (1, 144)
Eskenazi (1995)[16]	California, USA	1964-1967	2,292	Serum	28 wks	2-10 ng/mL vs. <2ng/mL	45 (-36, 126)
Haddow et al. (1998)[102]	Minnesota, USA	1980s	1,231	Serum	??	1-10 ng/mL vs. <0.5 ng/mL	104 (35, 173)
Hanke et al. (2001)[120]	Poland	1997-1998	183	Serum	20-24 wks	2-10 ng/mL vs. <2ng/mL	26 (CI not reported)
Jaakkola et al. (2001)[121]	Finland	1996-1997	389	Hair	Birth	>4μg/g vs. <0.75 μg/g	17 (-178, 145)
Kharrazi et al. (2004)[6]	California, USA	1992	2,777	Serum	15-19 wks	>0.235 ng/mL vs. <0.026 ng/mL 1 $\log_{10}$ unit	39.7 (-16.3, 95.7) 27 (-54, 1)
Jedrychowski (2008)[122]	Krakow, Poland	2000-2003	467	Serum	Cord	1 $\log_{10}$ unit	12.3 (-66.1, 90.7)
El-Mohandes (2009)[109]	Washington DC	2001-2003	700	Salivary	1st/2nd trimester and birth	Categorical (defined by authors)	161 (-19, 341)

Table 2: Summary of studies examining association between pre- and postnatal tobacco smoke exposure and childhood growth

Authors and Year	Study Year	Sample Size	Exposures	Exposure	Outcome	Remarks
Rona and Chinn, 1981 [123]	1972	~6500	Postnatal	Number of smokers in the home	Height at 7 years	Dose response between number of smokers and decrements in height.
Rantakallio, 1983 [124]	Unknown	Unknown	Prenatal	Fathers use of tobacco during pregnancy	Height	Decrements in height among women living with smoking partners.
Rona, et al., 1985 [125]	1972	~6500	Both	Self-reported smoking of both parents	Height at 5-11 years	Decrements in height associated with postnatal exposure among pre-and postnatal exposed infants
Chinn and Rona, 1991 [126]	1987	5002	Postnatal	Total cigarettes smoked in the home	Height at 5-11 years	Small decrements in height
Eskenazi and Bergmann, 1995 [119]	1960s	2,622	Prenatal	Serum cotinine and self-report	Height at 5 years	Decrements in height associated with increasing serum cotinine
Oken et al. 2005 [22]	1990s	746	Prenatal	Self-report of active and secondhand tobacco exposure	BMI and skinfold thickness at 3 years	Increased BMI and skinfold thickness among children born to active smokers. No association between SHS and BMI or skinfold thickness based on significance testing.
Leary et al. 200 6[21]	1991-1992	5689	Prenatal	Self-report of active and secondhand exposures	BMI and DXA at 10 years	Increased BMI, total fat, and truncal fat among prenatally exposed children. Magnitude of active associations larger, but less precise than secondhand associations.
von Kries et al., 2008 [118]	2005	5,899	Both	Self-report of maternal and paternal smoking	BMI at mean age of 6 years	Pre- and postnatal tobacco exposure associated with increased odds of overweight and obesity.

CHAPTER 3: METHODS

A. Study Description

A.1. Study Overview

The source of my data is an ongoing prospective birth cohort (Health Outcomes and Measures of the Environment [HOME] Study) that has been following 400 mothers and their children in the Cincinnati metropolitan area from early pregnancy to 5 years of age. The purpose of the HOME study is to study the association between low-level environmental toxicants and childhood health outcomes. A randomized trial is nested within this cohort to determine the efficacy of lead and injury hazard controls in the home.

Participants in the study were randomized to receive either injury prevention controls in the home or removal/remediation of lead hazards from the home. Lead hazard controls included removal of deteriorated paint surfaces, encapsulation of lead hazards, and cleaning; all of which were conducted by certified and licensed contractors. Mothers and infants in the injury reduction group did not receive interventions until the index child was 3 to 6 months old. Data from the randomized trial will be used to examine the effect of lead hazard controls on children's blood lead levels, intelligence, and behavioral problems in early childhood.

A.2. Eligibility Criteria

Eligibility criteria for participation in the HOME study included:

1. $\geq$18 years old
2. 16 weeks ($\pm$ 3 weeks) gestation
3. Living in a house, condominium, or apartment built before 1978;
4. Cannot be diagnosed with or receive treatment or medications for seizures, thyroid disorders, bipolar/manic depression, schizophrenia, cancer, HIV or AIDS
5. Have to live in and plan to deliver at participating OB practices in Brown, Butler, Clermont, Hamilton, or Warren counties.
6. Cannot have plans to move outside of study area at the time of delivery.
7. Must be fluent in English.

Participants were identified from 7 prenatal clinics associated with 3 hospitals in Cincinnati beginning in March of 2003. Women who were identified as being < 18 years of age or residing in housing built after 1978 were excluded. Recruitment letters were sent to the remaining eligible participants. After receiving the recruitment letter, women were contacted by telephone to determine eligibility and invited to participate if eligible. If they agreed to participate, women received informed consent in the mail to review before their next prenatal visit. Once a woman agreed to participate and signed the informed consent form, they were met by study personnel at their next prenatal visit to collect biologic samples (blood, serum, urine and saliva) and baseline interview information. A baseline home visit was scheduled for two to four weeks after the initial clinic visit to give the participant time to discuss the study with her family or friends.

A.3. Study Sample

Between March of 2003 and January of 2006 a total of 8,371 women were identified from seven prenatal clinics associated with three hospitals. We mailed letters to 5,512 women $\geq$ 18 years of age who were living in a home built before 1978 to see if they were eligible and interested in participating in our study. Among the 1,263 eligible women, 468 (37.1%) enrolled before the 19th week of pregnancy. Sixty-seven women (14.3%) dropped out before delivery, leaving 401 (85.7%) mothers who delivered 389 singletons, 9 children from sets of twins, and 3 still-born children.

To target children at increased risk for lead exposure, there was an over-sampling of African American mothers. African Americans comprise approximately 11% of the metropolitan Cincinnati population and the over-sampling resulted in the final sample consisting of 30% African Americans families.

B. Tobacco Smoke Exposures

B.1 Self-Reported Tobacco Smoke Exposure

Women were interviewed twice about secondhand and active tobacco smoke exposures for the periods between birth and 20 weeks (measured at 20 week home visit) and 20 weeks and birth (measured at 4 week postpartum visit). The interviewer asked women the average number of cigarettes they smoked per day, the number of smokers living in the home, and the number of cigarettes smoked per day in the house. I classified exposure during each time period as unexposed, secondhand exposure, and active exposure.

B.2. Prenatal Biomarkers of Tobacco Smoke Exposure

Serum biomarkers of tobacco smoke exposure were collected from women three times during pregnancy at 16 and 26 weeks gestation and within 24 hours of birth. Meconium samples were collected during the birth hospital stay.

Mothers provided three serum samples during pregnancy using a standardized venipuncture technique during routine clinic visits around 16 and 26 weeks gestation and within 24 hours of birth. Serum samples were stored at -20^{o}C until they were shipped to Centers for Disease Control and Prevention (CDC) for analysis.

Meconium specimens were collected from infant diapers. Hospital staff were asked to attach cellulose fiber inserts to the inside of the infants diapers. After the diaper was soiled, the diaper was stored in a labeled plastic bag. If meconium was present before the infant was put in a diaper, hospital staff collected the meconium in labeled storage containers and stored them. At least 5 meconium stools per neonate were obtained during the birth hospital stay. Meconium samples for each infant were pooled prior to storage. Both diapers and collection containers were stored at -20^{o}C until they were transported to the laboratory. All other biological samples were also stored at -20^{o}C until they were transported to the laboratory.

Serum and meconium samples were delivered to the Biomarker Laboratory Core at Cincinnati Children's Hospital and Medical Center by project staff at the end of each collection

day. Meconium and maternal serum samples collected for cotinine analysis were sent to the CDC for analysis.

B.2.1. Laboratory Analysis and Quality Control

Levels of cotinine were measured in serum using a previously described sensitive atmospheric-pressure ionization, tandem mass spectrometric method developed by Dr. Pirkle at the CDC.[42] High performance liquid chromatography-tandem mass spectrometry (HPLC-MS) is considered the gold standard to measure cotinine with the highest accuracy and precision. The limit of detection (LOD) for this assay was 0.015 ng/mL and had a coefficient of variation (CV) ranging from 3-4% at high concentrations (1 ng/mL) to 10% at low concentrations (100 pg/mL).

Approximately 0.5 grams of meconium were digested at room temperature in potassium hydroxide, which resulted in a free-flowing liquid. The digested material was then liquid-liquid extracted with methylene chloride and methylene chloride and ethanol. The solution was then back-extracted into hydrochloric acid and applied to a CleanScreen DAU column and processed as described by Ostrea et al.[47] HPLC-MS was then used to quantify the concentration of the nicotine NIC, COT, and 3HC using deuterated standards. Our laboratory results indicate that this method is mild enough to avoid metabolite decomposition while ensuring a high metabolite recovery. The LOD for these three compounds was 0.946 ng/gm for NIC, 0.070 ng/gm for COT, and 0.092 ng/gm for 3HC. Each analytical run included two blank samples and a low and high concentration quality control (QC) sample. The low-concentration QC samples had a CV between 10 and 30% while the high-concentration QC samples had a CV between 3 and 20%.

C. Infant and Childhood Growth Outcomes

C.1. Infant Birth Weight and Gestational Age

Trained research staff abstracted gestational age, birth weight from hospital charts and medical records. Gestational age was derived from last menstrual period (LMP), ultrasound, or Ballard scores.

C.2. BMI Measurements

Weight and length were measured at 4 weeks, 1, 2, and 3 years of age. Values were derived from the mean of 3 separate measurements that were taken in our study clinic. Length was measured with a length board at 4 weeks and 1 year and a stadiometer at 2 and 3 years. Weight was measured using an infant scale at 4 weeks and 1 year and a pediatric scale at 2 and 3 years. Body Mass Index (BMI), a measure of adiposity,[127, 128] was calculated by dividing weight in kilograms by height in meters squared. Age and sex-specific weight, height, and weight-for-height z-scores were derived from CDC growth charts.[129] Children were classified as overweight at 2 and 3 years of age if their BMI was greater than the 85th percentile for their age and sex.

C.3. Confounders

I used directed acyclic graphs (DAGs) to examine the role of potential confounders in both of my aims (Greenland and Brumback 2002; Greenland et al. 1999) (**Figures 1 and 2**). I did not adjust for confounding variables in the analysis examining the utility of meconium as a biomarkers since any confounding would be due to unmeasured genetic or metabolic factors (**Figure 1)**.

Table 3 outlines the details of my exposure, outcome, and confounding variables for these 2 aims. Confounders for Aim 1 and 2 included demographic, maternal, and perinatal factors. Demographic information, including maternal age, education, race, and marital status collected from interviews. Maternal and perinatal factors included depression, parity, and maternal weight and height. Maternal depression was assessed using the Beck Depression Inventory-II (BDI-II) which was administered during a home visit at approximately 20 weeks.[130] Parity was abstracted from medical records. Maternal weight (in kg) and height (in cm) were collected at the initial clinic visit at 16 weeks gestation.

Additional confounders were included for my Aim 2 analysis to capture confounding related to childhood activity and nutrition. These included household income which was

collected at the first prenatal care visit. Breast feeding duration was used as a proxy for early life nutrition. Breast feeding was assessed every 3 months using a phone or in-person interview that asked the mothers how much breast milk they fed their infants in each of the past 3 months.

I also examined the potential for residual confounding due to SES status. I attempted to control for this by including Home Observation and Measures of the Environment (HOME) Inventory scores in my statistical models. The HOME Inventory is a semi-structured interview that assesses the quality of the caregiving environment [131, 132] and was administered by trained interviewers during a home visit when the child was 1 year old. I did not adjust for gestational age at delivery since it lies on the causal pathway between prenatal tobacco smoke exposure and infant and childhood size.

C.4. Ethical Considerations

The Institutional Review Boards (IRB) of the University of North Carolina-Chapel Hill, Cincinnati Children's Hospital Medical Center, and CDC approved this study. The University Of Cincinnati College Of Medicine IRB was involved in the oversight of this study in the early stages of planning and implementation. All mothers provided written informed consent for themselves and their children prior to enrollment in the study. The informed consent signed at enrollment provided consent for follow-up, sample collection, and examinations until the child's 3rd birthday.

Figure 1: DAG representing the association between prenatal tobacco smoke exposure and meconium tobacco smoke metabolite concentrations.

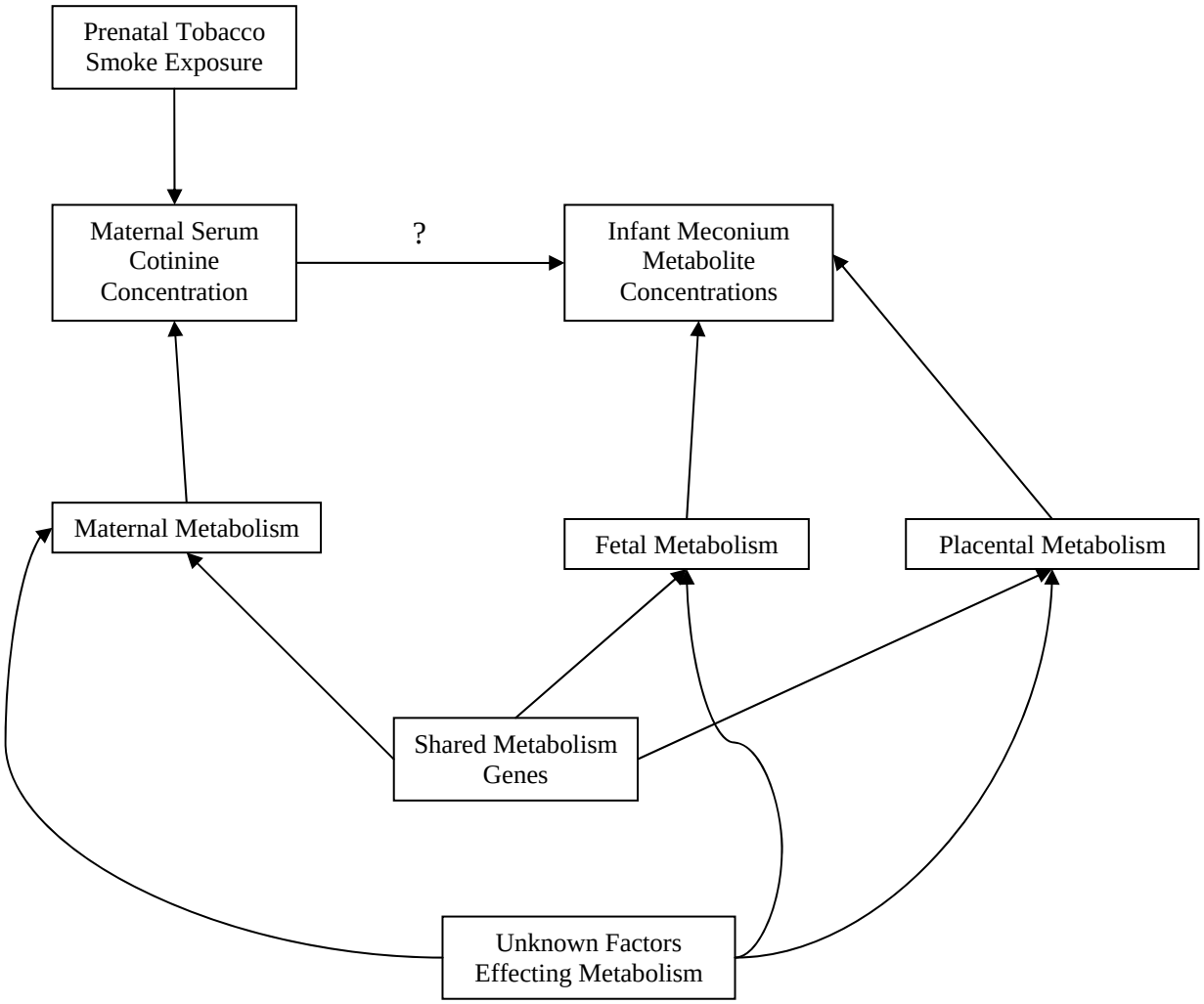
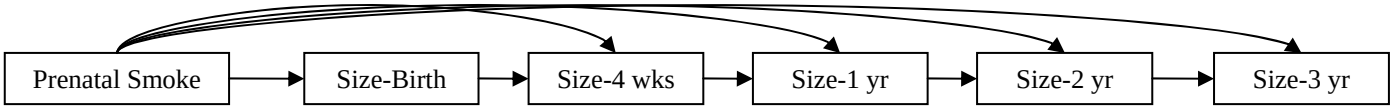
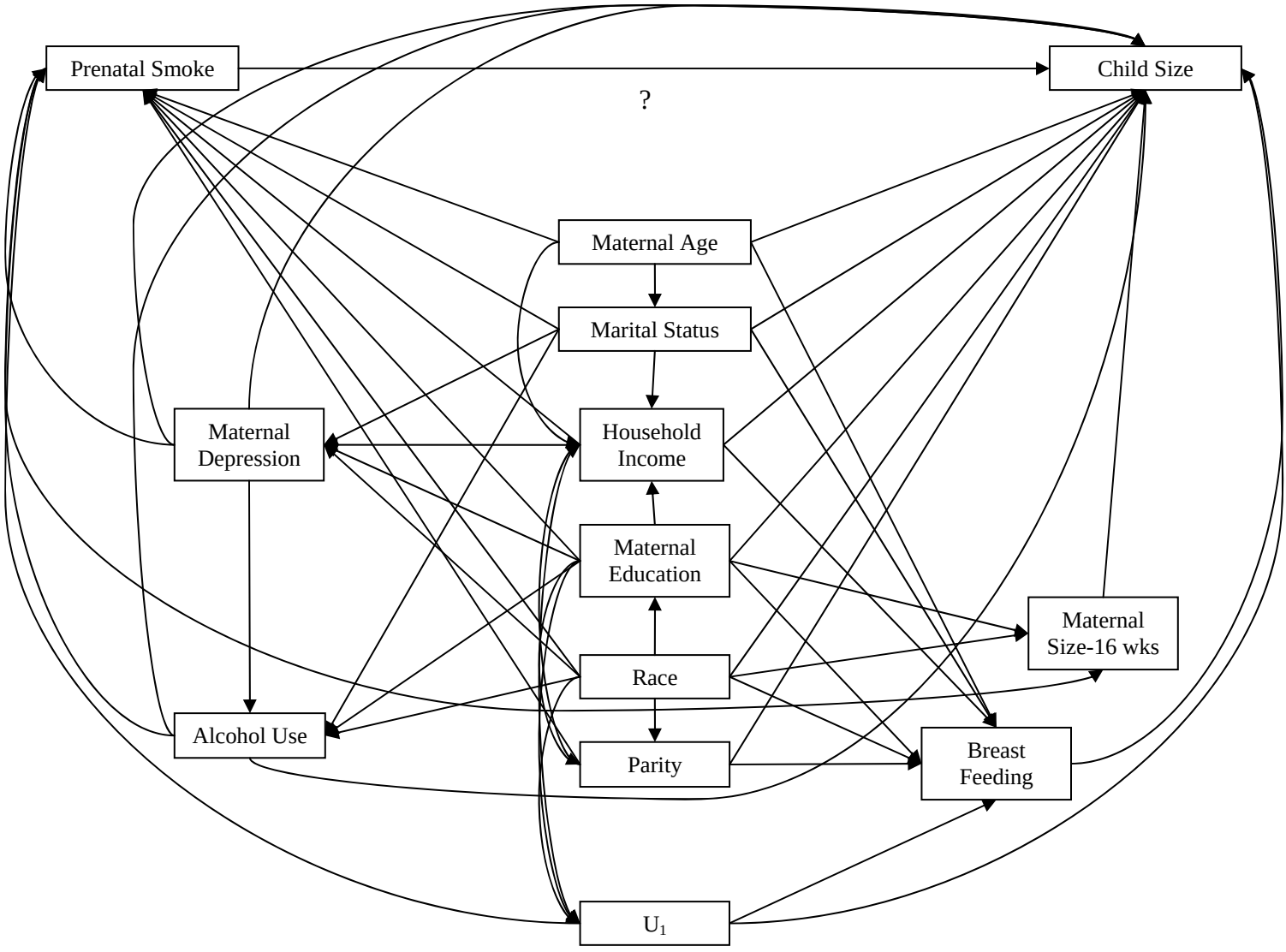


Figure 2: DAG representing the association between prenatal tobacco smoke exposure and childhood growth.





*-The top part of the DAG is reduced to the prenatal tobacco smoke and child size boxes for simplicity below. I assume that each variable's arrow will terminate at each size measurement.
U_1-Represents unknown SES factor that represents nutrition and activity factors.

Table 3: HOME Study Variables Used in Analyses

Variable	Source	Details	Coding	Aim
Outcome Variables				
Birth Weight	Med Records	In grams	Continuous	1 and 2
Birth Length	Med Records	In cm	Continuous	1 and 2
Birth Head Circumference	Med Records	In cm	Continuous	1 and 2
Weight	Anthropometrics at 4 weeks, 1, 2, and 3 years	In kg	Continuous	2
Length/Height	Anthropometrics at 4 weeks, 1, 2, and 3 years	In cm	Continuous	2
BMI	Kg/m^2	Derived for data at birth, 4 weeks, 1, 2, and 3 years	Continuous Categorical: Overweight: $\geq 85^{th}$ age and sex standardized percentile Normal weight: $<85^{th}$ age and sex standardized percentile	2
Exposure Variables				
Prenatal Serum Cotinine	16 and 26 week appointment, birth hospital stay	In ng/mL	Log_{10} transformed continuous. Categorical as no exposure, passive, and active	1 and 2
Postnatal Serum Cotinine	12 Months	In ng/mL	Log_{10} transformed continuous.	2
Meconium Cotinine	Birth hospital stay	In ng/gm	Log_{10} transformed continuous. Categorical as no exposure, passive, and active	1
Self-Reported Tobacco Exposure	20-week prenatal home visit and 4-week postpartum visit	Number of smokers in the home. Number of cigarettes smoked per day in the home Any active smoking as number of cigarettes per day.	Categorize as unexposed, second hand exposure, active exposure.	1
Confounders				
Maternal Age	Demographics	In years	<25, 25-34, >35	2
Maternal Race	Demographics		Non-Hispanic White, non-Hispanic Black,	2

Variable	Source	Details	Coding and other	Aim
Confounders (cont)				
Maternal Education	Demographics	In years	<12, 12, and >12	2
Marital Status	Demographics		Married and non-married/living alone	2
Child Sex	Birth records		Male vs. female	2
Maternal weight and height	Anthropometrics at 16 week visit	In kg and cm	Continuous	2
Depression	Beck Depression Inventory		Mild or minimal vs. moderate or severe	2
Alcohol use	Interview		Any or none	2
Parity	Birth records		0, 1, and >1	2
Breast feeding	Quarterly surveys	In years	Time varying as the number of years of feeding since the last anthropometric measurement	2
Household Income	Demographics	In dollars	<$40K, $40K-<$80K, and ≥$80K	2
Gestational Age	Medical Records	In weeks	Continuous Variable	1

D. Statistical Analyses

D.1. Statistical Analyses for Aim 1: Relationship between Serum and Meconium Biomarkers of Tobacco Smoke and Their Association with Infant Birth Weight

Aim 1: Examine the utility of meconium nicotine, cotinine, and OH-cotinine as a biomarker of prenatal tobacco smoke exposure.

Sub Aim 1.1: Determine if three serial serum cotinine concentrations and self-reported tobacco smoke exposures are positively associated with meconium tobacco smoke metabolites.

Sub Aim 1.2: Determine if meconium tobacco smoke metabolites represent cumulative exposure to gestational tobacco smoke.

Sub Aim 1.3: Compare the associations between serum and meconium biomarkers of prenatal tobacco smoke exposure and infant birth weight.

Aim 1 examined the utility of meconium tobacco smoke metabolites as a biomarker of prenatal tobacco smoke exposure. Statistical analyses were conducted on women who had at least 2 prenatal serum cotinine measurements and whose infants had a valid meconium measurement. Some analyses were further restricted to women with all 3 prenatal serum cotinine measurements or to women who completed both self-reports of prenatal tobacco smoke exposure. I started the statistical analysis by comparing women and infants with complete meconium, self-report and serum data to women with missing data.

I corrected for the right-skewed distribution of serum and meconium metabolite concentrations using the $\log_{10}$-transformation in analyses using continuous variables. In analyses involving meconium metabolite concentrations, results are presented for each metabolite (NIC, COT, and 3HC), as well as the sum of the three metabolites.

I created several measures of cumulative prenatal tobacco smoke exposure using either prenatal serum cotinine concentrations or self-reported tobacco smoke exposure (**Table 4)**.

First, I created a summary variable of women's self-reported prenatal tobacco smoke exposure among women with both tobacco smoke exposure interviews and infant meconium metabolite concentrations. This five category variable counted the number of periods that a woman had secondhand (0, 1, or 2 periods) or active (1 or 2 periods) tobacco smoke exposure. Women with 1 period of secondhand exposure could not have active exposure in the other period, whereas women with active exposure in 1 period could have secondhand or no reported tobacco smoke exposures in the other period.

Next, I used the mean of 2 or 3 individual serum cotinine measurements to create continuous and categorical summaries of women's prenatal tobacco smoke exposure between 16 weeks gestation and birth. I used categories of unexposed, (<LOD), secondhand exposure (LOD – 3 ng/mL), or active exposure (> 3 ng/mL).

Among women with all 3 serum cotinine and meconium measurements, I further quantified cumulative exposure to prenatal tobacco smoke by creating a summary variable that described the number of prenatal serum measurements that a woman was exposed to secondhand or active tobacco smoke. This seven category variable counted the number of measurements that a woman had secondhand (0, 1, 2, or 3) or active (1, 2, or 3) serum cotinine concentrations. Women in any of the secondhand categories could not have active exposure at their other measurements, while women in the active categories could have secondhand exposure or no exposure at their other measurements.

Finally, I investigated whether the timing of the serum measurements confounded the association between the number of secondhand or active serum cotinine measurements and meconium NIC concentrations. I did this by creating a 13 category variable that summarized the number and timing of secondhand and active serum measurements for the 16 week, 26 week, and birth serum cotinine measurement. I limited this analysis to NIC concentrations since they were detected most frequently and because the results with NIC were similar to COT and 3HC.

I calculated the geometric mean and standard deviation (GM and GSD) of meconium metabolite concentrations according to self-reported and serum cotinine concentration categories. I also calculated the proportion of infants with detectable meconium tobacco smoke metabolite concentrations according to self-reported and serum cotinine categories.

I used Locally Weighted Scatterplot Smoothing (LOESS) to graphically examine the relationship between the mean serum cotinine concentration and meconium tobacco smoke metabolite concentrations. LOESS uses locally weighted polynomials to fit subsets of data using ordinary least squares regression, giving more weight to points near the exposure value whose response is being estimated and less weight to points farther away.[133]

LOESS was used to examine the shape of the relationship between serum and meconium biomarker concentrations and infant size and gestational age. I used linear regression to estimate the association between $\log_{10}$-transformed serum and meconium biomarker concentrations and infant birth weight. I also estimated the association between each individual serum cotinine concentration (16 week, 26 week, and birth) and infant weight since many studies only can collect biomarkers at one time during pregnancy. Future investigators would be interested in comparing biomarkers that could be across the duration of pregnancy. I compared the magnitude and precision of meconium estimates to estimates using mean serum cotinine concentrations and estimates from the prior literature.

Table 4: Summary measures of prenatal tobacco smoke exposure using self-report or serum biomarkers

	Categories
Summary Self-Report	1: No exposure in either period
	2: Passive exposure in 1 period
	3: Passive exposure in both periods
	4: Active exposure in 1 period
	5: Active exposure in both periods
Mean Serum Cotinine	1: Unexposed (<LOD)
	2: Secondhand (LOD – 3ng/mL)
	3: Active (>3ng/mL)
Cumulative Serum Cotinine	1: Unexposed all 3 measures
	2: Passive exposure in 1 measure
	3: Passive exposure in 2 measures
	4: Passive exposure in 3 measures
	5: Active exposure in 1 measure
	6: Active exposure in 2 measures
	7: Active exposure in 3 measures

D.2. Statistical Analysis for Aim 2: Association between Prenatal Tobacco Smoke Exposure and Child Growth Over the First Three Years of Life

Aim 2: Estimate the association between prenatal secondhand and active tobacco smoke exposure and childhood BMI in the first three years of life.

Sub Aim 2.1: Compare the association between self-report and serum biomarkers of prenatal tobacco smoke exposure and BMI over the first three years of life.

Statistical analyses for Aim 2 began by comparing the demographic and exposure distributions of women-child dyads with missing and complete exposure and covariate data. Analyses included women with at least 2 prenatal serum cotinine measurements and complete covariate data.

Prenatal tobacco smoke exposure was quantified using the mean of at least 2 serum cotinine measurements and two self-reported measurements. I classified prenatal exposure into categorical and continuous variables. In categorical analyses using serum cotinine concentrations I classified women as unexposed (<0.015 ng/mL), secondhand exposure (0.015 to 3 ng/mL), and active exposure (>3 ng/mL). The threshold of 3 ng/mL was chosen based off of recent analyses of the National Health and Nutrition Examination Survey (NHANES) that found the previous threshold of 15 ng/mL resulted in a substantial proportion of smokers being classified as non-smokers.[134] I also created a continuous $\log_{10}$-transformed serum cotinine variable. All continuous cotinine analyses were repeated among women with no active tobacco smoke exposure during pregnancy (e.g., all 3 serum cotinine measurements $\leq$3 ng/mL). Self-reported prenatal tobacco smoke exposures were classified as unexposed for both periods of pregnancy, SHS exposure for one or two periods, and active exposure for one or two periods (could have SHS or no exposure in other period).

I started my analyses by cross-classifying self-reported and serum cotinine categories of prenatal tobacco smoke exposure. I assessed linearity between $\log_{10}$-transformed mean

prenatal and postnatal serum cotinine concentration and children's BMI at birth, 4 weeks, 1, 2, and 3 years using Locally Weighted Scatter Plot Smoothing (LOESS) analysis. LOESS uses locally weighted polynomials to fit subsets of data using ordinary least squares regression, giving more weight to points near the value whose response is being estimated and less weight to points further away [133]

Linear mixed models were used to examine the association between BMI and prenatal tobacco smoke exposure because of within-subject correlation of repeated measurements. Model construction began by modeling the association between weight, length, or BMI as a function of time (in years) since birth (birth coded as 0). Time was modeled with a linear, square, and cubic term. I explored using subject specific intercepts and time slope coefficients. However due to the homogeneity of growth trajectories in my sample the model fit better without these additional parameters. I included interactions terms between prenatal tobacco smoke exposure variables and time to allow the association between prenatal tobacco smoke exposure and childhood BMI to change at each follow-up visit.

:Logistic regression with generalized estimating equations was used to estimate the odds of overweight BMI at 2 or 3 years among by prenatal tobacco smoke exposure category (self-report or serum cotinine).

I compared estimated and compared the association between prenatal tobacco smoke exposure and childhood BMI using self-report or serum cotinine biomarkers. I estimated the precision of estimates using the confidence limit difference.[135]

All analyses were adjusted for maternal age, education, race, marital status, child sex, depression at 20 weeks gestation, parity, household income, breast feeding length and maternal weight/height/BMI. Breast feeding length was a time varying covariate that was coded as the number of weeks of breast feeding since the last child size measurement.

Several secondary analyses were conducted. First, trajectories of childhood growth were examined using weight, height, and weight-for-height z-scores. Weight, height, and

weight-for-height z-scores were computed using publicly available software from the National Centers for Health Statistics (NCHS). I estimated the mean weight, height, and weight-for-height z-score at birth and 1, 2, and 3 years of age according to prenatal tobacco smoke exposure category as well as the change in weight, height, or weight-for-height z-score among children with prenatal SHS or active tobacco smoke exposures compared to unexposed children (**Appendix 1**).

Second, I examined whether weight or height were responsible for associations between prenatal tobacco smoke exposure and BMI. I constructed linear mixed models as described above, but used continuous weight (in kg) or height (in cm) as the outcome in additional models (**Appendix 2**). Third, I included HOME Inventory scores in my statistical models to control for residual SES confounding. Finally, I restricted my analyses to the mothers and infants who completed all 4 follow-up visits to determine whether attrition biased my results (**Appendix 3**).

CHAPTER 4: RESULTS

A. Manuscript 1: Biomarkers of Prenatal Tobacco Smoke Exposure: The Correlation between Serum and Meconium and Their Association with Infant Birth Weight

A.1. Introduction

Fetal meconium is formed beginning in the 13th week of gestation from swallowed amniotic fluid, shed epithelial cells, and intestinal secretions.[24] Because meconium is metabolically inert, concentrations of drugs and other toxicants are thought to represent cumulative gestational exposure over the latter two-thirds of pregnancy. Ostrea and colleagues reported that meconium is a sensitive marker of gestational drug use in high-risk populations.[25, 47, 136] Others suggest that meconium may be a more sensitive matrix to measure gestational exposure to pesticides compared to maternal and infant hair and blood.[137, 138]

Several studies have detected and quantified tobacco smoke metabolites including nicotine (NIC), cotinine (COT), and trans-3'-hydroxycotinine (3HC) in infant meconium samples.[25, 27, 28, 48, 139] In humans, approximately 70 to 80% of NIC is metabolized into COT and 50% of COT is metabolized into 3HC.[140] Results from these studies indicate that NIC, COT, and 3HC concentrations are correlated with maternal report of tobacco smoke exposure. However, prior studies have been limited by use of self-reported or biomarkers of tobacco smoke exposure at birth to represent gestational exposures or have had few women with secondhand tobacco smoke (SHS) exposure.[25, 27, 29] To date, no studies have used serial self-reported or biomarkers of prenatal tobacco smoke exposure to determine if meconium tobacco smoke metabolites reflect the intensity and duration of prenatal tobacco smoke exposure.

Only two studies have examined the association between meconium tobacco smoke metabolite concentrations and childhood health outcomes.[26, 28] Gray and colleagues reported decrements in birth weight, length, and head circumference among women with detectable meconium tobacco smoke metabolite concentrations. Nuesslein et al. found a positive association between meconium cotinine concentrations and risk of respiratory infections in the first year of life. Both studies were relatively small and did not have other biomarkers of tobacco smoke exposure that allowed for comparison to meconium results.

We used data from the Health Outcomes and Measures of the Environment (HOME) Study to examine whether serial self-reported and serum biomarkers of prenatal tobacco smoke exposure were associated with meconium tobacco smoke metabolite concentrations.[141] We also examined whether meconium tobacco smoke metabolites represent cumulative gestational exposure to tobacco smoke. Finally, we evaluated whether the two biomarkers (meconium and serum) would produce similar results when investigating the association between prenatal tobacco smoke exposure and infant birth weight.

A.2. Methods

A.2.1. Study Sample

We used data collected from mothers and their infants participating in the HOME Study, an ongoing prospective birth cohort in the Cincinnati metropolitan area designed to examine low-level environmental toxicant exposure and the efficacy of injury and lead hazard controls in the home. From in March of 2003 to January 2006, women were identified from seven prenatal clinics associated with three hospitals. Eligibility criteria for the study included: $\leq$19 weeks gestation; $\geq$ 18 years old; living in a house built before 1978; negative HIV status; and not taking medications for seizure or thyroid disorders. We mailed letters to 5,512 women $\geq$ 18 years of age who were living in a home built before 1978 to see if they were eligible and interested in participating in our study. Additional eligibility criteria included: 13-19 weeks gestation at study enrollment; living in Brown, Butler, Clermont, or Warren counties, intention to continue prenatal

care and deliver at collaborating obstetric practices, HIV negative; and not receiving seizure, thyroid, or chemotherapy/radiation medications. Of the 1,263 eligible women, 468 enrolled in our study. Our analyses were restricted to singleton infants.

A.2.2.Tobacco Smoke Measurements

A.2.2.1. Self-Reported Exposure

Women were interviewed twice about secondhand and active tobacco smoke exposures for the periods between conception and 20 weeks (measured at 20 week home visit) and 20 weeks and birth (measured at 4 week postpartum visit). Trained interviewers asked the women the average number of cigarettes they smoked per day, the number of smokers living in the home, and the number of cigarettes smoked per day in the home for each time period. We classified the woman's exposure status during each time period as unexposed, exposed to secondhand tobacco smoke, and exposed to active tobacco smoke.

A.2.2.2. Serum and Meconium Biomarkers of Exposure

Women provided three serum samples at 16 weeks gestation, 26 weeks gestation, and within 24 hours of birth. All samples were stored at -20^{o}C until they were transported to the Centers for Disease Control and Prevention (CDC) laboratory for analysis, where they were stored at $\leq$ -20^{o}C. Serum samples were assayed for cotinine, a biomarker of nicotine exposure, using high performance liquid chromatography tandem mass spectroscopy (HPLC-MS/MS).[42] The limit of detection (LOD) for this assay was 0.015 ng/mL and had a coefficient of variation (CV) ranging from 3-4% at high concentrations (1 ng/mL) to 10% at low concentrations (100 pg/mL).

Meconium specimens were collected from infants during their hospital stay by placing cellulose fiber inserts inside infant diapers. After it was soiled, the diaper and insert were stored in a labeled plastic bag in a refrigerator. Meconium samples for each infant were pooled and subsequently stored at -20^{o} C until transported to CDC laboratories where they were stored $\leq$ -20^{o}C.

Briefly, for this analysis approximately 0.5 grams of meconium were digested at room temperature in 3 mL of 5N potassium hydroxide containing the internal standards. The digested material was extracted with methylene chloride and ethanol, back-extracted into hydrochloric acid, neutralized, buffered, and applied to a CleanScreen DAU column which was processed essentially as described by Ostrea et al.[47] HPLC-MS/MS was used to quantify the concentration of the NIC, COT, and 3HC relative to the deuterated internal standards. The LOD for these three compounds was 0.946 ng/gm for NIC, 0.070 ng/gm for COT, and 0.092 ng/gm for 3HC. Each analytical run included two blank samples and a low and high concentration quality control (QC) sample. The low-concentration QC samples had a CV between 10 and 30% while the high-concentration QC samples had a CV between 3 and 20%.

A.2.3. Infant Birth Weight and Covariates

Infant birth weight (in grams) was abstracted from hospital medical records and was analyzed as a continuous variable. Maternal age, race, education, and martial status were gathered at the first prenatal care visit. Maternal depression was assessed using the Beck Depression Inventory-II (BDI-II) which was administered during a home visit at approximately 20 weeks.[130] Parity was obtained from maternal medical records. Maternal weight (in kg) was collected at the initial clinic visit at 16 weeks gestation.

A.2.4. Statistical Analysis

We conducted our statistical analysis in two stages. First we examined the relationship between the various self-report and biomarkers of prenatal tobacco smoke exposure. Then, we examined and compared the association between meconium and serum biomarkers of tobacco smoke exposure and infant birth weight.

We started our statistical analysis by comparing women and infants with complete meconium, self-report, serum, and birth weight data to women with missing data. We corrected for the right-skewed distribution of serum cotinine and meconium tobacco smoke metabolite concentrations using the $\log_{10}$-transformation in analyses using continuous variables. Serum

and meconium cotinine values <LOD were randomly imputed from the left tail of the log_{10}-normal distribution.[142]

A.2.5.1. Relationship between Self-Report and Biomarkers of Tobacco Smoke Exposure

We compared the methods of classifying tobacco smoke exposures among mother-infant pairs with at least two prenatal serum cotinine measurements and a valid meconium measurement available. We created several measures of cumulative prenatal tobacco smoke exposure using either self-reported tobacco smoke exposure or prenatal serum cotinine concentrations.

First, we created a summary variable of self-reported prenatal tobacco smoke exposure over the course of pregnancy. This five category variable reflected the level and duration of exposure: unexposed, SHS exposure in one period, SHS exposure in both periods, active exposure in one period (the other period was unexposed or SHS exposure), and active exposure in both periods.

Next, we compared the three serum cotinine measurements from each woman. Because they were highly correlated (R~0.7-0.9), we calculated the avaialble serum cotinine measurements to create a continuous summary measure women's prenatal tobacco smoke exposure between 16 weeks gestation and birth. Form this, we also created categories of unexposed (<LOD), secondhand exposure (LOD – 3 ng/mL), and active exposure (> 3 ng/mL) over the course of pregnancy. The threshold of 3 ng/mL for active smoking was chosen based on results from the 1999-2004 National Health and Nutrition Examination Survey which compared self-reported smoking status and serum cotinine levels among a representative sample of the US population.[134]

Among women with all three serum cotinine and meconium measurements, we further quantified cumulative exposure to prenatal tobacco smoke by creating a summary variable that described the number of prenatal serum measurements that a woman was exposed to secondhand or active tobacco smoke. This seven category variable counted the number of

measurements that a woman had serum cotinine concentrations indicative of secondhand (zero, one, two, or three) or active (one, two, or three) tobacco smoke exposure. Women in any of the secondhand categories could not have had active exposure at another time point, while women in the active categories could have had another serum measurement indicative of secondhand or no exposure.

Finally, we investigated whether the timing of the serum measurements influenced the association between the number of serum cotinine measurements indicative of secondhand or active tobacco smoke exposure and meconium NIC concentrations. We did this by creating a 7 category variable that summarized the number and timing of serum cotinine concentrations consistent with secondhand and active tobacco smoke exposure at 16 weeks and birth. We limited this analysis to NIC concentrations because NIC was detected most frequently and the results with NIC concentrations were similar to those using COT and 3HC concentrations.

We calculated the geometric mean (GM) and corresponding 95% confidence interval (CI) of meconium tobacco smoke metabolite concentrations according to self-reported and serum cotinine concentration categories described above. We also calculated the proportion of infants with detectable meconium tobacco smoke metabolite concentrations according to self-reported or serum categories.

A.2.5.2. Association between Biomarkers of Prenatal Tobacco Smoke Exposure and Infant Birth Weight

The second stage of our analysis examined the association between biomarkers of tobacco smoke exposure and infant birth weight. We chose to examine birth weight because there is a well-established inverse relationship between serum cotinine concentrations and infant birth weight.[5, 6, 102, 108, 143] First, we analyzed the shape of the association between $\log_{10}$-transformed serum and meconium cotinine concentrations and birth weight using LOESS.[144]

Next, we estimated and compared the associations between continuous prenatal serum cotinine and meconium tobacco smoke metabolite concentrations and infant birth weight using

linear regression. Coefficients from these analyses represent the mean change in infant birth outcome for a 10-fold increase in mean prenatal serum cotinine or meconium tobacco smoke metabolite concentration. We also estimated the associations using meconium tobacco smoke metabolites and individual serum cotinine concentrations. In addition, we examined the association between categorical serum and meconium tobacco smoke metabolite concentrations and infant birth weight. Serum cotinine concentrations were categorized using the thresholds described above. Several different meconium tobacco smoke metabolite concentrations were used to discriminate secondhand from active tobacco smoke exposure. We compared the results for various exposure methods because many cohorts only have the resources to collect one exposure measurement. We examined the precision of the estimates by calculating the confidence limit difference (CLD) for each point estimate.[135]

In all of the analyses examining the association between prenatal tobacco smoke exposure and infant birth weight, we adjusted for confounders identified using a directed acyclic graph (DAG).[145] DAGs are a better method to assess the role of confounding variables compared to change in estimate and significance testing approaches.[146] Based on our DAG, we all models included maternal age (18-24, $\geq$35, and 25-34 years), maternal education (<12, 12, and >12 years), maternal race (non-Hispanic white, non-Hispanic black, and other), marital status (married vs. non-married), depression (minimally depressed [BDI-II score <14], mildly depressed [BDI-II Score 14-19], and moderately or severely depressed [BDI-II Score $\geq$20]), and maternal weight (in birth weight model) or height (in birth length model). We did not adjust for gestational age since it was an intermediary on the causal pathway between prenatal tobacco smoke exposure and infant birth weight.

Ethical Considerations

The Institutional Review Boards (IRB) of the University of North Carolina-Chapel Hill, Cincinnati Children's Hospital and Medical Center, and CDC approved this study. The Cincinnati Children's Hospital and Medical Center IRB was involved in the oversight of this

study. All mothers provided written informed consent for themselves and their children prior to enrollment in the study.

A.3. Results

Of the 468 women who enrolled in our study, 67 dropped out before delivery. We excluded 9 children from sets of twins and 3 still-born infants, leaving 389 infants. A total of 326 women and infants (83.8%) had at least two prenatal serum measurements and a meconium measurement available, many (n=284, 73.0%) had all three prenatal serum measurements and a meconium measurement. Complete self-reported tobacco smoke exposure and meconium data was available for 316 (81.2%) women. Women with complete data were better educated, non-Hispanic white, married, and 25-34 years of age compared to women with incomplete data (**Table 5**).

A.3.1. Relationship between Self-Report and Biomarkers of Tobacco Smoke Exposure

NIC, COT, and 3HC were detected in 80.1, 69.1, and 56.7% of infant meconium samples, respectively; 90.2% of samples had at least one detectable metabolite. Sixty-one percent of women had mean serum cotinine concentrations $\geq$ LOD and 88.9% had at least one detectable serum cotinine concentration between 16 weeks gestation and birth. Geometric mean meconium tobacco smoke metabolite concentrations were highest for NIC (GM: 2.40 ng/gm; 95% CI: 2.08, 2.78) and lower for COT (GM: 0.19 ng/gm; 95% CI: 0.15, 0.24) and 3HC (GM: 0.17 ng/gm; 95% CI: 0.13, 0.23). Seventy-five percent of women with mean serum cotinine concentration <LOD gave birth to infants with at least one detectable meconium tobacco smoke metabolite.

Meconium tobacco smoke metabolite concentrations were highly correlated with each other: NIC and COT (Pearson R=0.79), NIC and 3HC (Pearson R=0.72), and COT and 3HC (Pearson R=0.85). Meconium and serum metabolite concentrations were also highly correlated: NIC (Pearson R=0.59), COT (Pearson R=0.72), and 3HC (Pearson R=0.71). The CVs for our

meconium tobacco smoke metabolite assays (10-30%) were approximately 3 times higher than the CVs for our serum metabolite assays (3-10%).

Self-reported tobacco smoke exposures were positively associated with mean and detectable meconium tobacco smoke metabolite concentrations (**Table 6**). Compared to unexposed infants, women self-reporting SHS exposure gave birth to infants with slightly higher meconium nicotine concentrations and those reporting active smoking gave birth to infants with meconium nicotine concentrations 1 to 3 orders of magnitude higher. Meconium COT and 3HC concentrations were similar among women with self-report of secondhand or no tobacco smoke exposures, but higher among self-reported active smokers.

Infant meconium tobacco smoke metabolite concentrations were greater when mother's mean serum cotinine concentrations indicated secondhand and active tobacco smoke exposure rather than no exposure (**Table 6**). All three meconium tobacco smoke metabolites were detected in essentially all infants born to women with mean serum cotinine concentrations > 3 ng/mL.

Meconium tobacco smoke metabolite concentrations increased with the number of serum cotinine measurements indicative secondhand or active tobacco smoke exposure (**Table 6**). However, there was little difference in meconium tobacco smoke metabolite concentrations among infants born to women with one or two serum measurements indicative of SHS exposure (**Figure 3**). Infant meconium tobacco smoke metabolite concentrations were about 2 times higher in women with three serum measurements indicative of SHS compared to women with no tobacco smoke exposure. Relative to differences in meconium NIC concentrations, meconium COT and 3HC concentrations were higher among active smokers and women with three serum measurements indicative of SHS exposure compared to women with no exposure.

Secondhand or active tobacco exposures in later pregnancy resulted in greater increases in meconium NIC concentrations than exposures earlier in pregnancy (**Figure 4**). After adjustment for 26 week serum cotinine concentrations, meconium NIC concentrations

were higher among infants born to women with serum cotinine concentrations indicative of SHS exposure at birth only (GM: 2.71; 95% CI: 1.65, 4.43) compared to infants born to women with serum cotinine concentrations indicative of SHS exposure at 16 weeks only (GM: 1.77; 95% CI: 1.26, 2.48).

A.3.2. Association between Biomarkers of Prenatal Tobacco Smoke Exposure and Infant Birth Weight

LOESS analysis revealed a linear relationship between serum and meconium tobacco smoke metabolite concentrations and infant birth weight. Of the meconium tobacco smoke metabolites, NIC provided the largest and least precise point estimates compared to meconium COT and 3HC (**Table 7**). Of the individual serum cotinine concentrations, birth serum cotinine categories of prenatal tobacco smoke exposure were the largest and most precise point estimates. Meconium NIC concentrations provided estimates most similar to mean prenatal serum cotinine concentrations and birth serum cotinine concentrations. Categorical meconium COT and 3HC estimates provided attenuated associations relative to birth and mean serum cotinine categories and meconium NIC categories.

Estimates between SHS exposure and birth weight were similar when we evaluated different thresholds for classifying SHS and active exposure using meconium tobacco smoke metabolite concentrations. However, raising the threshold for active exposure increased NIC point estimates between active smoking and birth weight and raising the threshold for COT and 3HC decreased the point estimate between active smoking and birth weight.

A.4. Discussion

Meconium tobacco smoke metabolites were positively associated with self-report and serum biomarkers of prenatal tobacco smoke exposure. We observed a dose-dependent relationship between the number of serum cotinine measurements consistent with secondhand or active tobacco smoke exposure during the latter two-thirds of pregnancy and meconium tobacco smoke metabolite concentrations. Our results indicate that tobacco smoke metabolites

in meconium reflect the duration and intensity, but not timing, of gestational exposure to tobacco smoke.

Meconium COT and 3HC concentrations were higher and almost universally detected among infants born to active smokers compared to women with secondhand or no exposure. Meconium COT and 3HC concentrations may be a more sensitive biomarker of active prenatal tobacco smoke exposures, while meconium NIC concentrations may be a more sensitive marker of secondhand exposures since they were detected more frequently among infants with secondhand or no tobacco smoke exposure. An additional advantage to meconium NIC is the higher frequency of detection reduces the need to impute left-censored data.[142]

Meconium may be a more sensitive matrix to measure prenatal tobacco smoke exposure than serum if it reflects transient exposures that may not be captured by individual or serial serum measurements. However, non-detectable serum cotinine concentrations could be due to increased nicotine and cotinine metabolism and clearance during pregnancy.[140, 147] Thus, women with tobacco smoke exposures that are not detectable using serum cotinine might give birth to infants with detectable meconium tobacco smoke metabolites. We were not able to examine this hypothesis since we did not collect information about metabolic or genetic factors, like CYP2A6 enzyme activity, which may modify the relationship between tobacco smoke exposures and meconium tobacco smoke metabolites.[148, 149]

We detected a higher proportion of some meconium tobacco smoke metabolites than some previous studies.[27, 139] Our proportion of detectable meconium COT and 3HC was similar to Gray et al..[28] Another study reported almost universal meconium COT detection among their study participants.[26] Variations in study results could be due differences in meconium digestion/extraction or analytical chemistry methods.

Tobacco smoke exposures in later pregnancy may cause greater increases in meconium tobacco smoke metabolite concentrations relative to earlier exposures. This complicates the interpretation of meconium metabolite concentrations since they reflect the duration, intensity,

and timing of exposure. Differential accumulation of tobacco smoke metabolites in meconium over the course of pregnancy may be due to changes in blood volume, kidney and liver metabolism, placental perfusion, or increased quantities of amniotic fluid ingested by the infant later in gestation.[150] However, inferences regarding the timing of tobacco smoke exposures are based on a relatively small number of women and infants with different temporal patterns of exposure.

We are not aware of previous studies that attempted to validate meconium as a biomarker of prenatal tobacco smoke exposure using repeated serum cotinine measures for comparison. Ostrea et al. reported meconium nicotine concentrations increased with self-reported prenatal tobacco smoke exposure intensity.[25] Kohler et al. reported higher meconium NIC, COT, and 3HC concentrations among women with greater duration of active smoking during pregnancy compared to women who quit smoking earlier in pregnancy, but these results were based on only eleven women who quit smoking during pregnancy.[27] We were able to use serial serum measurements of prenatal exposure to accurately characterize exposure over the latter two-thirds of pregnancy.

Both serum cotinine and meconium tobacco smoke metabolite concentrations were inversely associated with birth weight. The magnitude and precision of the point estimates using meconium NIC concentrations was similar to serum cotinine concentration estimates within our cohort and to previous estimates of the association between prenatal serum cotinine concentrations and infant birth weight.[5, 6, 102, 108, 143] Categorical meconium COT and 3HC point estimates were smaller in magnitude relative to categorical meconium NIC and serum cotinine point estimates; however, point estimates using continuous meconium tobacco smoke metabolite or serum cotinine concentrations were very similar to one another. Investigators may wish to use serum cotinine measurements to quantify prenatal exposure since collecting and analyzing meconium samples will require additional resources.

There are some limitations to the presented results. First, we considered mean prenatal serum cotinine concentrations as the gold standard for prenatal tobacco smoke exposure in these analyses. Serum cotinine concentrations are a reasonable choice to compare a new biomarker of tobacco smoke exposure against since they are a more sensitive marker of secondhand exposure than self-report during pregnancy.[9, 23, 41] However, it would have been ideal to compare meconium to another long term biomarker of prenatal tobacco smoke exposure like hair nicotine or cotinine.

The small number of actively smoking women in our sample limited our ability to precisely estimate associations among active smokers. However, among the larger number of women with SHS exposure, we did observe similar patterns of association between the number serum cotinine measurements indicative of SHS exposure and meconium tobacco smoke metabolite concentrations.

Finally, women in our sample were from relatively high socioeconomic background, which is associated with decreased active and SHS exposure during pregnancy.[9, 23, 151] Thus, our results may not be generalizable to samples from populations with lower socioeconomic status who may have different exposure distributions.

There are several advantages and disadvantages to using meconium as a matrix to measure prenatal tobacco smoke exposure in epidemiological studies. First, meconium tobacco smoke metabolite concentrations reflect the duration and intensity of prenatal exposures, providing an accurate estimate of the dose of tobacco smoke constituents received by the infant in the latter part of pregnancy. In addition, meconium may be a good matrix to measure transient prenatal tobacco smoke exposures, as we detected nicotine in the meconium of infants born to women with undetectable serum cotinine concentrations and single serum cotinine measurement indicative of secondhand or active tobacco smoke exposure. However, meconium tobacco smoke metabolite concentrations do not allow classification of exposure during specific time periods of development. Furthermore, elevated meconium tobacco smoke

metabolite concentrations may be due to constant exposure over the entire course of pregnancy or high exposure in the latter parts of pregnancy. Second, meconium could be used as a matrix for biomarkers of exposure in research studies that enroll women at or shortly after parturition, but does require additional resources to collect and analyze. While both serum and meconium biomarkers provided similar estimates of association with birth weight, investigators should consider the additional resources necessary to collect and analyze meconium samples, especially if there are other well-developed biomarkers of exposure. Meconium digestion and analysis, done at the CDC laboratory, was more labor intensive and less efficient than serum cotinine assays, taking approximately 4 times as long to complete.

Meconium is a promising biological matrix to measure the duration and intensity of gestational exposure to environmental toxicants. Investigators planning to use meconium should consider whether meconium toxicant concentrations will provide additional exposure information not gleaned from other well-developed biomarkers of exposure. For studies of prenatal tobacco smoke exposure, meconium tobacco smoke metabolites do not provide additional exposure information that could not be captured by a single serum cotinine measurement. Additional research should determine meconium's ability to measure gestational exposure to other environmental toxicants. In addition, future studies should compare meconium and other validated biomarkers of exposure with infant or child health outcomes in well characterized exposure-outcome relations.

Table 5: Distribution of demographic variables among mothers/infants in HOME study

Variable	All Women N =389 (%)	Women with All Data N=315 (%) *	Women Missing Any Data N=74 (%)
Maternal Race			
White	237 (61.7)	209 (66.4)	28 (40.6)
Black	121 (31.5)	86 (27.3)	35 (50.7)
Other	26 (6.8)	20 (6.3)	6 (8.7)
Missing	5	0	5
Maternal Education (years)			
<12	41 (10.7)	23 (7.3)	18 (26.1)
12	54 (13.8)	41 (13.0)	13 (18.8)
>12	289 (74.5)	251 (79.7)	38 (55.1)
Missing	5	0	5
Marital Status			
Married	249 (64.8)	217 (68.9)	31 (44.9)
Single	135 (35.2)	98 (31.1)	38 (55.1)
Missing	5	0	5
Maternal Age Category (years)			
<25	96 (24.7)	63 (20.0)	33 (44.6)
25-34	231 (59.4)	197 (62.5)	34 (45.9)
35+	62 (15.9)	55 (17.5)	7 (9.5)
Missing	0	0	0

*Women had all 3 serum cotinine measurements, both self-reported tobacco smoke interviews, meconium measurement, and infant birth outcome data.

Table 6: Geometric mean infant meconium tobacco smoke metabolite concentration by prenatal tobacco smoke exposure.

		Meconium NIC		Meconium COT		Meconium 3HC	
	N	**% Detect**	**GM (95% CI)**	**% Detect**	**GM (95% CI)**	**% Detect**	**GM (95% CI)**
By Self-Reported Exposure							
Unexposed Both Periods	232	69.3	1.74 (1.50, 2.01)	60.3	0.10 (0.08, 0.12)	45.2	0.08 (0.06, 0.11)
SHS Exposure 1 Period	21	85.7	2.20 (1.35, 3.59)	95.2	0.27 (0.12, 0.62)	85.7	0.32 (0.13, 0.81)
SHS Exposure Both Periods	27	85.2	2.56 (1.67, 3.94)	77.8	0.20 (0.09, 0.40)	59.3	0.15 (0.07, 0.34)
Active Exposure 1 Period	15	86.7	4.34 (2.44, 7.73)	93.3	0.92 (0.35, 2.43)	86.7	0.91 (0.31, 2.68)
Active Exposure Both Periods	21	100	26.8 (16.6, 43.1)	100	15.9 (7.0, 36.2)	95.5	23.3 (9.5, 56.8)
By Mean Serum Cotinine							
Unexposed (<LOD)	131	70.6	1.50 (1.24, 1.82)	48.1	0.07 (0.05, 0.09)	33.8	0.05 (0.04, 0.07)
SHS Exposure (LOD – 3 ng/mL)	170	83.9	2.08 (1.76, 2.45)	79.4	0.16 (0.12, 0.21)	65.3	0.15 (0.12, 0.21)
Active Exposure (> 3 ng/mL)	37	94.7	21.3 (15.0, 30.1)	100	16.8 (9.7, 29.0)	97.4	21.3 (11.5, 39.3)
By Cumulative Serum Cotinine							
Unexposed all 3 Measures	61	71.2	1.32 (0.99, 1.76)	50	0.05 (0.03, 0.07)	30.0	0.03 (0.02, 0.05)
SHS Exposure in 1 Measure	61	65.5	1.57 (1.18, 2.10)	46.7	0.08 (0.05, 0.12)	40.0	0.07 (0.04, 0.11)
SHS Exposure in 2 Measures	41	71.8	1.70 (1.20, 2.42)	65	0.09 (0.05, 0.14)	40.0	0.07 (0.04, 0.12)
SHS Exposure in 3 Measures	92	89.2	2.41 (1.92, 3.03)	89.4	0.19 (0.14, 0.26)	72.5	0.18 (0.12, 0.27)
Active Exposure in 1 Measure	14	92.9	4.87 (2.71, 8.75)	100	2.77 (1.20, 6.37)	92.9	1.98 (0.75, 5.25)
Active Exposure in 2 Measures	3	100	24.6 (6.92, 87.3)	100	13.3 (2.20, 80.5)	100	20.6 (2.50, 170)
Active Exposure in 3 Measures	22	95.6	27.6 (17.5, 43.7)	100	20.6 (10.6, 40.0)	100	32.3 (15.1, 69.3)

*-Indicates any detectable meconium tobacco smoke metabolite.

**-Concentration in ng/gm

Table 7: Adjusted association between log_{10}-transformed serum and meconium biomarkers of tobacco smoke exposure and infant birth weight.*

	N	Change in Birth Weight (95% CI)	Confidence Limit Difference†
Meconium NIC			
Unexposed (<LOD)	62	Ref	---
SHS (LOD – 10 ng/gm)	212	-136 (-295, 24)	320
Active (>10 ng/gm)	28	-135 (-395, 126)	520
Continuous†	302	-62 (-178, 53)	231
Meconium COT			
Unexposed (<LOD)	97	Ref	---
SHS (LOD – 5 ng/gm)	171	-27 (-175, 120)	295
Active (>5 ng/gm)	34	-106 (-348, 135)	482
Continuous†	302	-61 (-132, 10)	143
Meconium 3HC			
Unexposed (<LOD)	135	Ref	---
SHS (LOD – 10 ng/gm)	139	-100 (-246, 46)	292
Active (>10 ng/gm)	28	-57 (-307, 192)	500
Continuous†	302	-30 (-96, 36)	132
Mean Serum COT			
Unexposed (<LOD)	122	Reference	---
SHS (LOD to 3 ng/mL)	148	-112 (-264, 41)	305
Active (> 3 ng/mL)	32	-189 (-462, 84)	545
Continuous†	302	-60 (-135, 16)	150
16 Week Serum COT			
Unexposed (<LOD)	101	Reference	---
SHS (LOD to 3 ng/mL)	162	-20 (-173, 132)	305
Active (> 3 ng/mL)	32	-109 (-386, 168)	554
Continuous†	295	-59 (-125, 7)	132
26 Week Serum COT			
Unexposed (<LOD)	126	Reference	---
SHS (LOD to 3 ng/mL)	138	20 (-135, 175)	305
Active (> 3 ng/mL)	29	-138 (-425, 150)	554
Continuous†	293	-41 (-104, 21)	125
Birth Serum COT			
Unexposed (<LOD)	121	Reference	---
SHS (LOD to 3 ng/mL)	131	-97 (-248, 54)	302
Active (> 3 ng/mL)	29	-190 (-462, 82)	545
Continuous†	281	-40 (-104, 24)	128

*-Adjusted for maternal age (18 to <25, 25 to <35, and 35+ years), race (non-Hispanic white, non-Hispanic black, and other), education (<12, 12, and >12 years), martial status (married and unmarried), depression (minimal, mild, and moderate/severe), parity (0, 1, and >1) and maternal weight (in weight models)/height (in length models).
†-Calculated by subtracting the lower 95% confidence limit from the upper 95% confidence limit
‡-Coefficients represent change in weight, length, head circumference, and gestational age for a 10-fold increase in metabolite concentration

Figure 3: Geometric mean meconium tobacco smoke metabolite concentration by duration and intensity of prenatal tobacco smoke exposure.

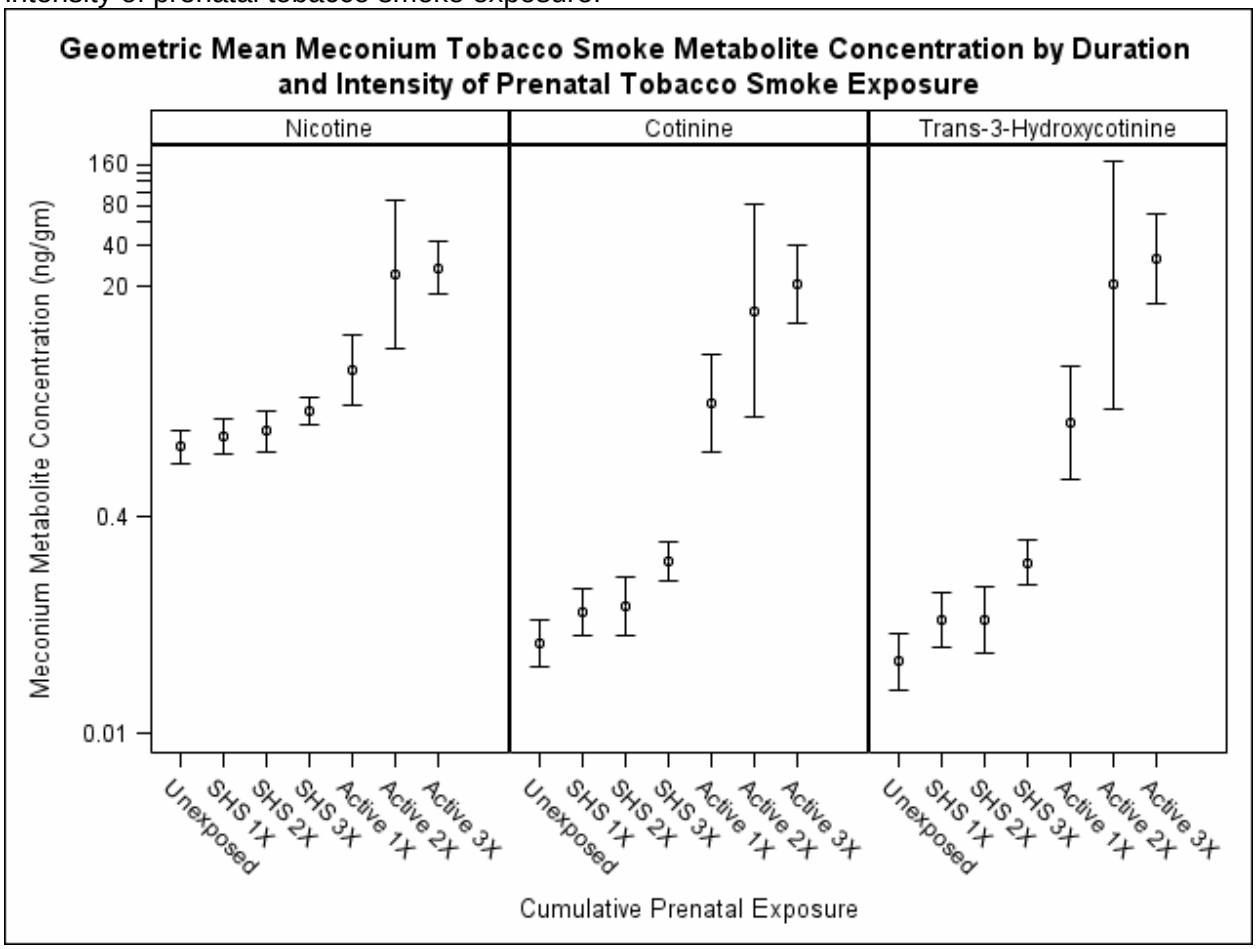
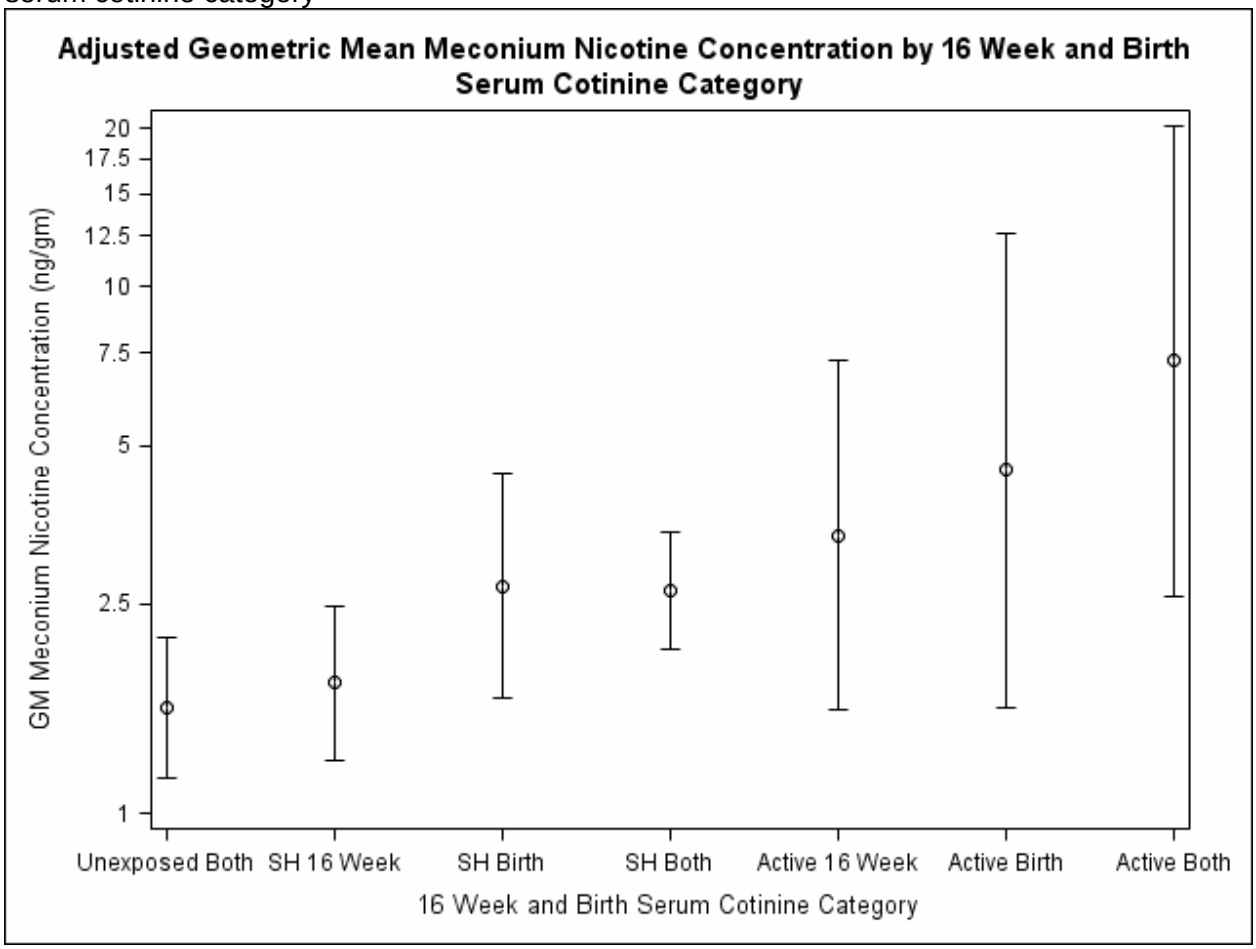


Figure 4: Adjusted* geometric mean meconium nicotine concentration by 16 week and birth serum cotinine category



*-Adjusted for 26 weeks serum cotinine category (<LOD, LOD – 3ng/mL, and > 3ng/mL)

B. Manuscript 2: Prenatal Tobacco Smoke Exposure and Early Childhood BMI

B.1. Introduction

Between 15 and 20% of United States children have overweight body mass index (BMI) and the proportion of children with overweight BMI is increasing in other countries.[152, 153] Elevated childhood BMI is associated with increased BMI and adiposity in adolescence and adulthood.[154, 155] Increased childhood BMI may also be a risk factor for metabolic disorders in early adulthood and coronary heart disease in later adulthood.[156, 157]

Several studies have observed excess adiposity and increased risk of overweight or obese BMI among children born to women who actively smoke during pregnancy compared to children born to non-smokers.[19, 21, 22, 158] A recent meta-analysis of 14 studies indicates that children born to active smokers have 1.5-times the risk of overweight or obesity as children born to non-smokers.[18] Increases in BMI and adiposity among children born to active smokers may be due to decreased weight and length at birth, rapid weight gain after birth, and decrements in height during childhood.[10, 15, 19, 82, 83, 159, 160]

Less is known about the association between prenatal SHS exposure and childhood BMI. We are aware of only two studies examining the association between prenatal secondhand tobacco smoke exposure and childhood BMI. Leary et al. reported elevated BMI, total body fat, and truncal fat among 10-year old children whose mothers had a partner who smoked during pregnancy.[21] Oken et al. did not report any association between self-reported prenatal secondhand tobacco smoke exposure and BMI at 3 years of age.[22]

Prior studies examining the association between prenatal active and secondhand tobacco smoke exposure and childhood BMI have relied on self-reported exposures. Self-reported secondhand tobacco smoke exposures may result in exposure misclassification that could bias the association between prenatal exposure and childhood BMI.[9, 23, 33] A prospectively

collected, valid biomarker of exposure might provide a more accurate estimate of the dose of tobacco smoke constituents received by the mother and fetus.

Given widespread exposure to secondhand tobacco smoke,[161] especially in newly industrialized countries,[13, 14] and the potential adverse health consequences of increased childhood BMI, we investigated the association between prenatal tobacco smoke exposure and early childhood BMI. In addition, we estimated and compared the associations between self-report and serum cotinine biomarkers of prenatal tobacco smoke exposure and early childhood BMI.

B.2. Methods

B.2.1 Study Sample

We used data collected from mothers and their children participating in the Health Outcomes and Measures of the Environment Study, an ongoing prospective birth cohort in the Cincinnati metropolitan area designed to examine low-level environmental toxicant exposure and the efficacy of injury and lead hazard controls in the home.[162] Between March of 2003 and January of 2006, women were identified from seven prenatal clinics associated with three hospitals. We mailed letters to 5,512 women $\geq$ 18 years of age who were living in a home built before 1978 to see if they were eligible and interested in participating in our study. Additional eligibility criteria included: $\leq$ 19 weeks gestation upon enrollment; living in Brown, Butler, Clermont, Hamilton, or Warren counties in Ohio; intention to continue prenatal care and deliver at collaborating obstetric practices; HIV negative; and not receiving seizure, thyroid, or chemotherapy/radiation medications. Of the 1,263 eligible women, 468 enrolled in our study. The current analyses were further restricted to singleton children.

B.2.2 Tobacco Smoke Exposure

Women were interviewed twice about secondhand and active tobacco smoke exposures for the periods between conception and 20 weeks (measured at 20 week home visit) and 20 weeks and birth (measured at 4 week postpartum visit) by trained research staff. Trained

interviewers asked the women the average number of cigarettes they smoked per day (if any), the number of smokers living in the home, and the number of cigarettes smoked per day in the home for each time period. Women were classified as unexposed if they reported no exposure for all of pregnancy. Women with active exposure during one or both periods were classified as active smokers. All others were classified as having SHS exposure.

B.2.2.1 Self-Reported Exposure

Birth weight and length were abstracted from the child's medical records. Subsequent weight and height measurements were obtained by study staff in the child's home or at our study clinic at 4 weeks and 1, 2, and 3 years of age. Each measurement was taken three times and the mean was used in the analysis. Weight was measured at 4 weeks and 1 year with an infant scale and at 2 and 3 years with a pediatric scale. Length at 4 weeks and 1 year was taken using a length board. At 2 and 3 years, height was obtained using a stadiometer; however, if the child was uncooperative at 2 years, I used a length board.[129] BMI was calculated by dividing weight in kilograms by height in meters squared.

B.2.2.2 Serum and Meconium Biomarkers of Exposure

Women provided blood samples around 16 weeks gestation, 26 weeks gestation, and within 24 hours of birth. All samples were stored at -20^{o} C until they were transported to the Centers for Disease Control and Prevention (CDC) laboratory for analysis, where they were stored at or below -20^{o} C. Serum from each sample was assayed for cotinine, a biomarker of nicotine exposure, using high performance liquid chromatography tandem mass spectroscopy (HPLC-MS/MS).[42] The limit of detection (LOD) for this assay was 0.015 ng/mL.

Prenatal serum cotinine concentrations were categorized as no exposure (<LOD), SHS exposure (LOD to 3 ng/mL), and active exposure (>3 ng/mL). The threshold of 3 ng/mL for active smoking was chosen based on results from the 1999-2004 National Health and Nutrition Examination Survey using self-reported smoking status and serum cotinine levels among a

representative sample of the US population.[134] These results showed that using a higher cutpoint of 15 ng/mL as the threshold for active smoking underestimates the proportion of active smokers. Prenatal serum cotinine concentrations were also analyzed as a continuous $\log_{10}$-transformed variable. In addition, we estimated the association between continuous prenatal serum cotinine concentrations and BMI among women with no prenatal active smoking (serum cotinine concentrations $\leq$3 ng/mL at each measurement).

B.2.3 Infant and Childhood Growth

B.2.3.1 Infant and Childhood Weight and Height Measurements

Birthweight and length were abstracted from the child's medical records. Subsequent weight and height measurements were obtained by study staff in the child's home or at our study clinic at 4 weeks and 1, 2, and 3 years of age. Each measurement was taken three times and the mean was used in the analysis. Weight was measured at 4 weeks and 1 year with an infant scale and at 2 and 3 years with a pediatric scale. Length at 4 weeks and 1 year was taken using a length board. At 2 and 3 years, height was obtained using a stadiometer; however, if the child was uncooperative at 2 years, we used a length board. BMI was calculated by dividing weight in kilograms by height in meters squared.

BMI was modeled as a continuous and dichotomous outcome. BMI was dichotomized to classify children as overweight at 2 or 3 years of age. We defined children as overweight if they had age and sex specific BMI $\geq$ the 85th percentile at 2 or 3 years of age. [163]

B.2.4 Confounders

We used directed acyclic graphs (DAGs) to examine the role of confounding by sociodemographic, perinatal, and childhood nutrition factors in our exposure-outcome relation.[145] Sociodemographic covariates were collected at the baseline clinic visit and included maternal age (<25, 25-34, and $\geq$35 years), race (non-Hispanic white, non-Hispanic black, and other), education (<12, 12, and >12 years), marital status (married and non-married), and household income (in increments of $10,000). Perinatal variables were maternal depression,

maternal BMI at 16 weeks gestation, and parity. Depression was measured at 20 weeks gestation with the Beck Depression Inventory (BDI-II).[164] Women were classified as minimally depressed (BDI-II score <14), mildly depressed (BDI-II score 14-19), or moderately/severely depressed (BDI-II score $\geq$20). Maternal BMI was modeled as a continuous variable from weight and height measurements collected at the 16-week prenatal clinic visit. Parity was abstracted from medical records and coded as 0, 1, and >1. Breastfeeding was collected quarterly during telephone interviews and included as a proxy of infant/childhood nutrition. We modeled breastfeeding as a time-varying covariate coded as the length of time (in fractions of years) in which the child received any breast milk since the last study visit. At birth, children were given a value of 0 years of breastfeeding.

The HOME Inventory, a semi-structured interview that assesses the quality of the caregiving environment, was administered by trained interviewers during a home visit when the child was 1 year old.[132] The HOME score, as a continuous variable, was evaluated as an additional proxy for socioeconomic status in a secondary analysis that examined the potential for residual confounding after controlling for household income and maternal education. We did not adjust for gestational age at birth since our DAG placed it on a causal pathway between prenatal tobacco smoke exposure and childhood growth.

B.2.5 Statistical Analysis

We descriptively compared the demographic characteristics of mothers and their children who had complete and missing covariate or exposure data. We also calculated the Pearson correlation coefficients between $\log_{10}$-transformed prenatal serum cotinine concentrations at each pair of time points.

We examined the shape of the relationship between continuous prenatal serum cotinine concentrations and children's BMI at each measurement using Locally Weighted Scatter Plot Smoothing (LOESS) analysis. LOESS uses locally weighted polynomials to fit subsets of data

using ordinary least squares regression, giving more weight to points near the exposure value whose response is being estimated and less weight to points farther away.[133]

Since our data involved repeated measurements on individuals, we used linear mixed models to examine the association between prenatal tobacco smoke exposure and BMI over the first 3 years of life.[165] Models were fit with time since birth (i.e., age in years), time-squared, and time-cubed terms. We allowed the association between prenatal tobacco smoke exposure and childhood size to vary over time by including interaction terms between tobacco smoke exposure and time variables. Tobacco smoke parameter estimates from our categorical models represent the mean change in BMI from the reference category, while estimates from models using continuous cotinine concentrations represent the mean change for each unit increase in $\log_{10}$-transformed (i.e., 10-fold increase) serum cotinine concentrations.

We used logistic regression with generalized estimating equations to estimate the association between secondhand and active prenatal tobacco smoke exposure and being overweight (BMI $\geq$ 85th percentile) at 2 or 3 years of age.[129] We generated odds ratios (OR) for being overweight given prenatal exposure to secondhand or active tobacco smoke compared to no exposure.

B.2.6 Secondary Analyses

We examined which component of BMI was responsible for associations between prenatal tobacco smoke exposure and BMI by constructing linear mixed models using categorical prenatal serum cotinine concentrations as the predictor variable and continuous weight or height z-scores at birth, 4 weeks, and 1, 2, and 3 years as the outcome. We calculated weight and height z-scores using publicly available software from the National Center for Health Statistics.[166] Our analyses were repeated using data from participants who had BMI measurements from all four visits to determine if children with incomplete data had excessive influence on our results. Finally, we constructed linear mixed models to analyze the association

between individual serum cotinine concentrations (16 week, 26 week, and birth) and BMI over the first 3 years of life.

B.2.7 Ethical Considerations

The Institutional Review Boards (IRB) of the University of North Carolina-Chapel Hill, Cincinnati Children's Hospital Medical Center, and CDC approved this study. The University Of Cincinnati College Of Medicine IRB was involved in the oversight of this study. All mothers provided written informed consent for themselves and their children prior to enrollment in the study.

B.3 Results

Of the 468 women who initially enrolled in our study, 67 dropped out before delivery. We excluded 9 non-singleton and 3 still-born children. Of the remaining 389 women and infants, at least two prenatal serum cotinine samples were collected and assayed from 384 (99%) women and self-reported prenatal tobacco smoke exposure was collected from 356 (92%) women. A higher proportion of women with complete self-report, serum cotinine, and covariate data at birth (N=292, 75%) were non-Hispanic white, better educated, wealthier, married, multiparous, and 25-34 years of age than women with missing data (**Table 9**). Of these women and infants, 202 (52%) had complete covariate data and follow up data at 3 years of age.

Nearly half of women (48%) with no self-report of prenatal tobacco smoke exposure had serum cotinine concentrations indicative of secondhand exposure (**Table 10**). Almost 84% of women who self-reported active smoking had corresponding serum cotinine concentrations. Pairs of prenatal serum cotinine concentrations taken at different times during pregnancy were highly correlated (Pearson R=0.7-0.8).

We observed an approximately linear relationship between $\log_{10}$-transformed prenatal serum cotinine concentration and weight, height, and BMI using LOESS analyses and

characterized $\log_{10}$-transformed serum cotinine concentrations as continuous variables in subsequent analyses.

After adjustment for confounders, children with prenatal active tobacco smoke exposure had higher mean BMI at 1, 2, and 3 years of age compared to unexposed children (**Table 11**). The pattern of association was similar using self-report or serum cotinine concentrations to classify exposure. The mean difference in BMI between unexposed and actively exposed children increased from 1 to 3 years of age. The HOME inventory score did not improve adjustment for socioeconomic confounding over maternal education and household income and was not retained in our models.

Children born to women self-reporting secondhand prenatal tobacco smoke exposure had BMI similar to unexposed children at all 5 measurements (**Table 11**). However, children born to women with serum cotinine concentrations indicative of SHS exposure had similar BMI at birth, 4 weeks, and 1 year, but higher BMI at 2 and 3 year of age compared to unexposed children (**Table 11**).

Children born to active smokers had increased odds of overweight BMI at 2 or 3 years of age compared to unexposed children (**Table 12**). The magnitude of association was greater for serum cotinine, but less precise than that for the self-reported association. Children with prenatal SHS exposure had increased odds for overweight BMI at 2 and 3 years of age when exposure was classified by serum cotinine concentrations, but not by self-report.

B.3.1 Secondary Analyses

As expected, at birth children with prenatal secondhand and active tobacco smoke exposure had lower weight (Mean Difference [MD] SHS vs. unexposed: -112, 95% CI: -268, 33; MD active vs. unexposed: -136; 95% CI: -405, 132) and shorter length (MD SHS vs. unexposed: -0.8, 95% CI: -1.5, -0.1; MD active vs. unexposed: -1.2; 95% CI: -2.4, 0) than unexposed children. Increased BMI at 2 and 3 years of age was the result of increased weight among prenatally exposed children at 2 and 3 years of age compared to unexposed children (**Figure**

5). In contrast, height at 1, 2, or 3 years of age was similar across categories of prenatal tobacco smoke exposure using self-report or serum cotinine concentrations (results for serum cotinine shown in Figure 1).

Our associations between prenatal serum cotinine concentrations and early childhood BMI were strengthened when we restricted analyses to the 194 children who completed all 4 follow-up visits. For example, at 3 years of age children with prenatal secondhand [MD: 0.53; 95% CI: 0.06, 1.00] and active [MD: 1.36; 95% CI: 0.47, 2.25] serum cotinine concentrations had higher BMI at 3 years compared to unexposed children.

Results from models separately examining 16 week, 26 week, and birth serum cotinine concentrations in relation to BMI were relatively similar to results using mean prenatal serum cotinine concentrations. Point estimates using individual or mean serum cotinine were similar in magnitude and precision for prenatal active tobacco smoke exposure. The point estimates for SHS were somewhat higher from serum cotinine concentrations taken at 26 weeks and birth compared to 16 week serum concentration estimates, but similar to mean prenatal serum cotinine concentration estimates.

B.4. Discussion

Children with prenatal active tobacco smoke exposure, classified using self-report or serum cotinine concentrations, had higher BMI and odds of overweight BMI at 2 or 3 years of age compared to unexposed children. Our results using serum cotinine concentrations were more suggestive of increased BMI among children with prenatal SHS exposure than the results using self-reported exposures. Self-reported secondhand tobacco smoke exposures were misclassified to a greater extent than self-reported active smoking, and accordingly, the point estimates were attenuated when compared to estimates based on serum cotinine concentrations.

Prior studies of prenatal tobacco smoke exposure and BMI have relied on self-reported tobacco smoke exposures and many studies have compared actively smoking women to non-smokers, without considering the impact SHS exposure, which, if misclassified as unexposed, may have contributed to an underestimate the impact of active prenatal tobacco smoke exposure on childhood BMI. Our results show that self-reported tobacco smoke measures fail to accurately quantify secondhand tobacco smoke exposures and may result in biased estimates of association.

Several investigators have reported increased BMI and risk of overweight BMI among 2-4 year old children born to active smokers.[18, 19, 22, 158, 167] ORs between prenatal active smoking and overweight BMI in prior studies range from 1.2 to 2.2.[19, 22, 158, 167] Our estimated association between active smoking and BMI were less precise than prior estimates, similar to Oken et al. and Adams et al. (OR: 2.2),[22, 158] and larger than Chen et al. and Whitaker et al. (OR: 1.2-1.5).[19, 167]

Consistent with our findings, Oken and colleagues did not report increased BMI among 3 year old children born to women with self-reported prenatal secondhand tobacco smoke exposure.[22] However, Leary et al. reported that 10 year old children born to women whose partners smoked regularly during pregnancy had BMI 0.1 standard deviations higher than unexposed children.[21] The discrepancy in results could be due to differences in tobacco smoke exposure questionnaire, temporal or geographic variations in secondhand exposure, or age of follow-up.

Increased weight at 2 or 3 years of age was responsible for increases in BMI among children born to women with serum cotinine concentrations consistent with secondhand and active tobacco smoke exposures. Children with prenatal tobacco smoke exposures were born lighter and shorter and grew heavier over the first 3 years of life compared to unexposed children. Attained height at 1, 2, or 3 years of age was similar between categories of prenatal tobacco smoke exposure.

Prenatal tobacco smoke exposure may influence childhood BMI by restricting fetal growth as a result of vascocontriction and hypoxemia.[82, 159, 160, 168] Restricted fetal growth may lead to rapid weight gain, resulting in increased BMI and adiposity during early childhood.[20, 160] In addition, tobacco smoke constituents may act on various hormonal systems that change metabolic programming.[169, 170] There has also been some suggestion that prenatal tobacco smoke exposure may alter infant appetite and leptin concentrations.[115, 171, 172]

The results of this study should be considered in light of several limitations. Confounding due to dietary, lifestyle, or socioeconomic factors may eliminate the observed elevation in BMI among children born to women with serum cotinine concentrations indicative of SHS exposure. Women who smoke or are exposed to higher levels of SHS during pregnancy are less likely to provide optimal diets and exercise for their children.[173, 174] Residual confounding may eliminate the positive association between prenatal secondhand tobacco smoke exposure and BMI at 2 and 3 years of age. We attempted to indirectly control for confounding due to dietary or lifestyle factors by including breast feeding duration and maternal BMI in our models. In addition, we controlled for other markers of socioeconomic status, including household income and maternal education which are associated with diet and exercise.[175, 176] We also used HOME Inventory scores as a proxy for socioeconomic, dietary, and exercise factors not captured by other variables. Our results were not substantially different when we included HOME Inventory scores in our models.

While serum cotinine is considered an ideal biomarker for tobacco smoke exposure, it only reflects exposure over the last 2 to 3 days, which introduces the potential for exposure misclassification.[30, 41] Women could have been exposed intermittently (e.g., only on weekends). However, we do not believe this is a substantial source of bias since serum cotinine concentrations were highly correlated and we used 2 or more prenatal serum samples from each woman to quantify prenatal exposure.

Increased BMI can result from increases in fat mass or fat-free mass. Other measures of body composition, including densitometry, bioelectric impedance, dual energy x-ray absorptiometry (DXA), and isotope dilution can derive these separate components of body composition and provide more accurate quantification of excess adiposity.[177] In addition, BMI is differentially correlated with direct measures of adiposity, with higher correlation among children $\geq 85^{th}$ BMI percentile.[178] While there are limitations to using BMI as a marker of early childhood adiposity, BMI is endorsed by the World Health Organization to measure and track body composition across the life-course.[179] Future research should examine the association between prenatal tobacco smoke exposures using direct measures of fat and fat-free mass and study children in later childhood when BMI measurements become more reflective of adult adiposity.[155]

A substantial proportion of women and their children dropped out of the study by 3 years of age. Attrition in our study was associated with socioeconomic factors, which are related to health and caregiving behaviors that influence childhood adiposity and tobacco smoke exposures. The association between prenatal serum cotinine concentrations and BMI was slightly strengthened when we restricted to children who completed all four follow-up visits.

Women in this study were required to be living in housing built before 1978. We do not believe this limits the generalizability of our study, since almost 70% of US housing units were built prior to 1978.[180] However, we analyzed a relatively homogenous sample of mothers who come from higher socioeconomic backgrounds which may reduce the generalizability of these results to samples drawn from populations with lower SES.

These results suggest that prenatal serum cotinine concentrations may be associated with increased BMI and odds of overweight BMI at 2 and 3 years of age. Given the high prevalence of tobacco smoke exposure and detrimental health consequences of increased BMI, additional research should be conducted examining the relationship between prenatal SHS exposures and childhood BMI and adiposity. Specifically, future studies should use validated

biomarkers of tobacco smoke exposure to reduce misclassification of SHS exposure and examine this association at later ages. The presented findings add to a growing body of literature suggesting that prenatal environmental insults-including secondhand tobacco smoke exposure- may impact the public's health by influencing the risk of later life health outcomes through excess childhood BMI.[181, 182]

Table 8: Distribution of demographic variables among mothers/infants in Health Outcomes and Measures of the Environment study

Variable	All Mothers and Infants N =389 (%)	Mothers and Infants with Complete Data N=292 (%)	Mothers and Infants with Missing Data N=97 (%)
Maternal Race			
Non-Hispanic White	237 (61.7)	191 (65.4)	46 (50.0)
Non-Hispanic Black	121 (31.5)	84 (28.8)	37 (40.2)
Other	26 (6.8)	17 (5.8)	9 (9.8)
Missing	5	0	5
Maternal Education (years)			
<12	41 (10.7)	27 (9.2)	14 (15.2)
12	54 (14.1)	36 (12.3)	18 (19.6)
>12	289 (75.3)	229 (78.4)	60 (65.2)
Missing	5	0	5
Marital Status			
Married	249 (64.8)	197 (67.5)	52 (56.5)
Single	135 (35.2)	95 (32.5)	40 (43.5)
Missing	5	0	5
Maternal Age Category (years)			
<25	96 (24.7)	60 (20.5)	35 (36.5)
25-34	231 (59.4)	181 (62.0)	50 (52.1)
35+	62 (16.0)	51 (17.5)	11 (11.5)
Missing	0	0	0
BDI at 20 Weeks			
Minimal Depression (0-13)	291 (7.6)	232 (79.4)	59 (71.1)
Mild Depression (14-19)	54 (24.4)	35 (12.0)	19 (22.9)
Moderate or Severe Depression (20-28)	30 (8.0)	25 (8.6)	5 (6.0)
Missing	14	0	14
Parity			
0	171 (44.2)	122 (41.8)	49 (51.6)
1	124 (32.0)	91 (31.2)	33 (34.7)
>1	92 (23.8)	79 (27.0)	13 (13.7)
Missing	2	0	2
Prenatal Serum Cotinine Category			
<LOD	140 (36.5)	114 (39.0)	26 (28.3)
LOD to 3 ng/mL	199 (51.8)	137 (50.4)	52 (56.5)
>3 ng/mL	45 (11.7)	31 (10.6)	14 (15.2)
Missing	5	0	5
Mean Maternal Weight at Baseline (SD)	75.0 (20.2)	74.8 (20.3)	75.7 (19.7)
Missing	5	0	5
Mean Maternal Height at Baseline (SD)	164.9 (6.8)	164.8 (6.7)	165.5 (7.2)
Missing	20	0	20
Mean BMI at Baseline (SD)	27.5 (6.8)	27.5 (6.9)	27.8 (6.4)
Missing	26	0	26
Mean Income in Thousands of Dollars (SD)	58 (43)	61 (43)	46 (41)
Missing	13	0	13
Mean Total Weeks of Breast Feeding (SD)	22.5 (23.4)	22.9 (23.3)	19.8 (24.3)
Missing	58	0	58

Table 9: Joint distribution of prenatal self-reported tobacco smoke exposure and serum cotinine concentration categories.

	No Self-Reported Exposure N=214 (%)	Self-Reported Secondhand Exposure N=42 (%)	Self-Reported Active Exposure N=36 (%)
Unexposed (<LOD)	108 (50.5)	5 (11.9)	1 (2.8)
SHS Exposure (LOD – 3ng/mL)	103 (48.1)	35 (83.3)	9 (25.0)
Active Exposure (> 3ng/mL)	3 (1.4)	2 (4.8)	26 (72.2)
Median Serum Cotinine Concentration (Min, Max)	0.015 (<LOD, 14.9)	0.134 (<LOD, 3.92)	35.3 (<LOD, 355.5)

*-Limited to women with complete covariate data.

Table 10: Adjusted mean and difference in BMI by change in prenatal tobacco smoke exposure*

Self-Reported Prenatal Tobacco Smoke Category	**N†**	**Birth (N=293)**	**4-Week (N=297)**	**1 Year (N=266)**	**2 Years (N=229)**	**3 Years (N=202)**
Mean among Unexposed	214	13.5 (13.2, 13.7)	14.0 (13.7, 14.2)	17.0 (16.8, 17.2)	16.7 (16.4, 17.0)	15.9 (15.6, 16.2)
SHS	43	0.0 (-0.4, 0.5)	0.0 (-0.4, 0.4)	-0.1 (-0.7, 0.4)	-0.1 (-0.6, 0.5)	0.0 (-0.5, 0.6)
Active	36	0.3 (-0.2, 0.8)	0.3 (-0.2, 0.8)	0.2 (-0.4, 0.8)	0.6 (0.0, 1.2)	1.1 (0.4, 1.7)

Categorical Prenatal Serum Cotinine Concentrations	**N†**	**Birth (N=300)**	**4-Week (N=303)**	**1 Year (N=273)**	**2 Years (N=236)**	**3 Years (N=209)**
Mean among Unexposed (<0.015 ng/mL)	116	13.4 (13.1, 13.7)	14.0 (13.7, 14.3)	17.3 (16.9, 17.66)	16.7 (16.3, 17.1)	15.8 (15.4, 16.2)
SHS (0.015-3 ng/mL)	153	-0.2 (-0.5, 0.2)	-0.2 (-0.5, 0.1)	-0.1 (-0.6, 0.3)	0.3 (-0.1, 0.7)	0.4 (0, 0.8)
Active (>3 ng/mL)	31	0.0 (-0.6, 0.6)	0.1 (-0.5, 0.6)	0.4 (-0.4, 1.1)	0.6 (-0.1, 1.4)	1.0 (0.3, 1.8)
Continuous Prenatal Serum Cotinine Concentrations						
Log_{10}-transformed mean cotinine	300	0.0 (-0.2, 0.2)	0.0 (-0.2, 0.2)	0.1 (-0.1, 0.3)	0.2 (0.0, 0.4)	0.3 (0.1, 0.5)
Log_{10}-transformed mean cotinine (Secondhand exposed women) ‡	269	-0.1 (-0.4, 0.2)	-0.2 (-0.5, 0.1)	-0.2 (-0.6, 0.2)	0.2 (-0.2, 0.5)	0.3 (-0.1, 0.6)

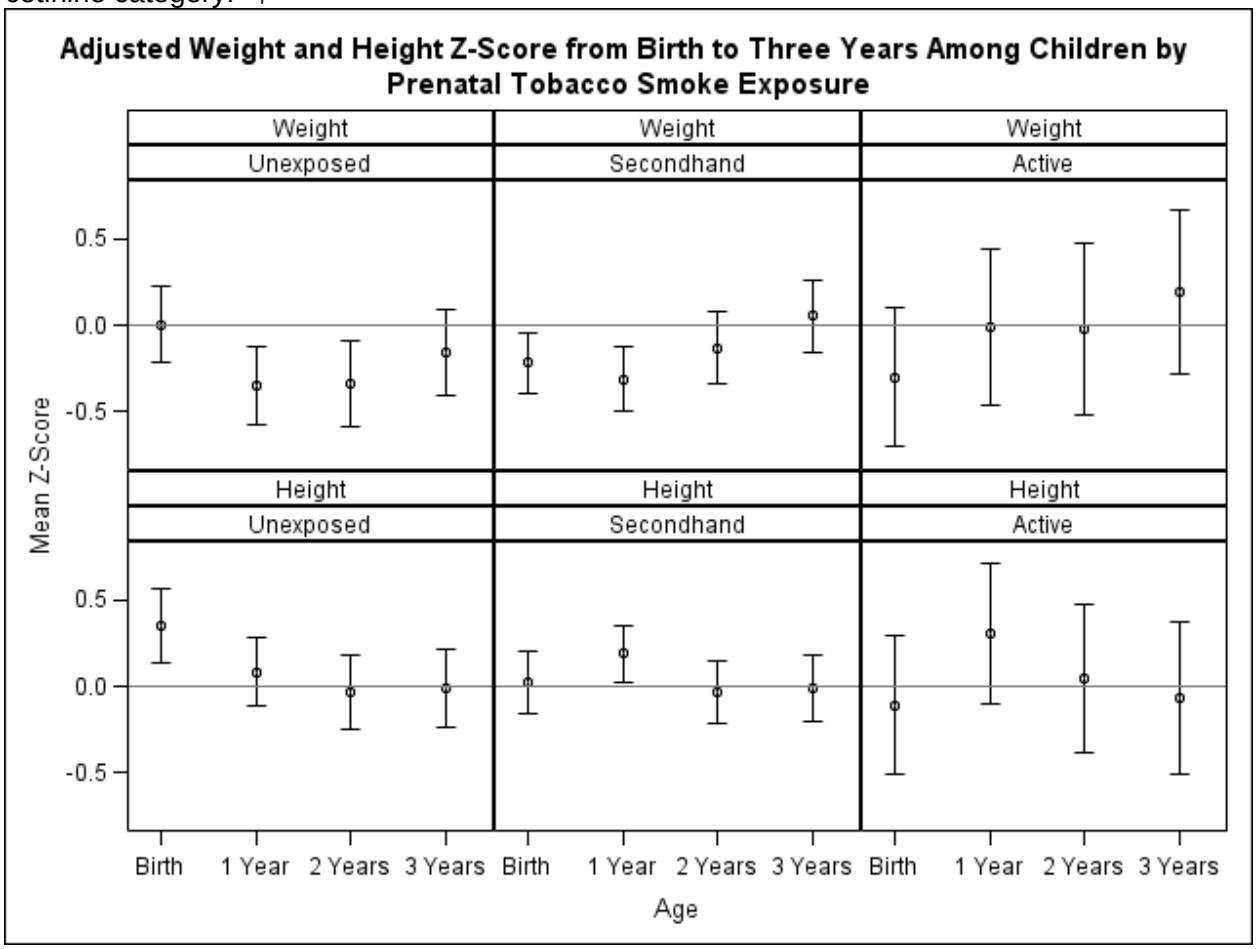
*-Adjusted for child age (in years), child age squared, child age cubed, maternal age (<25, 25-34, and >34 years), education (<12, 12, and >12 years), race (non-Hispanic white, non-Hispanic black, and other), marital status (married and non-married), depression at baseline home visit (moderately or severely depressed and mildly or minimally depressed), breast feeding duration since last visit (years), parity (0, 1, and >1), income ($10,000 increments), and maternal BMI (kg/m^2) at 16 weeks gestation.
†-Sample size at birth
‡-Women could not have cotinine concentration > 3 ng/mL in any serum measurement.
SHS-Secondhand smoke
§-Model with fit with birth, 4 week, and 1 year measurements.

Table 11: Unadjusted and adjusted association between categorical prenatal tobacco smoke exposure and overweight BMI at 2 or 3 years of age*

	Total	>85th Cases (%)	>85th Unadjusted OR (95% CI)	>85th Adjusted OR (95% CI)
Self-Reported Prenatal Tobacco Smoke Category				
Unexposed	307	43 (14.0)	Reference	Reference
SHS	56	9 (16.1)	1.2 (0.5, 3.1)	1.0 (0.3, 3.0)
Active	48	14 (29.2)	2.5 (1.0, 6.1)	1.9 (0.6, 6.1)
Categorical Prenatal Serum Cotinine Concentrations				
Unexposed (<0.015 ng/mL)	176	18 (10.2)	Reference	Reference
SHS (0.015-3 ng/mL)	209	40 (19.1)	2.1 (1.0, 4.5)	1.9 (0.8, 4.4)
Active (>3 ng/mL)	40	10 (25.0)	3.3 (1.1, 10.0)	2.4 (0.6, 9.7)

*-Adjusted for maternal age (<25, 25-34, and >34 years), education (<12, 12, and >12 years), race (non-Hispanic white, non-Hispanic black, and other), marital status (married and non-married), depression at baseline home visit (moderately or severely depressed and mildly or minimally depressed), breast feeding duration (years), parity (0, 1, and >1), income ($10,000 increments), and maternal BMI (kg/m^2) at 16 weeks gestation.

Figure 5: Mean weight and height z-score from birth to 3 years of age by prenatal serum cotinine category.* †



*-Adjusted for child age (in years), child age squared, child age cubed, maternal age (<25, 25-34, and >34 years), education (<12, 12, and >12 years), race (non-Hispanic white, non-Hispanic black, and other), marital status (married and non-married), depression at baseline home visit (moderately or severely depressed and mildly or minimally depressed), breast feeding duration since last visit (years), parity (0, 1, and >1), income ($10,000 increments), and maternal BMI at 16 weeks gestation.

CHAPTER 6: DISCUSSION

A. Summary of Findings

The presented findings addressed two questions using prospectively collected data from approximately 350 mothers and their infants participating in the HOME Study. First, are meconium tobacco smoke metabolite concentrations a useful biomarker of prenatal tobacco smoke exposure? Second, are prenatal serum cotinine concentrations associated with childhood BMI over the first 3 years of life?

The results of the first aim show that meconium tobacco smoke metabolites reflect the duration and intensity of prenatal tobacco smoke exposure. Infant meconium tobacco smoke metabolite concentrations were higher among women with self-reported and serum cotinine concentrations indicative of secondhand and active tobacco smoke exposure, in comparison to infant meconium metabolite concentrations among women unexposed to prenatal tobacco smoke. There were not substantial differences in meconium tobacco smoke metabolite concentrations among infants born to women with 1 or 2 serum or self-reported measurements of SHS exposure. This could be due to the poor sensitivity of self-report to capture secondhand exposures or little variability in meconium tobacco smoke metabolite concentrations among women with low-level SHS exposures.[9, 23]

My results suggest that secondhand or active tobacco smoke exposures later in pregnancy may result in greater increases in meconium metabolite concentrations compared to earlier exposures. Thus, elevated meconium tobacco smoke metabolite concentrations could be due to sustained exposure over the entire duration of pregnancy or high exposure near parturition. This further complicates the cumulative nature of meconium as biological matrix of

exposure since the rate of metabolite accumulation varies differentially across the course of pregnancy.

Since meconium is biologically inert, it may be a sensitive matrix for detecting transient environmental exposures. Two of my observations substantiate this. First, 75% of women with mean serum cotinine concentrations below the LOD had at least one detectable meconium metabolite concentration. Second, meconium metabolite concentrations were higher among infants born to women with only one serum cotinine concentration consistent with SHS exposure compared to women with all three serum cotinine concentrations below the LOD.

Prior studies validating the utility of meconium have not had serial measures of prenatal tobacco smoke exposures.[25, 27-29] Three studies have used maternal recall of prenatal tobacco smoke exposure[25, 28, 29] and one used urinary cotinine concentrations at birth and self-report.[27] Meconium tobacco smoke metabolites were detected in a higher proportion of infants than prior studies, indicating that our NIC, COT, and 3HC assays are more sensitive than previously developed methods. This is because our laboratory method was able to completely digest meconium into an aqueous solution.

I found that meconium tobacco smoke metabolites were inversely associated with infant birth weight, length, and head circumference. Furthermore, meconium point estimates were similar in magnitude to mean prenatal serum cotinine concentration associations and to estimates from previous studies using serum cotinine as a biomarker of exposure.[5, 6, 16, 102, 108, 122] Prior studies have estimated that SHS exposure is associated with reductions in birth weight ranging from 10 to 150 grams. A meta-analysis of 3 studies using serum cotinine levels estimated an 82 gram (95% CI: 37, 126) reduction in birth weight associated with prenatal SHS.[5]

In my second aim, I found that prenatal serum cotinine concentrations were associated with elevated BMI and odds of overweight BMI at 2 and 3 years of age. Elevated BMI resulted from non-uniform increases in weight from birth to 3 years of age among children born to

women with serum cotinine concentrations indicative of secondhand and active tobacco smoke exposure (**Appendix 1**). Prenatal active tobacco smoke exposures were associated with larger increases in BMI at 2 and 3 years of age compared to prenatal SHS. Consistent with non-differential exposure misclassification, the associations between self-reported SHS smoke exposure and BMI were attenuated towards the null compared to associations using serum cotinine concentrations to quantify SHS exposure.

A meta-analysis of 14 studies found children born to smokers were 1.50-times (95% CI: 1.36, 1.65) as likely to be obese or overweight as children born to non-smokers.[18] My estimated association is 47% higher than the meta-analysis estimate. The presented estimates were similar to two studies that have examined this association among 2-4 year old children (OR: 2.2),[22, 158] but larger than two other studies examining the same age children (OR: 1.2-1.5).[19, 167] However, the prior literature has not accounted for an association between prenatal secondhand tobacco smoke exposure and BMI, thus prior studies may have underestimated the association between active smoking and BMI.

The two prior studies examining the association between prenatal SHS exposure and childhood BMI have reported different findings. Oken et al. claimed no association between prenatal SHS exposure and BMI at 3 years of age.[22] However, they did not provide point estimates or confidence intervals in their paper. They appear to base their conclusion of no association based on non-significant p-values. Consistent with my results, Leary et al. reported that children born to women reporting prenatal SHS exposures had BMI 0.4 units higher than unexposed children at 10 years of age.[21] This prior estimate may actually be larger if self-reported SHS exposures were non-differentially misclassified in their study.

My results suggest that increases in childhood BMI associated with prenatal tobacco smoke exposures are due to changes in early childhood weight trajectories. Exposed children were born lighter and shorter and grew heavier over the first 3 years of life compared to

unexposed children. A prior study examining patterns of childhood growth over the first 8 years of life observed a similar phenomenon of increasing weight and decreased height among children born to smokers compared to children of non-smokers.[19] Tobacco smoke constituents may act on a variety of biological pathways to influence childhood growth. For example, nicotine may act as an appetite suppressant in utero and once removed after birth the infant may demand additional feeding.[183]

B. Strengths and Limitations

There presented results have strengths and limitations that should be considered when interpreting these results. While my first aim was relatively straightforward, there are concerns about generalizability, validity of biomarkers, and confounding.

Women and infants who had all self-report and biomarkers of prenatal tobacco smoke exposure were more likely to be higher SES than women with incomplete data. In addition, women in my sample were better educated and wealthier than the source population. Lower SES is associated with increased active and secondhand tobacco smoke exposure.[9, 23, 43, 184] These two biases limit the generalizability of my results to populations of women with lower socioeconomic backgrounds and greater prenatal tobacco smoke exposure. However, these biases should not affect my internal validity.

Picking a gold standard is a problem in any biomarker validation study and the choice of standard depends on the target tissue of interest (fetal or maternal) and timing and duration of exposure. In Aim 1, I assumed that maternal serum cotinine concentrations, which reflect recent tobacco smoke exposure, were the gold standard. This may not be appropriate since meconium reflects fetal exposure during the latter two-thirds of gestation. Maternal hair nicotine or cotinine concentrations may serve as a better gold standard for comparison to meconium tobacco smoke metabolite concentrations.[185] Given the low sensitivity of self-reported exposures[9, 23] and lack of hair cotinine concentrations in this study, serum cotinine concentrations were the most sensible gold standard.

While I had two different measures of prenatal tobacco smoke exposures, each measure has strengths and limitations. Self-reported measures of tobacco smoke exposures can be used to quantify exposure over longer periods of time; however, self-reported measures are known to misclassify women's secondhand exposure.[9, 23, 33, 39, 41] Unlike self-reported exposure, short-term markers like serum cotinine only reflect the last 2-3 days of exposure.[41] Thus, a woman with exposure on weekends (e.g., at bars or restaurants), may be misclassified depending on the timing of her serum measurement. Single serum measurements may be sufficient for classifying active smoking during pregnancy since active smoking tends to be relatively constant.[186, 187] However, repeated measurements may be necessary to accurately characterize SHS exposures since women may try to reduce their exposure after learning they are pregnant. Finally, pregnant women have increased CYP2A6 activity and kidney function resulting in increased NIC, COT, and 3HC metabolism. Thus, tobacco smoke exposures that would produce detectable serum cotinine concentrations in non-pregnant women may produce non-detectable concentrations in pregnant women.[147]

Residual confounding due to SES, dietary, or lifestyle factors may attenuate the association between serum and meconium metabolite concentrations and infant birth size and gestational age. However, the magnitude of attenuation was similar for both meconium and serum measures. Previous studies have observed an inverse association between prenatal serum cotinine concentrations and birth size even after adjustment for numerous perinatal and socioeconomic variables.[5, 11]

There were similar limitations in Aim 2 regarding exposure assessment, but most important were the role of selection bias, residual confounding, and the use of BMI as a measure of adiposity. Selection bias strengthened the association between prenatal tobacco smoke exposure and BMI at 3 years of age. I believe this is due to higher in birth weight and length among infant with complete follow-up. The association between prenatal tobacco smoke

exposure and birth weight and length was attenuated among infants with complete follow-up; whereas, infants with incomplete follow-up had greater decrements in weight and length associated with prenatal tobacco smoke exposure. The greater decrements in birth weight resulted in infants with incomplete follow-up having to catch up greater amounts of weight over the first 3 years of life as their similarly exposed peers to reach the same BMI. If women and children with incomplete follow-up are truly biasing my estimates, then the presented results in **Appendix 2** may be closer to the true association.

My observed estimates may be biased away from the null due to residual confounding from dietary and lifestyle factors. Prenatal tobacco smoke exposure is associated with lower SES, poor childhood diet, and less childhood activity.[9, 23, 43, 175, 176, 184, 188] I used HOME scores as a proxy of childhood diet and exercise. Additional adjustment for HOME scores did not substantially alter my results; however, the HOME scale may be an inappropriate proxy of dietary and exercise factors since it was originally designed to capture caregiving behaviors related to childhood neurodevelopment.[131, 189, 190] Prior studies examining the association between prenatal active tobacco smoke exposure and overweight BMI have observed little attenuation of their estimates after adjustment for children's diet or activity.[18]

Finally, I used BMI as an indirect measurement of adiposity. While cross-sectional measures of BMI and skin fold thickness are highly correlated,[127] elevated BMI can be a result of increased weight or decreased height. In addition, increased weight can be driven by gains in fat-free or fat mass. The correlation between BMI and fat mass is higher among overweight children compared to the correlation among normal weight children.[178] I did not have additional anthropometric measurements that would have allowed me examine what which one of these factors was responsible for the increase in weight among exposed children. However, I was able to utilize repeated measurements of weight and height to examine trajectories of early childhood growth.

C. Research and Public Health Implications

Interest and enthusiasm in using meconium as a biological matrix to measure gestational exposure to environmental toxicants has prompted the National Children's Study (NCS) to collect meconium from participating infants to quantify prenatal tobacco smoke and pesticide exposure.[24, 136, 137, 191] Bearer recommended that several steps be taken to enhance the usability of meconium in the NCS and other studies: 1) develop high-throughput methodology to measure a wide variety of environmental toxicants in meconium, 2) determine the relationship between maternal exposure and meconium metabolite concentrations of expsoure, and 3) estimate the relationship between meconium toxicant concentrations and infant birth weight and compare them to the association with other established biomarkers of that exposure. My first aim used prenatal tobacco smoke exposure as a system to complete these recommendations.

Dr. Bernert and his staff developed a high throughput assay that was able to detect NIC, COT, and 3HC in meconium. There were substantial hurdles in accomplishing this since meconium is a very difficult matrix to work with. Much effort was devoted to finding a way to liquefy the meconium without degrading tobacco smoke metabolites. Once dissolved into a liquid, the quantification of NIC, COT, and 3HC was relatively easy since they have very similar chemical structures. However, the quantification of other classes of environmental toxicants (e.g. phthalates or pesticides) in meconium may be more difficult since these compounds may have larger variations in chemical structure, each of which may require different analytical chemistry techniques.

Meconium NIC, COT, and 3HC concentrations can be used to approximate cumulative gestational tobacco smoke exposure. In addition, meconium metabolite concentrations were

inversely associated with infant birth size and these associations were similar to serum cotinine estimates.

Epidemiologists considering meconium as a biological matrix to measure gestational toxicant exposure should ponder several factors. First, meconium is easy to collect and does not require additional storage or shipping requirements compared to other biomarkers. In addition, meconium could be collected in studies that enroll women at delivery, thus eliminating the need to enroll women and gather biomarkers throughout pregnancy. However, collection of meconium samples may require additional cooperation from infant nursery staff to ensure that samples are not thrown away. Second, a meconium assay for the chemical of interest could take a substantial amount of time to develop and may turn out to be less accurate, precise, or sensitive than other well-established assays. In addition, quantification of tobacco smoke metabolites in meconium samples takes much longer serum samples. Third, in the case of tobacco smoke, meconium NIC appears to be a sensitive marker of tobacco smoke exposure in the 2nd and 3rd trimester of pregnancy. Meconium may be preferred in studies examining exposures with a high degree of temporal variability since meconium may capture exposures that serum or urine measurements would otherwise miss. However, using meconium to quantify prenatal exposure may result in exposure misclassification in studies of outcomes with time-sensitive windows of development since meconium reflects the duration and intensity of exposure. Fourth, the utility of meconium in a study depends on the exposure distribution of the source population. A sensitive biomarker like meconium may be unnecessary if the source population has relatively high exposure, especially if there are existing sensitive and well-developed assays. Finally, future studies would be advised to use meconium NIC because of the large proportion of infants with non-detectable COT and 3HC concentrations. This would make statistical analysis easier since biomarkers with a higher proportion of non-detectable values require more sophisticated techniques to handle left-censored data.[142, 192]

While meconium is a promising matrix to measure toxicant exposure, it is not necessary for most studies examining prenatal tobacco smoke exposure and infant or childhood health outcomes. Given the additional challenges of collecting, processing, and analyzing meconium, a single serum cotinine measurement collected during pregnancy or at birth would be sufficient to quantify prenatal exposure for most studies. However, meconium has the potential to be a useful biological matrix to measure environmental toxicants with a high degree of temporal or within-woman variability, including alcohol, pesticides, and phthalates. Future studies should determine if these chemicals can be quantified in meconium and whether meconium is able to capture exposures that were missed by urine, serum, or self-reported measurements of exposure.

Given the high prevalence of secondhand tobacco smoke exposures across the globe and the potentially devastating consequences of obesity, additional research examining the association between prenatal SHS and childhood BMI and adiposity is needed. The findings of this dissertation underscore the importance of using validated biomarkers of tobacco smoke exposure in future studies given the potential for self-reported prenatal SHS exposures to be misclassified.

The presented research confirms that prenatal secondhand and active tobacco smoke exposure is associated with decreased birth weight and length and suggests that secondhand tobacco smoke exposure may be associated with increased adiposity in children. A growing body of literature suggests that early alterations in early childhood growth lead to increased, adiposity, early adulthood cardiovascular disease markers, and adult cardiovascular morbidity and mortality.[77, 156, 193] Gestational exposures, like tobacco smoke, may increase disease risk in later life by decreasing birth weight and causing exposed children to undergo a period of rapid catch-up growth.

D. Conclusions

These results show that meconium is a promising biological matrix to measure gestational exposure to environmental toxicants. However, the utility of meconium in studies examining prenatal tobacco smoke exposure is questionable. While meconium NIC, COT, and 3HC metabolites are sensitive biomarkers of tobacco smoke exposure and highly correlated with prenatal serum cotinine concentrations, they provide similar associations with infant size and gestational age as serum cotinine associations. Meconium tobacco smoke metabolites do not provide additional information that could be obtained from a single serum cotinine measurement at birth.

The findings in Aim 2 add to a growing body of literature suggesting that prenatal tobacco smoke exposures may be associated with later life obesity. While additional research is needed to validate the presented findings; active and secondhand tobacco smoke exposures have been extensively studied and are causally linked to a variety of adverse infant, child, and adult health outcomes.[10, 11] Newly industrialized countries, like China and India, that have a high prevalence of active and secondhand tobacco smoke exposures,[12, 13] could learn from the successes and failures of tobacco regulation in Europe and North America. Both newly industrialized and developed nations should reduce exposures to active and secondhand tobacco smoke by banning smoking in workplaces and public, reducing the initiation of smoking, and providing cessation services to active smokers. Failure to take timely and nationally coordinated action may result in substantial morbidity and mortality among large portions of the population who are unwillingly exposed to tobacco smoke.

APPENDICES

APPENDIX A

A. Association between Prenatal Serum Cotinine Concentrations and Z-Scores of Weight, Height, and Weight-for-Height

Adjusted weight, height, and weight-for-height z-scores were calculated using a publicly available SAS program.[129] I observed a similar pattern of association (**Table A1**) between prenatal serum cotinine categories and z-scores as those presented above, with the exception of the association between prenatal active tobacco smoke exposure and weight-for-height z-scores. Children with prenatal active exposure had increased weight-for-height z-scores 0.2 to 0.5 units higher at all five measurement occasions from birth to 3 years of age.

Mean z-scores were calculated at birth and 1, 2, and 3 years of age by prenatal tobacco smoke exposure category (**Figure A1**). Children with prenatal secondhand or active tobacco smoke exposure were lighter and shorter at birth than unexposed children. Weight increased among both groups of exposed children, but the magnitude of increase was less among children with prenatal SHS exposure. Mean height z-scores remained unchanged between exposure groups across the first 3 years of life. By age 3, children with active prenatal tobacco smoke exposure had weight-for-height z-scores 0.5 standard deviations (95% CI: 0.0, 1.0) higher than unexposed children.

These results substantiate my previous findings using untransformed weight and height values. In addition, they provide evidence that active and secondhand prenatal tobacco smoke exposures may alter early childhood weight trajectories, resulting in increased BMI (or weight-for-height) by 3 years of age.

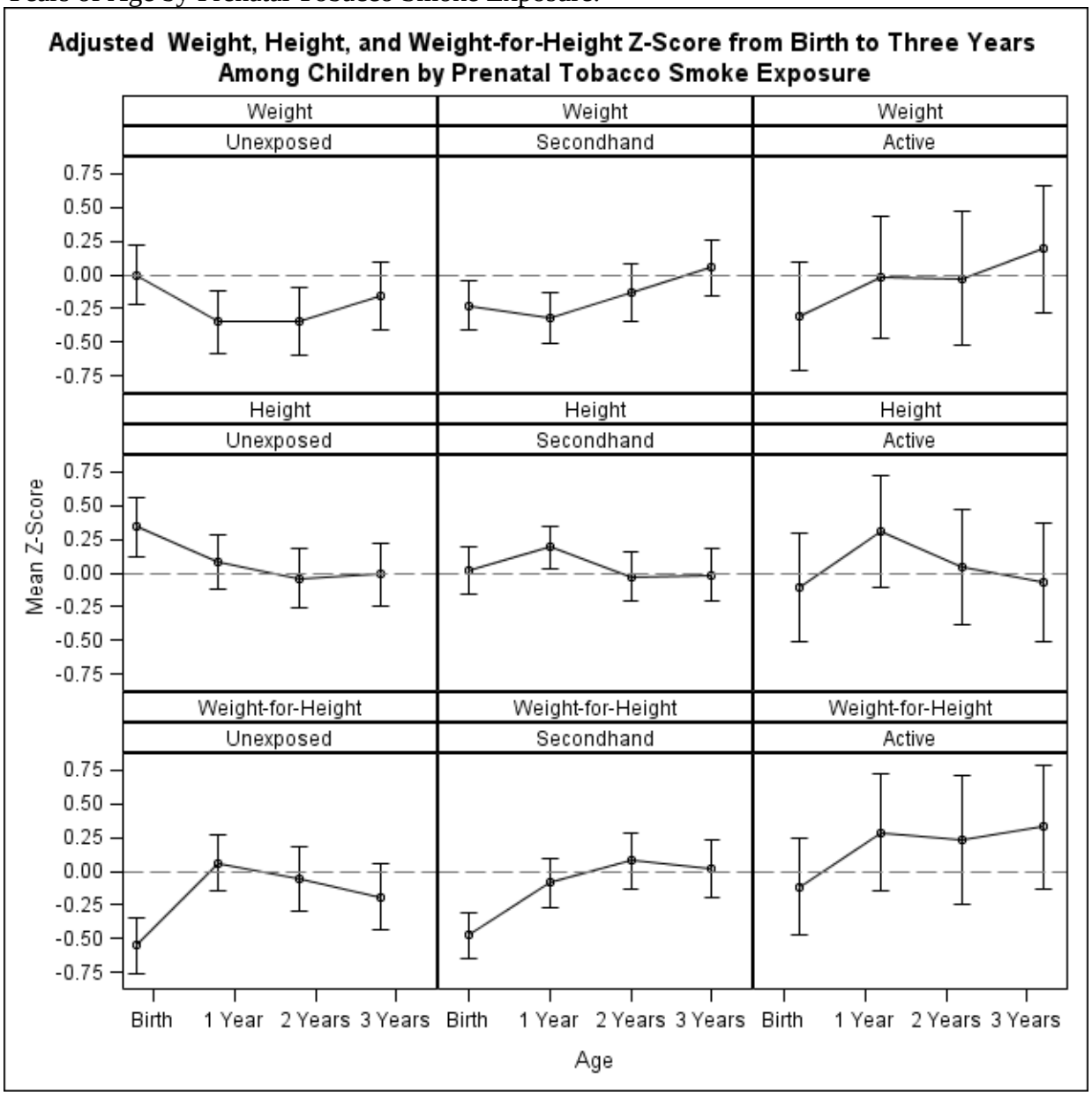
Table A1: Adjusted mean difference in weight, height, and weight-for-height z-scores by change in mean prenatal serum cotinine concentrations *

	N (%)†	Birth (N=300)	4-Week (N=303)	1 Year (N=273)	2 Years (N=236)	3 Years (N=209)
Weight Z-Score Models						
Categorical Prenatal Serum Cotinine Concentrations						
Unexposed (<0.015 ng/mL)	116 (38.8)	Ref	Ref	Ref	Ref	Ref
SHS (0.015-3 ng/mL)	152 (50.8)	-0.2 (-0.5, 0.0)	-0.2 (-0.5, 0.1)	0.0 (-0.3, 0.3)	0.2 (-0.1, 0.5)	0.2 (-0.1, 0.5)
Active (>3 ng/mL)	31 (10.4)	-0.3 (-0.8, 0.2)	-0.2 (-0.7, 0.2)	0.3 (-0.2, 0.9)	0.3 (-0.3, 0.9)	0.4 (-0.2, 0.9)
Continuous Prenatal Serum Cotinine Concentrations						
Log_{10}-transformed mean cotinine	299	-0.1 (-0.2, 0.0)	-0.1 (-0.2, 0.0)	0.0 (-0.1, 0.2)	0.1 (-0.1, 0.2)	0.1 (-0.1, 0.2)
Height Z-Score Models						
Categorical Prenatal Serum Cotinine Concentrations						
Unexposed (<0.015 ng/mL)	116 (38.8)	Ref	Ref	Ref	Ref	Ref
SHS (0.015-3 ng/mL)	152 (50.8)	-0.3 (-0.6, -0.1)	-0.3 (-0.5, 0.0)	0.1 (-0.2, 0.4)	0.0 (-0.3, 0.3)	0.0 (-0.3, 0.3)
Active (>3 ng/mL)	31 (10.4)	-0.5 (-0.9, 0.0)	-0.4 (-0.8, 0.1)	0.2 (-0.3, 0.7)	0.1 (-0.4, 0.6)	-0.1 (-0.6, 0.5)
Continuous Prenatal Serum Cotinine Concentrations						
Log_{10}-transformed mean cotinine	299	-0.1 (-0.3, 0.0)	-0.1 (-0.2, 0.0)	0.0 (-0.1, 0.2)	0.0 (-0.1, 0.1)	0.0 (-0.2, 0.1)
Weight-for-Height Z-Score Models						
Categorical Prenatal Serum Cotinine Concentrations						
Unexposed (<0.015 ng/mL)	116 (38.8)	Ref	Ref	Ref	Ref	Ref
SHS (0.015-3 ng/mL)	152 (50.8)	0.1 (-0.2, 0.3)	0.0 (-0.2, 0.2)	-0.1 (-0.4, 0.1)	0.1 (-0.2, 0.4)	0.2 (-0.1, 0.5)
Active (>3 ng/mL)	31 (10.4)	0.4 (0.0, 0.8)	0.4 (0.0, 0.8)	0.2 (-0.3, 0.7)	0.3 (-0.2, 0.8)	0.5 (0.0, 1.0)
Continuous Prenatal Serum Cotinine Concentrations						
Log_{10}-transformed mean cotinine	299	0.1 (0.0, 0.2)	0.1 (0.0, 0.2)	0.0 (-0.1, 0.1)	0.1 (-0.1, 0.2)	0.1 (0.0, 0.3)

*-Adjusted for maternal age (<25, 25-34, and >34 years), education (<12, 12, and >12 years), race (non-Hispanic white, non-Hispanic black, and other), marital status (married and non-married), depression at baseline home visit (moderately or severely depressed and mildly or minimally depressed), breast feeding duration since last visit (years), parity (0, 1, and >1), income ($10,000 increments), and maternal BMI (kg/m^2) at 16 weeks gestation.

†-Sample size at birth

Figure A1: Adjusted Weight, Height, and Weight-for-Height Z-Scores from Birth to Three Years of Age by Prenatal Tobacco Smoke Exposure.



*-Adjusted for maternal age (<25, 25-34, and >34 years), education (<12, 12, and >12 years), race (non-Hispanic white, non-Hispanic black, and other), marital status (married and non-married), depression at baseline home visit (moderately or severely depressed and mildly or minimally depressed), breast feeding duration since last visit (years), parity (0, 1, and >1), income ($10,000 increments), and maternal weight(kg)/height(cm)/BMI (kg/m^2) at 16 weeks gestation.

APPENDIX B

B. Association between Prenatal Serum Cotinine Concentrations and Weight, Height, and BMI Among Children with Complete Follow-Up

Table A2: Adjusted mean difference in weight, height, and BMI by change in mean prenatal serum cotinine concentrations among children with complete follow-up *

	N (%)†	Birth (N=194)	4-Week (N=194)	1 Year (N=192)	2 Years (N=194)	3 Years (N=194)
Weight Models						
Categorical Prenatal Serum Cotinine Concentrations						
Unexposed (<0.015 ng/mL)	83 (42.8)	Ref	Ref	Ref	Ref	Ref
SHS (0.015-3 ng/mL)	95 (49.0)	-58 (-236, 121)	-68 (-252, 117)	-5 (-384, 375)	242 (-269, 752)	376 (-264, 1015)
Active (>3 ng/mL)	16 (8.2)	-18 (-367, 331)	48 (-310, 406)	515 (-182, 1211)	749 (-186, 1683)	1333 (171, 2496)
Continuous Prenatal Serum Cotinine Concentrations						
Log_{10}-transformed mean cotinine	194	-49 (-144, 45)	-40 (-136, 57)	29 (-145, 202)	80 (-148, 308)	223 (-58, 505)
Log_{10}-transformed mean cotinine (Passively exposed women) ‡	180	-82 (-251, 86)	-100 (-273, 72)	-181 (-495, 133)	-95 (-502, 312)	0 (-487, 486)
Height Models						
Categorical Prenatal Serum Cotinine Concentrations						
Unexposed (<0.015 ng/mL)	83 (42.6)	Ref	Ref	Ref	Ref	Ref
SHS (0.015-3 ng/mL)	96 (49.2)	-0.5 (-1.3, 0.3)	-0.4 (-1.1, 0.3)	0.1 (-0.8, 1.0)	-0.1 (-1.1, 1.0)	-0.1 (-1.3, 1.1)
Active (>3 ng/mL)	16 (8.2)	-0.7 (-2.2, 0.8)	-0.4 (-1.9, 1.0)	1.1 (-0.7, 2.9)	1.2 (-0.8, 3.2)	1.1 (-1.1, 3.4)
Continuous Prenatal Serum Cotinine Concentrations						
Log_{10}-transformed mean cotinine	195	-0.3 (-0.7, 0.1)	-0.2 (-0.6, 0.2)	0.1 (-0.3, 0.6)	0.1 (-0.4, 0.7)	0.1 (-0.5, 0.6)
Log_{10}-transformed mean cotinine (Passively exposed women) ‡	181	-0.4 (-1.1, 0.3)	-0.4 (-1.1, 0.3)	-0.1 (-0.9, 0.8)	-0.1 (-1.1, 0.8)	-0.4 (-1.4, 0.6)
BMI Models						
Categorical Prenatal Serum Cotinine Concentrations						
Unexposed (<0.015 ng/mL)	82 (42.4)	Ref	Ref	Ref	Ref	Ref
SHS (0.015-3 ng/mL)	95 (49.2)	0.0 (-0.4, 0.4)	0.0 (-0.4, 0.4)	0.1 (-0.4, 0.5)	0.4 (-0.1, 0.9)	0.5 (0.1, 1.0)
Active (>3 ng/mL)	16 (8.3)	0.2 (-0.6, 1.0)	0.3 (-0.4, 1.0)	0.8 (-0.1, 1.6)	0.9 (0.0, 1.7)	1.4 (0.5, 2.2)
Continuous Prenatal Serum Cotinine Concentrations						
Log_{10}-transformed mean cotinine	193	0.0 (-0.2, 0.2)	0.0 (-0.2, 0.2)	0.1 (-0.1, 0.3)	0.2 (-0.1, 0.4)	0.3 (0.1, 0.5)
Log_{10}-transformed mean cotinine (Passively exposed women) ‡	180	0.0 (-0.4, 0.4)	0.0 (-0.4, 0.3)	-0.1 (-0.5, 0.3)	0.1 (-0.3, 0.5)	0.3 (-0.1, 0.7)

*-Adjusted for maternal age (<25, 25-34, and >34 years), education (<12, 12, and >12 years), race (non-Hispanic white, non-Hispanic black, and other), marital status (married and non-married), depression at baseline home visit (moderately or severely depressed and mildly or minimally depressed), breast feeding duration since last visit (years), parity (0, 1, and >1), income ($10,000 increments), and maternal BMI (kg/m^2) at 16 weeks gestation.

†-Sample size at birth

‡-Women could not have serum cotinine concentration > 3 ng/mL at any measurement

APPENDIX C

C. Adjusted association between mean and individual serum cotinine concentrations and BMI at 2 and 3 years of age

Table A3: Adjusted association between 16 week, 26 week, and birth serum cotinine concentrations and BMI at 2 or 3 years of age*

	2 Year BMI		3 Year BMI	
	Estimate (95% CI)	CLD	Estimate (95% CI)	CLD
Mean Serum				
LOD	Ref		Ref	
Passive	0.3 (-0.1, 0.7)	0.8	0.4 (0.0, 0.8)	0.8
Active	0.6 (-0.1, 1.4)	1.5	1.0 (0.3, 1.8)	1.5
Continuous	0.2 (0.0, 0.4)	0.4	0.3 (0.1, 0.5)	0.4
16 Week				
LOD	Ref		Ref	
Passive	0.0 (-0.4, 0.5)	0.9	0.2 (-0.2, 0.6)	0.9
Active	0.7 (-0.1, 1.5)	1.6	1.2 (0.4, 2.0)	1.6
Continuous	0.0 (-0.1, 0.2)	0.4	0.2 (0.0, 0.4)	0.4
26 Week				
LOD				
Passive	0.3 (-0.1, 0.7)	0.8	0.5 (0.1, 1.0)	0.8
Active	0.8 (0.0, 1.5)	1.5	1.2 (0.4, 2.0)	1.5
Continuous	0.2 (0.0, 0.3)	0.3	0.2 (0.1, 0.4)	0.3
Birth				
LOD				
Passive	0.3 (-0.1, 0.8)	0.9	0.3 (-0.1, 0.7)	0.9
Active	0.8 (0.0, 1.7)	1.7	1.1 (0.3, 1.9)	1.7
Continuous	0.2 (0.0, 0.3)	0.4	0.3 (0.1, 0.4)	0.4

*-Adjusted for child age (in years), child age squared, child age cubed, maternal age (<25, 25-34, and >34 years), education (<12, 12, and >12 years), race (non-Hispanic white, non-Hispanic black, and other), marital status (married and non-married), depression at baseline home visit (moderately or severely depressed and mildly or minimally depressed), breast feeding duration since last visit (years), parity (0, 1, and >1), income ($10,000 increments), and maternal BMI (kg/m^2) at 16 weeks gestation.

†-Model with fit with birth, 4 week, and 1 year measurements.

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