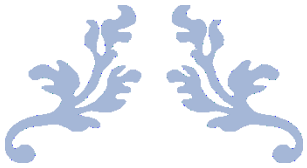

EARLY LIFE EXPOSURES AND DEVELOPMENT OF ADHD IN CHILDHOOD

by

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ABSTRACT

Attention deficit hyperactivity disorder (ADHD) is one of the most common neurodevelopmental disorders that imposes an enormous cost to individual, family and society across lifespan and generations in the U.S. This dissertation sought to systematically examine three potentially important early life factors in relation to ADHD, specifically, maternal cholesterol levels, early childhood lead exposure, and maternal acetaminophen use, using the data of mother-infant pairs already enrolled and followed in the Boston Birth Cohort (BBC), a high risk, predominantly urban low income minority population.

First, I investigated the prospective association of maternal cholesterol levels measured within a few days of delivery with the risk of offspring ADHD diagnosis among 1479 mother-infant pairs of the BBC. A low maternal high-density lipoprotein level (≤60 mg/dL) was associated with an increased risk of ADHD. A "J" shaped relationship was observed between triglycerides and ADHD risk. These associations were more pronounced among boys.

Second, I investigated the prospective associations between early childhood lead exposure and ADHD diagnosis and its potential effect modifiers among 1479 mother-infant pairs in the BBC. I found that the elevated lead levels (5-10μg/dL) in early childhood was associated with a 66% increased risk of ADHD. Boys were more vulnerable than girls at a given lead level. This risk of ADHD was reduced by half if the mother had adequate high-density lipoprotein level or low stress.

Third, I examined the prospective association between maternal plasma biomarkers of acetaminophen intake measured within a few days of delivery and offspring ADHD diagnosis

among 1180 mother-infant pairs of the BBC. There were significant dose-response associations between ADHD diagnosis and each maternal acetaminophen biomarker; and such associations were specific to ADHD, rather than other neurodevelopmental disorders.

These findings not only raise a new mechanistic perspective for understanding the origins of ADHD but also shed new light on the sex difference in ADHD and point to opportunities for early risk assessment and primary prevention of ADHD.

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ABBREVIATIONS

ADHD: attention deficit hyperactivity disorder

BBC: Boston Birth Cohort

ASD: autism spectrum disorder

LTP: long-term potentiation

HDL: high-density lipoprotein

LDL: low-density lipoprotein

TG: Triglycerides

TC: total cholesterol

NAPQI: N-acetyl-p-benzoquinone imine

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

Chapter 1 INTRODUCTION

1.1 BACKGROUND

Attention deficit hyperactivity disorder (ADHD) is one of the most common neurodevelopmental disorders in the U.S.; its prevalence has increased significantly from 7.0% to 10.2% among children ages 4-17 years during the past two decades.[1] ADHD is defined as a chronic neurobehavioral disorder,[2-4] which can be categorized into three major presentations: predominantly hyperactive/impulsive, predominantly inattentive, and combined.[5] Previous studies have primarily shown that ADHD is much more common among males, while the range of rates varies greatly by studies.[6,7] Approximately 66% to 85% of ADHD children will carry their disorder into adolescence and adulthood.[8,9] The high prevalence along with a tendency for the disorder to persist into later life have serious short-term and long-term consequences. At a young age, children diagnosed with ADHD have been shown to be more likely to miss school, encounter learning difficulties, have tense relationships with others, engage in more spontaneous sexual activities, and suffer from more motor vehicle accidents and/or unintentional injuries.[4,8,10-13] As they age into adulthood, individuals with ADHD not only have a higher risk of oppositional, conduct, and substance abuse disorders, but also tend to have a poorer psychological adaptation to the social environment, and as a result, their chances to hold a job and expand their professional career have been shown to be significantly affected by their disorder.[14] Additionally, adults diagnosed with ADHD have been shown to be likely to have comorbidities associated with a range of other mental and substance-related disorders.[15] The most recent estimation of the annual cost of ADHD to society, including costs related to health care utilization, medication, education, crime, and unemployment is $14,500 USD per individual ($42.5 billion USD in total).[16] Moreover, as the prevalence of ADHD diagnosis has been

increasing by nearly 5% each year since 2003,[17] the annual health care costs attributable to ADHD have been rising in parallel with the increasing prevalence of ADHD. [8,16,18]

Unfortunately, the current understanding of this highly prevalent and costly disorder is insufficient. The exact cause of ADHD is still unknown, not to mention the biological mechanisms behind the sex difference in ADHD risk. Gene variants, brain structural abnormalities, and neurotransmitter deficiency and deregulation are potential etiological mechanisms.[19,20] However, no study to date has identified any specific gene that could explain a large amount of variation in the probability of ADHD development. On the other hand, multiple social and environmental risk factors can potentially influence the development of ADHD, including family-related factors,[21-33] maternal obesity,[34] maternal smoking,[26,35,36], maternal drinking,[26] low birthweight and preterm birth,[37] and exposure to phthalates,[38] bisphenol A,[39] organophosphates,[40] polychlorinated biphenyls,[41,42] and lead. [41,43-45] My recent analyses on maternal stress and child ADHD has lent further support to the role of early life psychosocial factors in child risk of ADHD. However, it has been difficult to identify which factors are in the causal pathways of ADHD.[46] This is partly due to limitations in previous clinical and epidemiological studies of ADHD, including a lack of accurate ADHD measurement, biased sample selection, unmeasured confounders, and cross-sectional or retrospective study designs.

To avoid those limitations, my studies described here used a large, prospective birth cohort design to examine several understudied, but potentially important and modifiable, early life risk factors of ADHD, taking into account multiple pre-/perinatal and early childhood factors previously reported as risk factors for ADHD.[46] While a comprehensive review of each of the previously studied risk factors of ADHD is beyond the scope of this dissertation, below is a review of the major risk factors of interest, specifically maternal cholesterols (an exmaple of

maternal nutrition), early childhood blood lead levels (environmental toxin), and maternal acetaminophen levels (maternal medication). Table 1-1 provides a snapshot of the available published findings for each of these factors.

Table 1-1 Summary of current findings.

Topic	Author, year	Population	Study design	Sample size	ADHD measurement	Finding	Limitation
Maternal cholesterols	Rodriguez, 2008[34]	North European	Cohort	12556	Teacher-rated score	Risk of ADHD for both maternal overweight and large weight gain during pregnancy is two folds of normal weight mother	No biomarker of cholesterols measured; no clinical ADHD diagnosis
Early childhood lead	Braun, 2006[43]	United States	Cross-sectional	4704	Parent-reported ADHD and reported stimulant medication use	Higher current blood lead levels were independently associated with ADHD. No sex or prenatal smoking modification	Late lead measurement; no clinical ADHD diagnosis
	Froehlich, 2009[44]	United States	Cross-sectional	2588	Structured diagnostic interview with a caregiver; caregiver reports of ADHD medication use and previous diagnosis	Higher current blood lead levels were independently associated with ADHD; the multiplicative interaction between high current lead and prenatal smoking	Late lead measurement; self-reported ADHD diagnosis; did not test the lead-sex interaction
	Wang, 2008[47]	China	Matched case-control	630 ADHD 630 non-ADHD	Structured diagnostic interview to children, one of their parents, and their teachers	Even current blood lead less than 10 μg/dL is associated with higher risk of ADHD; sex did not modify the association	Late lead measurement
Maternal acetaminophen	Ystrom, 2017[48]	Norway	Cohort	112973	Electronic medical record	There is a significant positive association between long-term maternal use of acetaminophen during pregnancy and ADHD even after adjusting for potential confounders	Self-reported use; lack of dose quantification
	Stergiakouli, 2016[49]	England	Cohort	7796	Maternal reports of behavioral problems using Strengths and Difficulties	The multiple behavior difficulties in offspring were associated with prenatal acetaminophen exposure	Outcome is not assessed by health care professionals; self-reported

					Questionnaire (SCQ) at age 7 years		use; lack of dose quantification
	Avella-Garcia, 2016[50]	Spain	Cohort	2195	Teacher-rated ADHD related symptoms	Prenatal acetaminophen exposure was significantly associated with more hyperactivity /impulsivity symptoms	Outcome is not assessed by health care professionals; self-reported use; lack of dose quantification
	Liew, 2014[51]	Denmark	Cohort	64322	Parental report of ADHD symptoms at age 7; electronic medical record after age 5; use of ADHD medications	Hyperkinetic disorders and ADHD like behaviors in offspring were significantly associated with prenatal acetaminophen use	Self-reported use; lack of dose quantification
	Thompson, 2014[52]	New Zealand	Cohort	871	Parental report of ADHD symptoms at age 7 and both parent- and child-report at the age of 11	Prenatal acetaminophen exposure was significantly associated with increased risk of ADHD symptoms at age 7 and 11	Outcome is not assessed by health care professionals; self-reported use; lack of dose quantification
	Brandlistuen, 2013[53]	Norway	Sibling-controlled cohort	2919 same-sex sibling pairs	Maternal reports of behavior and temperament problems using questionnaire at the age of 3	Long-term prenatal acetaminophen exposure is significantly associated with adverse developmental outcomes at the age of 3	Outcome is not assessed by health care professionals; self-reported use; lack of dose quantification

The maternal nutrient condition at each reproductive stage can have a profound impact on the development and well-being of the offspring.[54] During pregnancy, fetal neurodevelopment is dependent on stable and optimal levels of nutrients from the mother. Recent studies have reported prenatal exposure to maternal metabolic syndrome could influence children's neurodevelopment outcomes, including autism spectrum disorder (ASD) and ADHD.[34,55,56] As the major biomarker of metabolic syndrome, the maternal cholesterol profile might play essential roles in maintaining fetal neurodevelopment. To date, the role of the maternal cholesterol profile in child ADHD has been mainly unexplored yet biologically plausible because of its essential

roles in development and maintenance of the neural system.[57,58] Moreover, maternal cholesterol levels increase with gestational age during healthy pregnancy and are transferred to the fetus via the placenta;[59] this synchronized increase indicates that the cholesterols are essential for the proper development of the fetus.[59] However, no published study was found to investigate the relationship between maternal cholesterol profiles and childhood ADHD.

While lead has been extensively studied in relation to neurodevelopmental outcomes, its association with ADHD as a clinical entity has not been well studied. To date, only three large studies have investigated this association.[43,44,47] These studies showed a strong dose-response association between lead levels and risk of ADHD. However, there were multiple drawbacks in these existing studies. First, the outcome assessment may have been flawed: all existing large sample studies defined ADHD cases via caregiver/school report, stimulant medication use record, or diagnostic interview by the researcher. No large study used the clinical specialist diagnosis as their case definition. Second, none of the studies investigated if lead affected boys and girls differently or had a large enough sample size to test the potential interaction between lead and sex on ADHD. Lastly, while both prenatal and postnatal lead exposures may affect ADHD risk, most study designs were either cross-sectional or retrospective. Most studies examined childhood lead exposure, with a mean age of measurement ranging from 7-14 years,[60] and the time of the lead measurement was either at the same time or after the ADHD diagnosis. There has not been a large longitudinal study designed to investigate the prospective association between early life lead exposure (before the age of 2) and the development of ADHD in childhood.

Acetaminophen is a widely used and highly recommended medication for fever and pain relief during pregnancy. The percentage of pregnant women who use acetaminophen during pregnancy

is over 65% in the U.S. and over 50% in Europe.[61,62] Since 2013, multiple independent research studies analyzing five prospective cohorts from Europe and New Zealand have consistently shown a positive association between maternal intake of acetaminophen during pregnancy and increased risk of ADHD and its related symptoms.[48-53] However, the Society for Maternal-Fetal Medicine and the Food and Drug Administration both have issued statements regarding their belief that the evidence from those studies is inconclusive for showing a causal relationship between prenatal acetaminophen use and ADHD in the offspring.[63,64] Their primary criticisms include self-reported exposure, lack of dose quantification, unmeasured confounders, and lack of adjustment for multiple testing.[63] However, given its widespread usage, it is too risky to ignore any potential unknown side effects of this drug considering the health consequences of exposed fetus.[65] Given the infeasibility of conducting any randomized trial, a well-designed prospective birth cohort with measurements of acetaminophen blood biomarker levels is needed. Currently, no published such study exists.

In summary, to date, there remains insufficient knowledge regarding the role of maternal blood cholesterol profiles, early childhood blood lead levels, and maternal blood acetaminophen levels in the development of ADHD. Likewise, although remarkable sex difference in ADHD has been well observed, the cause for male dominance in ADHD is still unknown. Additionally, there is a paucity of prospective birth cohort studies designed to investigate the influences of these factors on the risk of physician-diagnosed ADHD in the U.S., especially among high risk, low-income urban minority populations. To fill in these significant research gaps, I used the data of mother-infant pairs already enrolled and followed from birth up to age of 21 years in the Boston Birth Cohort (BBC), along with maternal cholesterols levels measured in blood samples collected 1-3 days postpartum, early childhood blood lead levels measured before the age of 2 years, and

maternal acetaminophen levels measured in blood samples collected 1-3 days postpartum. The study participants of the BBC are primarily drawn from an urban, low-income and minority setting, which has much higher rates of maternal obesity[66], elevated lead exposure[67], and ADHD as compared to U.S. general populations. As detailed in the subsequent chapters, this dissertation leveraged the BBC's extensive molecular, epidemiological, and clinical databases and biospecimen repository to address the following novel specific aims.

1.2 SPECIFIC AIMS AND HYPOTHESES

Aim 1. To investigate the role of maternal cholesterol (total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglycerides (TG)), independently and jointly, in the development of ADHD in childhood, adjusting for pertinent pre- and peri-natal and childhood factors. In addition, I aimed to investigate whether the associations differ by sex.

Hypotheses: Maternal dyslipidemia during pregnancy can increase the risk of ADHD in childhood, and there is a sex difference in the association.

Aim 2a. To investigate the role of early childhood lead exposure, independently and jointly, in the development of ADHD in childhood, adjusting for pertinent pre- and peri-natal and childhood factors. In addition, I aimed to investigate whether the associations differ by sex.

Hypotheses: Lead exposure during early childhood can independently and jointly increase the risk of ADHD in childhood, and there is a sex difference in the association.

Aim 2b. To investigate the potential protective effects of optimal maternal cholesterol levels in reducing the risk of ADHD associated with lead exposure.

Hypotheses: Optimal maternal cholesterol levels during pregnancy can mitigate the adverse effect of lead exposure on the risk of ADHD in childhood.

Aim 3a. To investigate the role of maternal plasma levels of acetaminophen metabolites, independently and jointly, in the development of ADHD in childhood, adjusting for pertinent pre- and peri-natal and childhood factors.

Hypotheses: Maternal plasma levels of acetaminophen metabolites during the perinatal period can increase the risk of ADHD in childhood.

Aim 3b. To investigate the potential protective effects of optimal maternal cholesterol levels in reducing the risk of ADHD caused by maternal acetaminophen exposure.

Hypotheses: Optimal maternal cholesterol levels during pregnancy can mitigate the adverse effects of maternal acetaminophen exposure on the risk of ADHD in childhood.

1.3 CONCEPTUAL FRAMEWORK

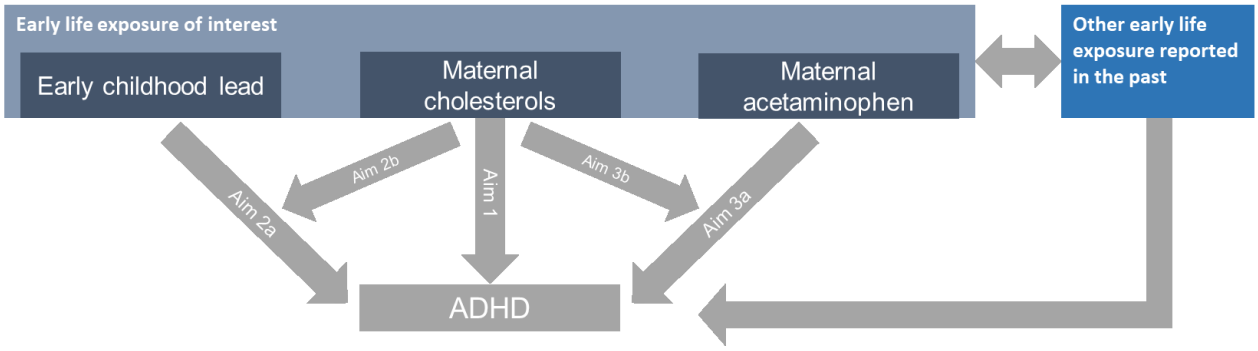

Figure 1-1 Conceptual framework.

Figure 1-1 illustrates the conceptual framework for the three studies presented in this dissertation. This framework serves as a visual aid that supports the rationale for the specific aims mentioned above. By linking multiple early life risk factors (the dark blue boxes) with

childhood ADHD diagnosis (the bottom grey box), this framework illustrates the potential actors in the early life origin of ADHD development using a life-course perspective.

1.4 DISSERTATION OVERVIEW

This dissertation is arranged as follows. Chapter 1 provides the background and significance of this topic, major research gaps, and specific aims designed to fill in the research gaps on this topic. It also provides an overview of the entire dissertation structure. In Chapter 2, the current understanding of ADHD and the major risk factors of interest are described in detail. Chapter 3 provides a description of the measurements and methods used related to each specific aim. Chapter 4 presents the manuscript that was developed in support of Aim 1 with the title "Do maternal cholesterol levels affect attention deficit hyperactivity disorder in offspring? A prospective birth cohort study". Chapter 5 presents the manuscript that was developed in support of Aim 2 with the title "A prospective birth cohort study on early childhood lead levels and attention deficit hyperactivity disorder: new insight on sex differences." Chapter 6 presents the manuscript that was developed in support of Aim 3 with the title "Maternal biomarkers of acetaminophen use and offspring attention deficit hyperactivity disorder." Chapter 7 addresses the implications of findings presented in this dissertation in terms of public health and clinical research. Chapter 8 synthesizes the major findings across each specific aim and discusses the strengths and limitations of the studies that are presented in the dissertation.

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Chapter 2 LITERATURE REVIEW

2.1 BACKGROUND AND SIGNIFICANCE

Attention deficit hyperactivity disorder (ADHD) is a neurodevelopmental disorder, which is highly prevalent in the U.S. The prevalence of ADHD has increased significantly from 7.0% to 10.2% among children aged 4-17 years during the past two decades.[1] This chronic neurobehavioral disorder is characterized by inattention, hyperactivity, or impulsiveness.[2-4] Although stimulant medications are used as first-line treatment for ADHD, their potential side effects short and long-term on health outcomes are of concern.[5,6] For example, taking these medications for ADHD can cause sleep disturbances, reduced appetite, and suppressed growth, which has been shown to impact on ADHD children's development and quality of life.[7] Like autism spectrum disorder (ASD), ADHD diagnosis is also disproportionately high among boys,[8-11] with a three times higher risk compared to girls per most recent estimates.[10,12] ADHD can affect multiple aspects of a child's life, such as school performance, social involvement, and overall quality of life.[13-17] In the school setting, children diagnosed with ADHD tend to leave their seat frequently, talk incessantly, play loudly, and call out answers before question are stated completely. It is also harder for ADHD children to organize tasks and sustain attention during schoolwork or extracurricular activities.[18] Due to their disruptive and aggressive behaviors, ADHD children are likely to be alienated by their classmates as early as the first day of school.[19] Thus, ADHD children are more liable to miss school, encounter learning difficulties, have tense relationships with others, engage in more spontaneous sexual activities, and suffer from more motor vehicle accidents and unintentional injuries.[4,10,13,20-22]

Approximately 66% to 85% of ADHD children will carry their disorder into adolescence and adulthood.[10,23] The high and rising prevalence of ADHD along with a tendency for it to persist into later life has serious short- and long-term consequences. As those diagnosed as children get

older, ADHD adults not only have an increased risk of oppositional, conduct, and substance abuse disorders but also have a poorer psychological adjustment. As a result, their chances to hold a job and advance their professional career are significantly affected by their ADHD.[24] Additionally, ADHD adults are likely to share comorbidities with many other mental and substance-related disorders.[25] These coexisting disorders not only aggravate the deterioration linked with ADHD during adulthood but also inflate the economic burden.[26] For instance, ADHD students contribute to a higher annual cost to the U.S. education system due to special education placement, grade retention, and disciplinary incidents.[27] Moreover, studies have shown that those with ADHD had higher annual medical costs than those without ADHD due to higher utilization of hospitalization, primary care visits, outpatient mental health visits, and pharmacy fills.[18] Claims data indicate that the excess medical costs of ADHD were $31.6 billion in the U.S. in 2000.[28] The most recent estimation of the annual total cost of ADHD to society, including costs related to health care utilization, medication utilization, education, crime, and unemployment, is $14,500 per individual and $42.5 billion in total.[29] Moreover, as the prevalence of ADHD diagnosis has been increasing by nearly 5% each year since 2003,[8] the annual health care costs attributable to ADHD and related ambulatory care visits are becoming synchronized with the increasing prevalence of ADHD.[10,29,30]

2.2 DEFINITION AND DIAGNOSIS

ADHD is defined as a behavioral disorder characterized by symptoms of inattention, impulsivity, and hyperactivity.[2] Based on these characteristics, ADHD is categorized into three major presentations: predominantly hyperactive/impulsive, predominantly inattentive, and combined.[31] Moreover, one presentation might shift to another one as the disorder progresses over time.[32] Currently, there are no reliable neuroimaging markers for diagnosing ADHD.[33-35] Clinicians in

the U.S. currently use the Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-5) to diagnose ADHD.[31] The DSM-5 criteria for ADHD in children up to age 16 are "six or more symptoms of inattention" (symptoms present over six months) and/or "six or more symptoms of hyperactivity-impulsivity" (symptoms present over six months).[31] For an individual aged 17 years and older, the criteria are "five or more symptoms of inattention" (symptoms present over six months) and/or "five or more symptoms of hyperactivity-impulsivity" (symptoms present over six months).[31] Based on these two types of symptoms, the DSM-5 categorizes ADHD into three presentations: predominantly hyperactive-impulsive, predominantly inattentive, and a combined presentation.[31] Moreover, those symptoms should clearly interfere with or reduce the quality of functioning in two or more settings, such as social, academic or occupational; a symptom would not be counted if it only occurs in one of these settings.[31] In addition, a symptom would not be counted if it occurs exclusively in other mental disorders or is better explained by another mental disorder.[31] Noteworthy, the ASD diagnosis is no longer an exclusion criterion for ADHD diagnosis.[31]

In 2002, Vanderbilt ADHD Diagnostic Rating Scale (VADRS), a toolkit for assessment and treatment of ADHD in children between the ages of 6 and 12 in primary care settings, was developed jointly by American Academy of Pediatrics and National Initiative for Children's Healthcare Quality.[36-38] This toolkit consists of two versions: a parent version with 55 questions and a teacher version with 43 questions.[37] Both the parent and teacher versions consists of two sections: symptom assessment and performance impairment.[37] The VADRS has screening items corresponding to the ADHD diagnostic criteria in DSM-IV.[38,39] Having at least 6 positive responses towards either the core 9 inattentive symptoms or core 9 hyperactive symptoms, or both would meet ADHD diagnosis criteria. [37] Because of its strong psychometric capacity in

ADHD assessment, [39,40] VADRS is a commonly used diagnostic scale for physicians.[41] Moreover, VADRS also includes symptoms screening items for three common comorbidities for ADHD, including oppositional defiant disorder, conduct disorder, and anxiety/depression.[39] By calculating the scores for each comorbidity domain, VADRS could provide possibility of comorbidity according to the recommended threshold.[39]

In addition to clinical diagnosis, behavior rating scales have become great additions for providing information about children's symptoms in different settings, such as home and school.[42] Because they are cost-efficient and easily administrable, the Child Behavior Checklist–Attention Problem (CBCL-AP) subscale [43] and Conners Rating Scale-Revised (CRS-R)[44] are the commonly used ADHD assessment tools for children and adolescents in schools and communities.[42,45] While CBCL-AP has strong discriminatory power for screening ADHD among children and adolescents, CRS-R is more suitable for assessing ADHD and related behavioral problems.[42] For instance, the CRS-R has an ADHD index, different length versions for parents and teachers, and various subscales for various behavioral domains, such as oppositional disorders, cognitive problems or inattention, and hyperactivity subscales.[42] Based on meta-analysis results, the sensitivity and specificity for ADHD diagnosis are moderate for both the CBCL-AP and CRS-R.[42] Furthermore, the Conners' Abbreviated Symptom Questionnaire (ASQ), an abridged version of the CRS-R, is considered to be the most effective diagnostic tool for assessing ADHD because of its high accuracy and conciseness.[42]

2.3 CURRENT UNDERSTANDING OF ADHD ETIOLOGY

Brain Structure and Function: Brain structural abnormalities, and neurotransmitter deficiency and deregulation have been found to be potential etiological mechanisms.[46,47] Scientists have

observed global reduced volume and/or functionality of gray, especially in the right lentiform nucleus and extending to the caudate nucleus,[48] and white matter among ADHD patients.[49] Moreover, ADHD patients have also shown smaller volume and/or reduced activity in the prefrontal cortex (PFC), caudate, and cerebellum areas, which are primarily in charge of attention, thoughts, emotions, behaviors, and actions.[50,51] The functionalities across those areas are sensitively controlled by neurotransmitters, such as dopamine (DA) and norepinephrine (NE), through multiple receptors.[52-57] Specifically, too little DA/NE release will cause an individual to become easily distracted and impulsive, while too much release will cause misguided attention and responses.[58] Several studies have found reductions in DA and/or NE functioning in those diagnosed with ADHD.[49,50,59-61] In contrast, several other studies have identified a hyperactive DA response in ADHD.[56,62,63] These findings suggest a U-shaped relationship between the functioning level of DA/NE and ADHD symptoms, that is, the complex etiology of ADHD involves both hypoactive and hyperactive DA/NE systems.[58]

Genetic Factors: One meta-analysis of 20 twin studies from the U.S., Australia, Scandinavia, and the European Union indicated that ADHD is a highly heritable psychiatric disorder with a mean heritability estimate of 76%.[47] Although this study showed that the genes coding for SNAP25, DRD4, SLC6A3, HTR1B, SLC6A4, and DBH might play pivotal roles in the etiology of ADHD, single nucleotide polymorphisms (SNPs) in these genes and many biologically plausible genes did not show genome-wide significance (i.e., a P-value of $<5\times10^{-8}$) in the International ADHD Genetics (IMAGE) project that included 909 family trios.[64] Moreover, grouped SNPs only showed weak associations with ADHD. These findings indicate that the highly heritable condition presumed of ADHD cannot be fully explained by genetics.

Epigenetics and gene-environment interactions should be investigated in future studies to expand the understanding of the role of genetics in the etiology of ADHD.

Environmental Factors: Growing evidence suggests that environmental factors may also play a major role. The longitudinal, randomized control treatment trials, the quasi-experimental, and genetically informative studies have all shown that negative parenting, maltreatment, and poverty are strongly associated with the risk of ADHD, especially among a genetically susceptible population.[65-68]. In addition to those factors, other well-recognized environmental risk factors include family-related factors,[65-77] maternal obesity,[78] maternal smoking,[70,79,80] maternal drinking,[70] low birthweight and preterm birth,[81] and exposure to phthalates,[82] bisphenol A,[83] organophosphates,[84] polychlorinated biphenyls,[85,86] and lead.[85,87-89] My recent analyses on maternal stress and child ADHD lend further support for the role of early life psychosocial factors in child risk of ADHD. It has been difficult to identify which among them are definitively causal;[90] this is in part due to the limitations of previous clinical and epidemiological studies of ADHD, including lack of accurate ADHD measurements, biased sample selection, unmeasured confounders, and non-longitudinal designs. For instance, multiple studies using a genetically sensitive design have shown that the effect of maternal smoking is mostly confounded by genetic or other unidentified environmental factors.[91,92] Furthermore, the possibility of reverse causality could not be excluded. For instance, a longitudinal study using a twin design found that it is more likely that the child's ADHD symptoms are the causes for mother-son hostility rather than that hostility is the cause of ADHD.[93] Moreover, another study showed that ADHD children identified from a nonclinical setting were more likely to show a sex difference in levels of impairment.[94] Since it is not feasible or ethical to use randomized controlled trials (RCTs) to

examine the potential environmental risks and their modifications on ADHD, a large, prospective birth cohort design is needed to overcome these confounding and reverse causality issues.

Sex Difference: Like ASD, ADHD diagnosis is also disproportionately high among boys,[8-11] with a three times higher risk compared to girls per most recent estimates.[10,12] The results from two recent meta-analyses indicated that this sex difference in prevalence is caused by "potential confounding effects of referral bias, comorbidity, developmental patterns, diagnostic procedures, and rater source."[94,95] For instance, ADHD girls were shown to have a more severe intellectual impairment, lower level of hyperactivity, and less externalizing behaviors compared to ADHD boys.[94] Those differences strongly indicate that sex-specific biological mechanisms are underlying the neurodevelopmental factors and interaction with environmental factors. However, there is still no well-established biological theory to help unravel the exact etiology of sex difference in ADHD.

In the following, I will further elaborate on the three early life factors that this dissertation will focus on.

2.3.1 Maternal nutrition

The maternal nutrient condition at each reproductive stage can have a profound impact on the development and well-being of offspring.[96] During pregnancy, fetal neurodevelopment is dependent on stable and optimal levels of nutrients from the mother. A suboptimal maternal nutrient condition during critical developmental periods could drastically increase the risk of multiple adverse neurodevelopmental problems, such as neural tube defects and schizophrenia.[97] Previous studies have shown that maternal nutrient imbalance or deprivation, especially during the fast growth period that demands a high nutrient supply, can seriously affect the structure and function

of the fetal brain.[98,99] Thus, maternal nutritional status during pregnancy is biologically plausible to influence neurodevelopment in the offspring.

Recent studies have reported that prenatal exposure to maternal metabolic syndrome could influence a child's neurodevelopment outcomes, including ASD and ADHD.[78,100,101] A study using BBC data showed a strong association between maternal obesity and diabetes and increased risk of ASD.[100] Moreover, a vast longitudinal study, using prospective pregnancy cohorts from the Nordic Network, showed that both overweight moms and moms with excessive weight gain during gestation had an over 2-fold higher risk of having ADHD children.[78] However, these studies did not specifically examine a major component of metabolic syndrome, the maternal cholesterol profile, and it remains unclear what is the role of maternal cholesterols in fetal neurodevelopment.

Cholesterol plays multiple essential functions in the human body. [102] First, it is the building block for synthesizing steroids or cortisone-like hormones, such as vitamin D and the sex hormones testosterone, estrogen and cortisone.[102] Those steroids or hormones, in turn, regulate development and metabolism. Second, it assists in digestion and absorption of fat-soluble vitamins including vitamin A, D, E and K.[102] Third, it is the critical component for stabilizing cell membranes and facilitating inter-cellular communication. Lastly, it plays crucial roles in myelin sheath formation, which is a neuron in charge of aiding the route of electrical impulses.[102] As such, a lack of abundant cholesterol might on its own lead to memory loss and focus problem.

High-density lipoproteins (HDL) and low-density lipoproteins (LDL) are both complex particles composed of multiple fat-transporting proteins.[103] As shown in Figure 2-1**,** HDL plays essential roles in transporting excess cholesterol from the periphery to the liver via a reverse cholesterol transport mechanism.[103-105] HDL can carry cholesteryl ester to hepatocytes, steroid-producing

cells, and adipocytes through Scavenger receptor class B member 1 (SR-BI), a receptor for HDL.[103] In addition to HDL, LDL is another crucial player in reverse cholesterol transport. LDL could exchange cholesteryl esters (CE), triglycerides (TG) and phospholipids (PL) with HDL via Cholesteryl ester transfer protein (CETP). Next, the LDL receptors (LDLR) recognize and take up LDL in hepatocytes.[103] The central nervous system contains nearly 25% of the un-esterified cholesterol in the entire body, while it only accounts for 2% of bodyweight.[106] Those sterols primarily reside in two locations in the brain: 1) plasma membranes of glial cells and neurons; 2) specialized membranes of myelin.[106]

While the cholesterol in the CNS is primarily synthesized locally in the brain, the evidence for cholesterol transfer from maternal plasma into the brain of a fetus or newborn is limited.[106] The formation and excretion of 24S-hydroxycholesterol out of the brain is the primary mechanism for eliminating excessive cholesterol and keeping a steady state in the brain.[106] Indirect findings show that a significant amount of cholesterol recycling occurs among glial cells and neurons during neurodevelopment and neuron repair and remodeling.[106] Ligands, such as apolipoproteins E and AI, and membrane transport proteins, such as HDL and LDL, may be involved in the sterol recycling process for both the brain and other parts of the body.[107,108] Although there is no direct transport of sterol across the brain, studies suggest that an imbalance of cholesterol in the body may alter internal sterol recycling within the CNS, which would affect the integrity of both neurons and myelin.[106] Moreover, another biological study showed that diabetes could cause a wide-spectrum of changes in sterol regulatory element-binding protein 2 (SREBP-2) and its downstream cholesterol synthetic genes expression in brain.[109] As shown in Figure 2-2, those changes would result in a low production of brain cholesterol and its precursors, which in turn could lead to disruptions in synaptic formation and function.[109] Thus, dysregulated brain

cholesterol metabolism presents another potential biological pathway leading to neurodevelopmental disorders.

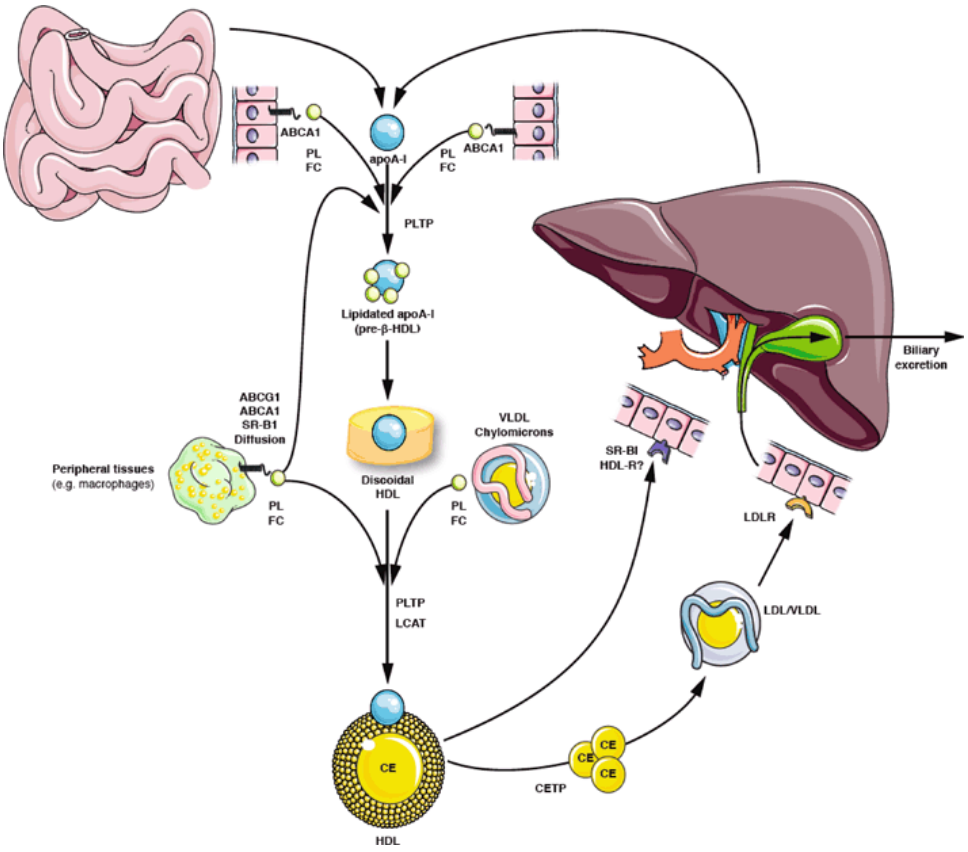

Figure 2-1 Role of HDL and LDL in lipoprotein metabolism. (adapted from [103])

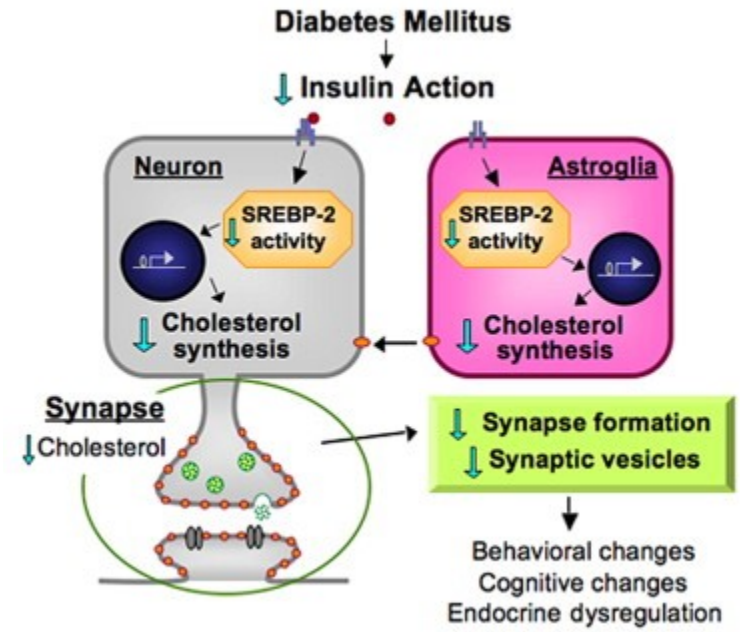

Figure 2-2 Diabetes and Insulin in Regulation of Brain Cholesterol Metabolism (adapted from [109])

In sum, maternal cholesterols have important biological functions as outlined above. In fact, maternal cholesterol levels increase with gestational age during normal pregnancy and are transferred to the fetus via the placenta; [110] and this increase in normal pregnancy indicates that the cholesterols are essential for the proper development of the fetus.[110] Moreover, the clinical cut points for abnormal levels of cholesterol may not apply to the unique physiological conditions during pregnancy, given it was derived for preventing cardiovascular diseases in adults. As such, a prospective birth cohort study is critically needed to investigate the association between maternal cholesterol levels and offspring ADHD risk.

2.3.2 Environmental toxins

Maternal exposure to chemical agents and pollutants have a potential negative impact on the nervous system of the developing fetus. Moreover, environmental toxins could further have a postnatal influence through the daily use of contaminated soil, water and air.[111] For instance, exposure to pesticides, herbicides, and polychlorinated biphenyls has been associated with increased risk of perinatal mortality, growth restriction, and intellectual function.[112] Specifically, growing evidence indicates a link between the risk of ADHD and multiple environmental toxin exposures, including phthalates,[82] bisphenol A,[83] organophosphates,[84] polychlorinated biphenyls,[85,86] and lead.[85,87-89]

Lead will be the primary research focus of this dissertation. Lead has the potential to damage multiple organ systems within the human body across the lifespan. [113] The toxicity of lead exposure on the central neuron system makes it extremely harmful during infancy and childhood, which is the critical period for neurodevelopment.[114] As the understanding of lead's toxicity advances, the threshold to define lead toxicity has been revised from as high as 30 μg/dL in 1975 to 5 μg/dL in 2012 to the current understanding that there is no safe level of exposure for the

fetus or young child.[115-119] High exposures to lead could result in grave outcomes, including neurological impairments, coma, and even death.[118] Even under low levels of lead exposure, increased risks have been identified for multiple outcomes including intellectual reduction,[120,121] executive functioning impairment,[122] and socio-behavioral problems.[123] Furthermore, accumulating literature suggests that lead exposure is associated with the core symptoms of ADHD.[113]

Although lead has been extensively studied for neurodevelopmental outcomes, its association with ADHD as a clinical entity has not been well studied. Currently, only three large sample sized studies have investigated this association.[87,88,124] Two large cross-sectional studies using different time periods of National Health and Nutrition Examination Survey (NHANES) data consistently showed a dose-response relationship between childhood blood lead level and ADHD diagnosis.[87,88] The adjusted odds ratios between the highest lead group and the lowest lead group were 4.1 (95%CI, 1.2–14.0)[87] and 2.3 (95%CI, 1.5–3.8),[88] respectively. This dose-response trend was still observed for the group of children with blood lead levels lower than 2 μg/dL.[87] A relatively large matched case-control study in China also observed a similar dose-response relationship.[124] However, there have been multiple drawbacks in these existing studies. First, the definition of ADHD in the sizable studies was mainly based on caregiver/school report, stimulant medication use record, or diagnostic interview by the researcher. No large study used the clinical specialist diagnosis as their case definition. Second, none of these studies investigated the lead-sex interaction or had a large enough sample size to test the potential interaction. Lastly, while there has been plenty of evidence to support the link between the effects of both prenatal and postnatal lead exposure and risk for ADHD, most studies only examined the consequences of postnatal lead exposure, when the mean age of measurement ranged from age 7-14 years,[113] such

that the time of measurement was either at the same time or after the ADHD diagnosis. So far, there has been no large longitudinal study to investigate ADHD in relation to blood lead levels measured before the age of 2.

The neurotoxicity induced by lead is determined by both age[125] and lead level.[126] Compared to adults, children absorb more lead into the brain due to higher potential intake from the environment and an underdeveloped blood-brain barrier.[127,128] The lead-induced damage in the developing brain preferentially occurs in the prefrontal cortex (PFC), hippocampus, and cerebellum,[129-131] while the brains of ADHD individuals also show a reduction in the volume and activity of the PFC and cerebellum.[58] Although the exact neurotoxicological pathways induced by lead exposure are still understudied, current biological studies suggest that lead disrupts the hippocampus region through interacting with the NMDA receptor both synaptically and extra-synaptically.[132] Figure 2-3 summarizes the detailed mechanisms of synaptic interactions between lead and the NMDA receptor.[132] When the Pb^{+2} ion enters the hippocampus synaptic region, it binds to the NMDA receptor with a much higher affinity compared to glutamate.[132] The Pb-NMDA complex formed by this binding causes a low release of the Ca^{+2} ion. When there is a lack of Ca^{+2} ion, the Ca^{+2} dependent pathways, such as calmodulin-II (CAM-II), neuronal nitric oxide synthase (n-NOS) and cAMP response element-binding protein (CERB), are inhibited, which can lead to long-term potentiation (LTP) dysfunction.[133] Figure 2-4 summarizes the lead-NMDA receptor interactions in the extra-synaptic region.[132] In the extra-synaptic region, the picomolar level of Pb^{+2} can efficiently substitute a micromolar level of Ca^{+2} on the NMDA receptor's NR2 B subunit, which induces an inflow of Ca^{+2}.[134] However, in this case, the increased level of Ca^{+2} can activate both protein kinase-C (PKC) and calmodulin (CAM) mRNA

expression.[134] Overactivation of these two pathways can cause an imbalance between apoptosis factors and antioxidants factors, which leads to neuron cell death.[134]

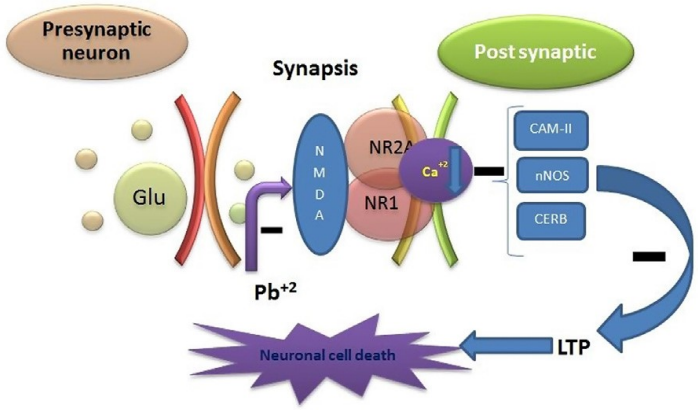

Figure 2-3 The synaptic interaction between lead and the NMDA receptor. (adapted from [132])

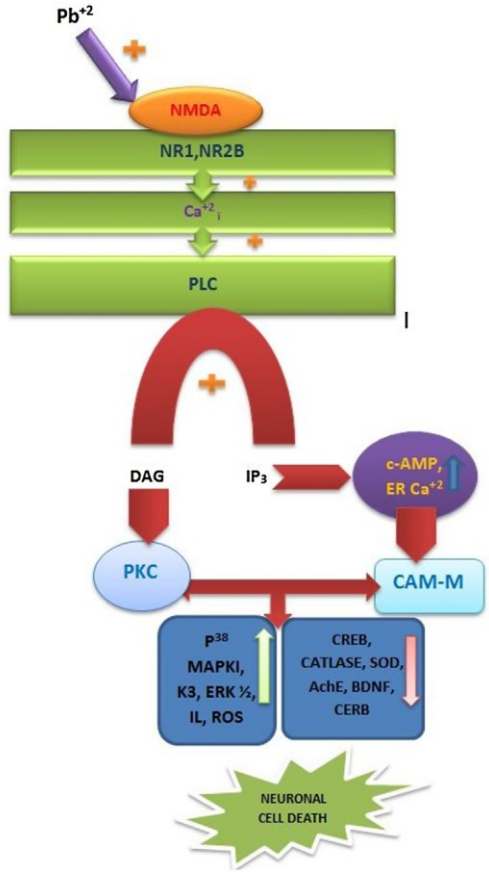

Figure 2-4 The extra-synaptic interaction between lead and the NMDA receptor. (adapted from [132])

Although lead exposure in children has declined in the U.S. since the ban on leaded gasoline.[117] lead has remained an important risk factor for certain children for two important reasons. First, accumulating evidence has revealed that even low-level lead exposure still has adverse effects on neurodevelopment. In agreement with this finding, the blood lead level of concern was reduced from 30 μg/dL in 1975 to 5 μg/dL in 2012 in the U.S.[118,135] Even further, accumulating new findings together with recent CDC guidelines suggest that there is no threshold for the adverse health effects of lead exposure.[117,136] Second, there still is a profound disparity of lead exposure in the U.S. A study conducted in South Carolina showed that the soil lead concentration was much higher in the urban areas because of more potential lead sources, such as road networks and industries.[137] In relation to this, low-income and racial/ethnic minority individuals including children younger than 6 years old have a much higher risk of lead exposure because they tend to live in urban areas and in neighborhoods that are closer to these lead sources;[137] this is not a matter of choice; these are the urban areas in which low-income individuals can afford to and hence are forced to live. Consistently, many other studies have also found that low-income minority populations are more likely to live in the highly lead-contaminated regions and have higher median blood lead concentrations, particularly among children, as a result.[137-140] The Flint, Michigan drinking water crisis is a clear example of the deep disparity of lead exposure in the U.S. Before the water source was switched, the population of Flint, which is home to many low-income minority populations, had already suffered multiple risks due to high lead exposure, including poor nutrition, condensed poverty, and older housing, which also increases the potential for lead exposure.[141] Data shows that there was already 2.4% of children who had elevated blood lead levels (>5 μg/dL) before the water source switch, while the percentage outside of Flint was 0.7%.[141] With a limited alternative water supply, the already higher

proportion of children with elevated blood lead levels was shown to be doubled after the water crisis.[141] The latest CDC report shows that the percentage of children in the U.S. with a confirmed blood lead level higher than 10 μg/dL increased from 7.6% to 13.4% from 2009-2011.[142] In the BBC, all of the cord blood samples had detectable levels of lead. The high prevalence of lead exposure along with exposures to other psychosocial and environmental toxins could have an enormous negative impact on children's neurodevelopment, particularly among most vulnerable segments of populations in the U.S.[141]

In sum, early life exposure to lead remains a clinical and public health concern in the US, especially, among disadvantaged populations. The role of early life exposure to lead in ADHD is biologically plausible, but not well-established. As such, a prospective birth cohort study is critically needed to investigate the association between early life exposure to lead and ADHD risk. Such investigation will be most relevant and revealing among high-risk U.S. populations such as urban, low income, minority children.

2.3.3 Maternal medication use

The average childbearing age in North America and Europe overlaps with the typical age of drug misuse.[143] According to the 2013 National Survey on Drug Use and Health in the U.S., 5.4% of pregnant women aged 15 to 44 in 2012-2013 reported as current illicit drugs users.[144] Given the underestimated nature of self-reported illicit drug use into consideration,[145] the true prevalence of drug misuse is likely much higher. Due to challenges associated with lifestyle changes in the face of addiction as well as both a lack of timely awareness about conception and the potential toxicity of drugs on the fetus, women might keep using harmful medications before and during pregnancy.[111] The potential fetal toxicity as a result of maternal illicit drug and medication exposure has been well-established.[143] One study provided evidence for the transplacental

transfer of gestational drug exposure by identifying detectable levels of illicit drugs, medications, tobacco ingredients, and alcohol metabolites from neonatal hair samples.[146] Heroin and methadone exposure during pregnancy have both been linked to an increased risk of neonatal abstinence syndrome, preterm birth, low birthweight, and even perinatal mortality.[143] At the same time, the prenatal use of anticonvulsants, a group of medications prescribed for the treatment of epileptic seizures, could lead to multiple congenital disabilities, including neural tube defects and developmental delays.[147]

Accumulating literature suggests that early child neurodevelopment could be highly influenced by prenatal prescribed and over-the-counter medication exposure.[148,149] One example is acetaminophen. Since 2013, multiple independent research studies analyzing five prospective cohorts from Europe and New Zealand have consistently shown a positive association between maternal intake of acetaminophen during pregnancy and risk of ADHD and its related symptoms in offspring.[150-154] The most recent study investigated the relationship between maternal acetaminophen intake during pregnancy and risk of ADHD in offspring further adjusting for familial risk for ADHD and acetaminophen-related indications.[150] This study, using the Norwegian Mother and Child Cohort Study (MoBa), collected data on maternal acetaminophen use through MoBa questionnaires at week 18, week 30, and 6 months after delivery. Children's ADHD diagnosis (2246 ADHD cases identified out of 112973 children) was based on the electronic medical record. Cox proportional hazard model results showed that short-term maternal acetaminophen use during pregnancy was negatively associated with the risk of ADHD, while long-term use was strongly associated with the risk of ADHD after adjusting for major known risk factors and other potential confounders. However, both the Society for Maternal-Fetal Medicine and the Food and Drug Administration both have issued statements indicating a

belief that the findings from these current studies are still too inconclusive to draw any causal inference between prenatal acetaminophen use and ADHD in the offspring.[155,156] Their primary criticisms include the use of self-reported exposure, lack of dose quantification, unmeasured confounders, and lack of model adjustment for multiple testing.[155] Given the infeasibility of conducting any randomized clinical trial, a well-designed prospective birth cohort with acetaminophen levels measured in maternal blood samples will be needed to address the noted concerns about previous studies and improve the understanding of acetaminophen's effects during pregnancy. Currently, no such study of this kind exists.

Acetaminophen is a widely used and commonly recommended medication for fever and pain relief for mother during pregnancy[157] and for babies in early life.[158] The percentage of pregnant women who use acetaminophen during pregnancy is over 65% in the U.S. and over 50% in Europe.[157,159] Starting from the early 1980s, acetaminophen replaced the carcinogenic and toxic phenacetin, which can be metabolized into acetaminophen in the human body.[160] The liver is the primary location for metabolism of acetaminophen.[161] As illustrated in Figure 2-5, the main metabolites of acetaminophen include unchanged acetaminophen, acetaminophen glucuronide, acetaminophen sulfate, and hepatotoxic N-acetyl-p-benzoquinone imine (NAPQI).[162] Under a therapeutic dose, the majority of acetaminophen is converted into nontoxic glucuronide (52-57% of urine metabolites) and sulfate (30-44%) conjugates.[162] However, 5-10% of acetaminophen is processed into highly toxic metabolite NAPQI, which is responsible for the major hepatotoxicity of acetaminophen.[162] The process of NAPQI detoxification is a glutathione-dependent process. Within the glutathione capacity, NAPQI is ultimately excreted in the urine as acetaminophen mercapturate.[161,163] In contrast, under supra-therapeutic doses of acetaminophen, the production of toxic NAPQI drastically increase to over 15% of total metabolites.[162] As the depletion of

glutathione proceeds, the undetoxified NAPQI leads to energy production loss, ion channel disturbance, and cell death.[161,163,164]

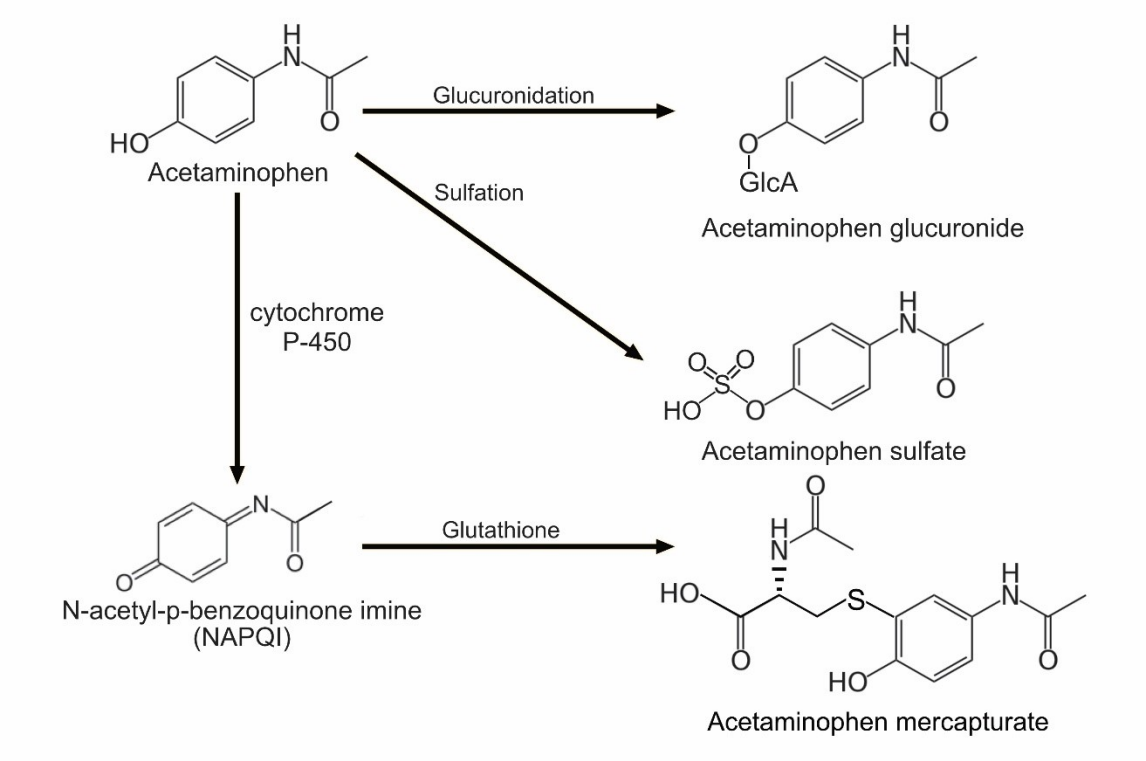

Figure 2-5 Pathways of acetaminophen metabolism.

Although the causality and biological mechanisms underlying the maternal acetaminophen and child ADHD association remain to be determined, its potential neurotoxicity is plausible according to previous findings. First, acetaminophen can be transferred through the placenta and stays in the infant's circulation much longer than in adults.[165] One study showed that maternal intake of phenacetin (which can be converted into acetaminophen rapidly in adults) containing tablets 5.5 hours before delivery can lead to detectable acetaminophen and its metabolites in an infant's 47-hour urine.[165] The prolonged detection of acetaminophen among children is due to

their undeveloped livers, which is in charge of acetaminophen metabolism.[166] On one hand, children's low metabolic capacity makes it safer for them to use acetaminophen, while on the other hand, it makes the fetus more vulnerable to maternal metabolized toxic NAPQI during pregnancy. The challenge that remains is that, while current opinions supporting acetaminophen's safety are based on findings related to the low toxic burden of the liver, kidney, and intestines in the short-term,[167,168], the long-term neurodevelopmental outcomes related to acetaminophen exposure have remained to be clarified.[169]

Second, as illustrated in Figure 2-6, the therapeutic effect of acetaminophen involves inhibition of prostaglandin production.[170] Prostaglandin H2 is the precursor of prostaglandin, which is converted from arachidonic acid by membrane-bound enzyme cyclo-oxygenase (COX).[171] COX exists in two major isoforms: COX-1 and COX-2.[172] COX-1 isoform is detectable in most tissues, while COX-2 isoform is only detectable in neuronal tissues under normal physiological conditions.[173] The therapeutic effect of acetaminophen can selectively inhibit COX-2.[174] As a result, the inhibition of prostaglandin production by acetaminophen primarily occurs in the brain.[174] However, prostaglandin synthesis in the brain involves multiple essential biological processes underlying the function and development of neural systems, such as long-term potentiation,[175] learning,[176] and cerebellar development.[170] While acetaminophen has some therapeutic effects, these disruptions in neuronal development and regulation caused by acetaminophen provide an additional plausible explanation for its potential neurotoxicity.

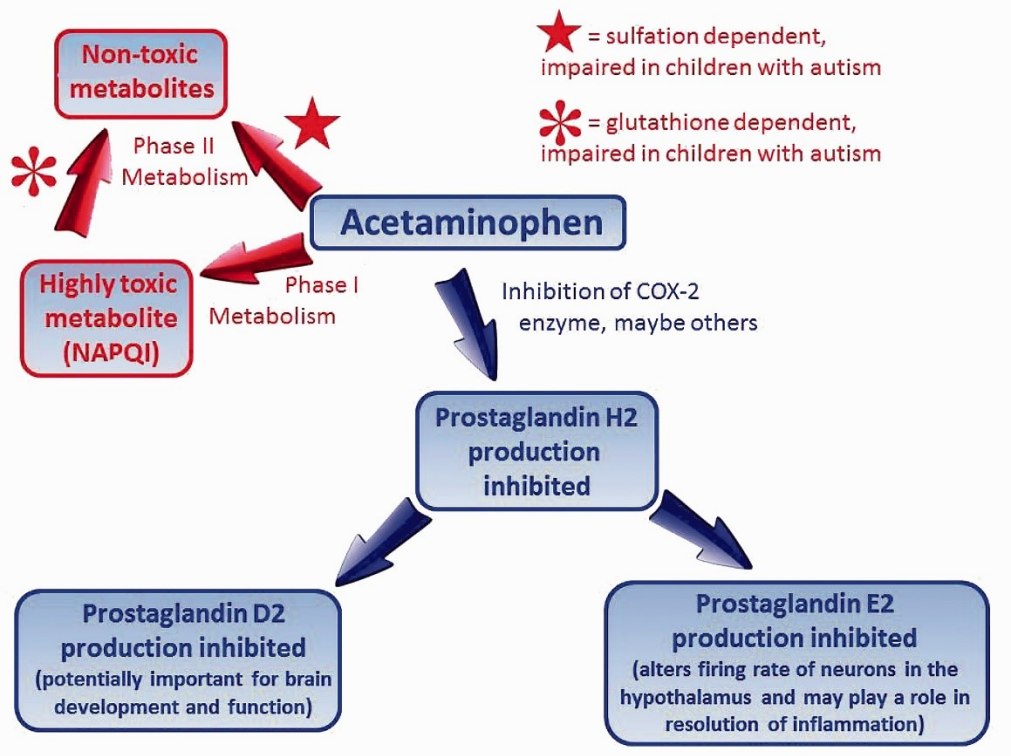

Figure 2-6 Action and metabolism of acetaminophen in babies and children. (adapted from [169])

Third, accumulating studies have shown that acetaminophen not only rapidly enters the cerebrospinal fluid but also shows a profound influence on adult brain function. Again, except for its therapeutic effect, acetaminophen can also reduce adults' response to stimuli and social rejection, and make them less likely to be aware of mistakes made during simple tasks.[177-180] These impacts on neural function also provide indirect evidence in support of acetaminophen's potential neural toxicity.

In sum, long-term exposure to maternal acetaminophen metabolites during pregnancy combined with a lack of metabolic capacity within the fetus might lead to both direct toxic damage from maternal NAPQI exposure and potential disruption in neurodevelopment due to prostaglandin inhibition. Considering the widespread use of acetaminophen in pregnant and peripartum women and growing concerns about its potential adverse effect on the developing brain and ADHD,

Prospective birth cohort study using objective biomarkers of exposure is critically needed to investigate the association between maternal acetaminophen and offspring ADHD risk.

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Chapter 3 METHODOLOGY

3.1 DATA SOURCE AND STUDY SAMPLE

The Boston Birth Cohort (BBC) is an ongoing prospective birth cohort, which was initiated by Dr. Xiaobin Wang at the Boston Medical Center (BMC) in 1998. This cohort was initially designed to support a molecular epidemiological study to capture the risk factors for low birthweight and prematurity among an urban, low-income, minority population in the Boston region. Since 1998, the BBC has used a rolling enrollment. To date, the BBC has successfully recruited over 8500 mother-infant pairs at birth; the participation rate was about 90% among eligible mothers approached by the research staff. Since 2003, a subset of children of BBC who continue to receive primary pediatric care at BMC are being enrolled in a postnatal follow-up study: Children's Health Study.[1-3] Besides collecting extensive demographic and environmental exposure assessments, the BBC has significantly benefited from the implementation of EMR since 2003 and the clinical data warehouse since 2005, which allows to access electronic medical records of the study children, including inpatients, outpatients, the emergency room, the operating room and billing, along with physician diagnoses based on the International Classification of Diseases, Ninth Revision or Tenth Revision (ICD-9 or ICD-10) for each postnatal visit were obtained from each child's EMR from 2003 through 2016.

The analyses presented here use data from the Children's Health Study (n~=3000). The sample size for each aim is presented in Table 3-1, and a power calculation is provided in

Table 3-2.

Table 3-1 Sample size calculation.

	N	Any ADHD diagnosis	Neurotypical	Other developmental disorder diagnosis
Sample size for Aim 1				
Maternal cholesterol biomarkers	1479	303	1176	
Sample size for Aim 2				
Early childhood lead exposure (before age 2 years)	1479	299	1180	
Sample size for Aim 3				
Maternal acetaminophen metabolites	1180	188	604	388

Table 3-2 Power calculation.

Alpha (α)	Effect Size for acetaminophen (ψ)	Power	Ratio of non-ADHD to ADHD children	N1	N2
0.05	0.21	0.7385	5.44	176	957
0.10		0.8301	5.44	176	957

Note: The power calculation for acetaminophen (Aim 3b) is presented above. Aim 3b for maternal blood unchanged acetaminophen was chosen to demonstrate the power since this sub-aim has the smallest sample size (n=1133). Due to a lack of appropriate data from other sources, BBC data was used for the power calculation. The mean difference in maternal unchanged acetaminophen levels between non-ADHD children and those with ADHD was 0.21 (inverse transformed intensity). The prevalence of ADHD in the BBC is 12%. Using a two-tailed alpha of 0.05 and 0.10, detecting an effect size of 0.21, with an N2/N1 ratio of 5.44 yielded a power of ~ 0.7385-0.8301%.

3.2 DATA COLLECTION

After obtaining informed consent and recruiting mothers into the BBC within a few days of delivery, a standard questionnaire interview was used to collect data on maternal demographics, smoking status, drug use, alcohol consumption, and several other variables. A medical abstraction form was used to review and collect clinical-related data from the maternal and newborn medical records, including parity, pre-pregnancy weight and height, gestational weight gain, pregnancy-related complications, intrauterine infection, and birth outcomes, such as gestational age and birthweight. Maternal blood samples collected shortly after delivery were

analyzed for maternal plasma HDL, LDL, TG, and acetaminophen metabolites. ADHD diagnosis and early childhood blood lead levels were obtained from the EMR at every postnatal clinical visit since 2003.

The study was approved by the Institutional Review Boards of the Johns Hopkins Bloomberg School of Public Health and Boston University Medical Center.

3.3 MEASURES

3.3.1 Primary outcomes

The primary outcome of this dissertation is ADHD. ADHD cases in this study are defined by clinician diagnosis based on ICD codes in the EMR. Specifically, ICD-9 codes 314.0-314.9 or ICD-10 codes F90.0-F90.9 documented by developmental-behavioral specialists or general physicians in each child's EMRs. The developmental-behavioral specialists included developmental-behavioral pediatricians, pediatric psychologists, pediatric neurologists, and child psychiatrists; general physicians were pediatricians and family medicine physicians. While there is a possibility of under- or misdiagnosis of ADHD, the validity of such diagnosis should be high in the BBC, given that most of the ADHD diagnoses in the BBC were made by developmental-behavioral specialists (301 out of 418 ADHD diagnosis). Prescribed medications documented in the EMR can further verify the ADHD diagnoses.

The ICD-9 codes have been listed in the Diagnostic and Statistical Manual of Mental Disorders (DSM) since 1980. The Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-5) is the latest DSM version published, on May 18, 2013, which lists both ICD-9 and ICD-10 codes for transitional purposes.[4] Starting from October 1, 2015, all entities covered by HIPAA (Health Insurance Portability and Accountability Act of 1996) must use ICD-10 codes.[5]

Clinicians in the U.S. currently use the DSM-5 to diagnose ADHD, superseding the DSM-IV-TR published in 2000.[4] The diagnostic criteria for ADHD in the DSM-5 are similar to those in the DSM-IV. For example, the same 18 symptoms are divided into two symptom domains (inattention and hyperactivity-impulsivity), of which at least six symptoms in each domain are required for diagnoses of children less than 17 years of age. However, several changes were made in the DSM-5: 1) criterion items now are applicable across the life span; 2) the cross-situational requirement has been strengthened to "several" symptoms in each setting; 3) the onset criterion has been changed from "symptoms that caused impairment were present before age 7 years" to "several inattentive or hyperactive-impulsive symptoms were present prior to age 12"; 4) subtypes have been replaced with presentation specifiers that map directly to the prior subtypes; 5) a comorbid diagnosis of autism spectrum disorder is now allowed; and 6) a symptom threshold change has been made from six required symptoms to five for adults, both for the inattention and for the hyperactivity–impulsivity domain. Finally, ADHD is placed in the neurodevelopmental disorders chapter in DSM-5 to reflect brain developmental correlates with ADHD.[4,6] Because the study population of this dissertation is younger than age 17 years, the transition from DSM-IV to DSM-5 is not expected to have an impact on my ADHD diagnosis determination.

The primary outcomes are listed in

Table 3-3. Figure 3-1 presents the distribution of diagnosis age for the first and last ADHD diagnosis. Age of diagnosis information can be used for sensitivity analysis by using survival analysis or more stringent ADHD case criteria (such as excluding the last diagnosis age younger than 6 years old).

Table 3-3 List of primary outcomes.

Name	Case definition	N	Non-case definition	N
Any ADHD diagnosis	Having at least one ADHD clinician diagnosis from any of the postnatal visits	418	Not having any ADHD clinician diagnosis through all the postnatal visits	2680
Any specialist diagnosis	Having at least one ADHD specialist diagnosis from any of the postnatal visits	301	Not having any ADHD clinician diagnosis through all the postnatal visits	2680
Any general physician diagnosis	Having at least one ADHD general physician diagnosis from any of the postnatal visits	117	Not having any ADHD clinician diagnosis through all the postnatal visits	2680
Any ADHD diagnosis with strict case and non-case definition	Having at least one ADHD specialist diagnosis from any of the postnatal visits	301	Not having any ASD, ADHD, DD clinician diagnosis through all the postnatal visits	1800

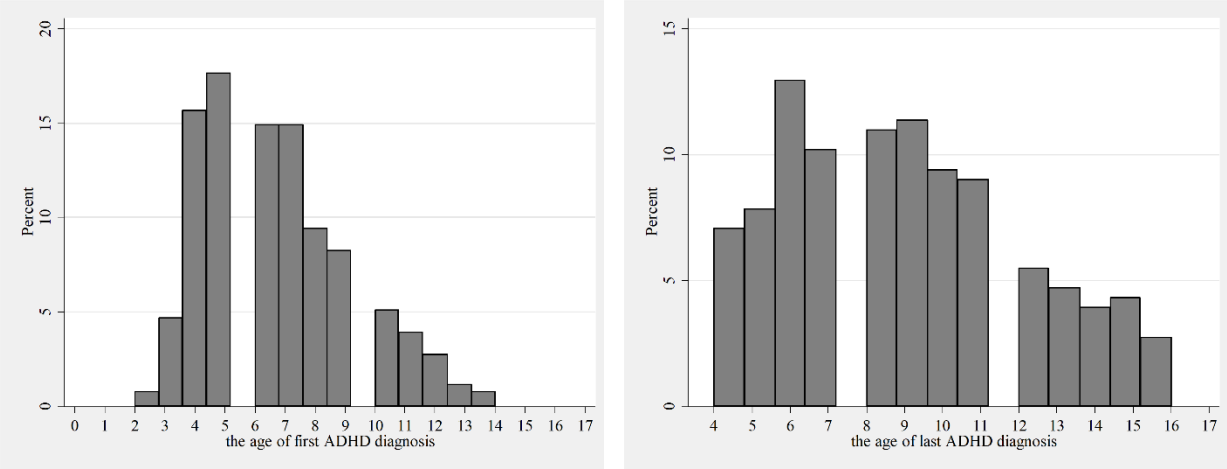

Figure 3-1 The age distributions of first and last ADHD diagnosis.

3.3.2 Primary exposures

The primary exposures are listed in Table 3-4. Maternal plasma total cholesterol, HDL, LDL, TG, and biomarkers of acetaminophen use were measured using nonfasting blood samples

obtained between 24 to 72 hours after delivery. Early childhood blood lead levels were obtained from the EMR of postnatal clinical visits.

Table 3-4 List of primary exposures.

Primary exposures	N	Distribution, median (IQR)
Aim 1		
Maternal total cholesterol (mg/dL)	2126	213.8 (175.6-254.1)
Maternal HDL (mg/dL)	2125	60.3 (49.9-72.7)
Maternal LDL (mg/dL)	2127	121.0 (95.9-150.4)
Maternal TG (mg/dL)	2124	176.7 (134.6-231.2)
Aim 2		
Early childhood lead (μg/dL)	2276	2 (1-3)
Aim 3		Percentage detectable
Unchanged acetaminophen	1412	100%
Acetaminophen glucuronide	1412	62.6%
3-(N-Acetyl-L-cystein-S-yl) acetaminophen	1412	55.0%

3.3.3 Other covariates

Table 3-5 provides a list of pertinent pre- and peri-natal and child factors that could potentially confound the relationship between maternal cholesterol, early childhood lead, maternal acetaminophen and ADHD risk; these factors will be adjusted for in the multivariate models.

Table 3-5 Pertinent pre- and peri-natal and child factors to be adjusted in the models.

Variables	Definition	Type
Pre- and peri-natal factors		
Maternal age	Maternal age at the time of enrollment	Continuous
Parity	Number of previous deliveries not including index pregnancy - nulliparous vs. multiparous	Binary
Maternal education	Below college degree vs. college or more	Binary
Maternal race/ethnicity	Black, White, Hispanic and Other	Categorical
Smoking during pregnancy	Whether mother ever smoked 3 months before pregnancy/during pregnancy - never, quit, continuous	Categorical
Intrauterine infection	Maternal intrauterine infection during pregnancy	Binary
Child factors		
Sex	Child's sex	Binary
Delivery type	C-section vs. vaginal	Binary
Gestational age	Preterm (<37 weeks) vs. term delivery	Binary
Birthweight	Low birthweight (<2500 g) vs. normal birthweight	Binary
Breastfeeding	Bottle fed or both vs. exclusively breastfed	Binary

3.4 DATA ANALYSIS

3.4.1 General analytical approach

Descriptive data analyses: The **primary outcomes** are binary (any ADHD diagnosis). The **primary exposures** of interest, such as maternal total cholesterol, HDL, LDL, TG (Aim 1), early childhood lead (Aim 2) and maternal acetaminophen metabolites (Aim 3), were analyzed as continuous, categorical, or binary variables. For key exposures, I first delineated their ranges and distributions and then determined appropriate transformations (i.e., natural logarithm transformation) to render the distributions approximately Gaussian as well as to stabilize the variance, if necessary. Group comparisons used ANOVA for continuous variables and Chi-square or Fisher's exact tests for categorical variables.

Consideration of pertinent covariates: Due to different biological theories and previous literature findings, the selection of pertinent covariates in each aim was different. The initial analysis started with a saturated model, which included all the potential covariates and interaction terms. After that, I gradually eliminated insignificant terms with the help of model comparison tools such as the likelihood ratio test.

3.4.2 Analysis for Aim 1

Aim 1 intended to investigate the role of maternal cholesterols (total, HDL, LDL, TG), independently and jointly, on the development of ADHD in childhood, adjusting for pertinent pre- and peri-natal and childhood factors. I further investigated whether the associations differed by sex. I hypothesized that maternal dyslipidemia during pregnancy can increase the risk of ADHD in childhood and that there is a sex difference in the association.

The relationship between maternal cholesterol biomarkers and ADHD was explored using a lowess plot with and without adjustment for other covariates, with each exposure considered a

continuous variable. With the visual aid of the lowess plot, each cholesterol biomarker was grouped into categorical variables using both clinical cut-off points and other cut-points (e.g., tertiles, quintiles, quartiles).

Generalized linear models (GLM) were used to analyze exposure-outcome associations systematically. GLM represent a large model class with well-established methods for model fitting and statistical inference.[7] I chose appropriate models from the class depending on the data type of the outcomes. For example, I used logistic regression for a binary outcome. The logit-transformed event probability was assumed to be a function of exposure and covariates: $\ln(\Pr(Y_i = \text{ADHD})/\Pr(Y_i = \text{non-ADHD})) = \beta_0 + \beta_1 E_i + \beta_c C_i + \varepsilon_i$, where Y_i is the outcome for subject i, E_i and C_i are exposures and a set of covariates for subject i, and β_1 *and* β_c are the corresponding regression coefficients. After accounting for confounding covariates, the exposure-outcome associations can be studied using a maximum likelihood estimate, hypothesis test, and confidence interval of β_1. The odds ratio (OR) can be estimated based on $\exp(\beta_1)$, which, for a common binary outcome, relative risk (RR) can be estimated as $RR=OR/[(1-P_0)+(P_0*OR)]$, where P_0=prevalence of the outcome in the unexposed group.[8]

With the help of the GLM method, a set of sequential models were executed in STATA. Below is the detailed sequential analytical plan:

Aim 1:

Analytical goal $\ln(\frac{\Pr(Yi = ADHD)}{\Pr(Yi = non-ADHD)}) = \beta_0 + \beta_1 E_i + \beta_c C_i + \beta_2 E_i * sex + \varepsilon_i$													
Independent effect of HDL													
Crude					HDL								
Adjusted					HDL		covariates						
Adjusted+interaction					HDL		covariates		interaction				
Independent effect of LDL													
Crude					LDL								
Adjusted					LDL		covariates						
Adjusted+interaction					LDL		covariates		interaction				

Independent effect of TG											
Crude					TG						
Adjusted					TG		covariates				
Adjusted+interaction					TG		covariates		interaction		
Independent effect of total cholesterol											
Crude					total cholesterol						
Adjusted					total cholesterol		covariates				
Adjusted+interaction					total cholesterol		covariates		interaction		
Joint effect of cholesterol											
					Based on findings						

3.4.3 Analysis for Aim 2

Aim 2a intended to investigate the role of early childhood lead exposure on the development of ADHD in childhood, adjusting for pertinent pre- and peri-natal and childhood factors. I further investigated whether the associations differed by sex. Aim 2b intended to investigate the potential protective effects of optimal maternal cholesterols in reducing the risk of ADHD associated with lead exposure. My hypotheses were as follows: lead exposure during early childhood can independently increase the risk of ADHD in childhood, and there is a sex difference in the association. Additionally, optimal maternal cholesterol during pregnancy can mitigate the adverse effect of lead on the risk of ADHD in childhood.

The relationship between early childhood lead exposure and ADHD was explored using a lowess plot with and without adjustment for other covariates, with each exposure considered to be a continuous variable. With the visual aid of the lowess plot, lead levels were grouped into categorical variables using multiple cut-off points (e.g., tertiles, quintiles, quartiles).

As described previously, the GLM was also used as the primary analytical method for Aim 2. The sequential analysis plan is provided below:

Aim 2a:

Analytical goal	$\ln(\frac{Pr(Yi = ADHD)}{Pr(Yi = non - ADHD)}) = \beta_0 +$	$\beta_1 E_i$	$+ \beta_c C_i$	$+ \beta_2 E_i * sex + \varepsilon_i$
Independent effect of early childhood lead				
Crude		early childhood lead		
Adjusted		early childhood lead	covariates	
Adjusted+interaction		early childhood lead	covariates	interaction

Aim 2b:

Analytical goal	$\ln(\frac{Pr(Yi = ADHD)}{Pr(Yi = non - ADHD)}) = \beta_0 +$	$\beta_1 E_i$	$+ \beta_c C_i$	$+ \beta_2 E_i * cholesterol + \varepsilon_i$
Test the interaction between optimal cholesterol and lead on ADHD				
Early childhood lead* optimal cholesterol		Early childhood lead	covariates	interaction

3.4.4 Analysis for Aim 3

Aim 3a intended to investigate the role of maternal blood acetaminophen metabolites, independently and jointly, on the development of ADHD in childhood, adjusting for pertinent pre- and peri-natal and childhood factors. I further investigated whether the associations differed by sex. Aim 3b intended to investigate the potential protective effects of optimal maternal cholesterols in reducing the risk of ADHD associated with maternal acetaminophen exposure. My hypotheses were as follows: maternal acetaminophen exposure during the perinatal period could independently and jointly increase the risk of ADHD in childhood, and there is a sex difference in the association. Additionally, optimal maternal cholesterol during pregnancy can reduce the adverse effect of maternal acetaminophen exposure on the risk of ADHD in childhood.

The relationship between each maternal acetaminophen metabolite and ADHD was explored using a lowess plot with and without adjustment for other covariates, with each maternal acetaminophen metabolite considered to be a continuous variable. Based on previous findings regarding the proportions of acetaminophen metabolites typically found in blood samples,[9] I

further calculated a variable to reflect overall "acetaminophen burden" by combining all of the acetaminophen metabolite levels with a weight based on their proportions in the acetaminophen metabolic pathway [acetaminophen burden=(unchanged acetaminophen/5%+ acetaminophen glucuronide/50%+ 3-(N-Acetyl-L-cystein-S-yl) acetaminophen/5%)/60%].[9] With the visual aid of the lowess plot, each maternal acetaminophen metabolite level was grouped into categorical variables using multiple cut-off points (e.g., tertiles, quintiles, quartiles).

As described previously, the GLM was also used as the primary analytical method for Aim 3. The sequential analysis plan is provided below:

Aim 3a:

Analytical goal	$\ln(\frac{\Pr(Yi = ADHD)}{\Pr(Yi = non-ADHD)}) = \beta_0 +$	$\beta_1 E_i$	$+ \beta_c C_i$	$+ \beta_2 E_i * sex + \varepsilon_i$
Independent effect of unchanged acetaminophen				
Crude		unchanged acetaminophen		
Adjusted		unchanged acetaminophen	covariates	
Adjusted+interaction		unchanged acetaminophen	covariates	interaction
Independent effect of acetaminophen glucuronide				
Crude		acetaminophen glucuronide		
Adjusted		acetaminophen glucuronide	covariates	
Adjusted+interaction		acetaminophen glucuronide	covariates	interaction
Independent effect of 3-(N-Acetyl-L-cystein-S-yl) acetaminophen				
Crude		3-(N-Acetyl-L-cystein-S-yl) acetaminophen		
Adjusted		3-(N-Acetyl-L-cystein-S-yl) acetaminophen	covariates	
Adjusted+interaction		3-(N-Acetyl-L-cystein-S-yl) acetaminophen	covariates	interaction
Independent effect of acetaminophen burden				
Crude		acetaminophen burden		
Adjusted		acetaminophen burden	covariates	
Adjusted+interaction		acetaminophen burden	covariates	interaction

Aim 3b:

Analytical goal	$\ln\left(\frac{\Pr(Yi = ADHD)}{\Pr(Yi = non - ADHD)}\right) = \beta_0 + \beta_1 E_i + \beta_c C_i + \beta_2 E_i * cholesterol + \varepsilon_i$											
Test the interaction between optimal cholesterol and maternal acetaminophen metabolite on ADHD												
maternal acetaminophen metabolite * optimal cholesterol							maternal acetaminophen metabolite		covariates		Interaction	

3.5 REFERENCES

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Chapter 4 AIM 1: A PROSPECTIVE BIRTH COHORT STUDY ON MATERNAL CHOLESTEROL LEVELS AND OFFSPRING ATTENTION DEFICIT HYPERACTIVITY DISORDER: NEW INSIGHT ON SEX DIFFERENCES

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4.1 ABSTRACT

Growing evidence suggests that maternal cholesterol levels are important in the offspring's brain growth and development. Previous studies on cholesterols and brain functions were mostly in adults. We sought to examine the prospective association between maternal cholesterol levels and the risk of attention deficit hyperactivity disorder (ADHD) in the offspring. We analyzed data from the Boston Birth Cohort, enrolled at birth and followed from birth up to age 15 years. The final analyses included 1479 mother-infant pairs: 303 children with ADHD, and 1176 neurotypical children without clinician-diagnosed neurodevelopmental disorders. The median age of the first diagnosis of ADHD was seven years. The multiple logistic regression results showed that a low maternal high-density lipoprotein level (≤60 mg/dL) was associated with an increased risk of ADHD, compared to a higher maternal high-density lipoprotein level, after adjusting for pertinent covariables. A "J" shaped relationship was observed between triglycerides and ADHD risk. The associations with ADHD for maternal high-density lipoprotein and triglycerides were more pronounced among boys. The findings based on this predominantly urban low-income minority birth cohort raise a new mechanistic perspective for understanding the origins of ADHD and the gender differences and future targets in the prevention of ADHD.

Keywords: high-density lipoprotein; triglyceride; sex difference; ADHD

4.2 INTRODUCTION

In the U.S., attention deficit hyperactivity disorder (ADHD) is one of the most common neurodevelopmental disorders in children; its prevalence has risen from 7.0% to 10.2% among children aged 4-17 years during the past two decades[1] representing a nearly 5% increase each year since 2003.[2] ADHD is characterized by inattention, hyperactivity, or impulsiveness,[3-5] and is three times more common among males than females.[6] Approximately 66% to 85% of children diagnosed with ADHD will carry their disorder into adolescence and adulthood.[7,8] A 2007 estimation of the annual cost of ADHD in the U.S., including the cost of related health care utilization, medication, education, crime, and unemployment, was $14500 per child ($42.5 billion in total).[9] While ADHD medications have shown to be effective in controlling ADHD symptoms, they neither preclude the rising incidence of ADHD nor cure ADHD, not to mention that they are also the causes for additional costs and potential side effects.[2] Given its high prevalence and continuously rising trend, the impact of ADHD on individual families and society is expected to increase dramatically.[7,9,10]

At present, our knowledge regarding the biological mechanisms of ADHD development and effective ways to prevent ADHD is insufficient. While research has identified several potential etiological mechanisms, such as gene variants, brain structural abnormalities, and neurotransmitter deficiency and dysregulation,[11,12] much more work is needed to fully understand the early life determinants of ADHD and significant sex differences in ADHD risk. There is an urgent need to identify modifiable early life risk factors for ADHD, which are essential to the primary prevention efforts. Well-recognized environmental risk factors for ADHD include parent-related factors,[13-25] low birthweight and preterm birth,[26] exposure to organophosphates,[27] polychlorinated biphenyls,[28,29] and lead.[28,30-32] Besides those factors,

multiple recent studies indicate that maternal metabolic profiles may also influence offspring's neurodevelopment. For example, findings in the Boston Birth Cohort showed a strong association between maternal obesity and diabetes and increased risk of autism in childhood. [33] A large longitudinal study, using prospective pregnancy cohorts from the Nordic Network, showed that both overweight moms and moms with excessive weight gain during gestation had an over 2-fold higher risk of having ADHD children. [34] However, no study has investigated the role of maternal dyslipidemia (a condition often associated with obesity or metabolic syndrome) in offspring's ADHD development.

Maternal cholesterol levels are biologically plausible to influence neurodevelopment in the offspring. [33-40] Besides cholesterol's key functions, such as hormone synthesis, fat-soluble vitamin digestion and absorption, cell membrane stabilization, and inter-cellular communication, it is essential for normal brain development, especially during in-utero and early childhood. [36,41,42] Nearly 70% to 80% of brain cholesterol is present in myelin. [43] While fetal cholesterol can be synthesized endogenously [38], the placenta also delivers cholesterol from maternal circulation to the fetus through multiple cholesterol-carrying lipoproteins, such as low-density lipoproteins (LDL), high-density lipoproteins (HDL) and very low-density lipoproteins (VLDL). [39,40] It was estimated that up to 20% of fetal cholesterol in the first trimester is derived from maternal cholesterol via the placenta. [38]

During normal pregnancy in humans, maternal blood cholesterol levels increase with gestational age to meet the increasing demands of fetal growth and development, especially with regards to the fetal brain.[44-46] Conceivably, dysregulation in the amount and the type of cholesterol during critical developmental windows could lead to suboptimal neurodevelopment, and subsequently, ADHD symptoms in childhood. However, this possibility remains to be explored. To our

knowledge, existing cholesterol studies in humans have mainly focused on mental health outcomes in adults, in which HDL levels have been found to be associated with multiple cognitive impairments and neurodegenerative diseases.[47-49] Particularly, there is a lack of prospective birth cohort study to investigate the inter-generational impact of cholesterol on ADHD.

To fill in the aforementioned knowledge gaps, in this study, we sought to examine the prospective association between maternal cholesterol levels 24-72 hours after delivery and the development of ADHD in the offspring using a longitudinal birth cohort design. Findings from such a study have important clinical and public health implications. The current clinical guidelines for optimal cholesterol levels have been set for non-pregnant women based on cardio-metabolic outcomes, aiming to control cholesterol levels. However, the requirements for optimal nutrition, including cholesterols, are higher during pregnancy due to the increasing demands of the uterus, placenta, and fetal growth. Furthermore, no guidelines for cholesterol levels have been established for pregnant women in the context of fetal brain growth and long-term neurodevelopmental outcomes.

4.3 MATERIALS AND METHODS

4.3.1 Study Sample

The Boston Birth Cohort (BBC) has successfully recruited mother-infant pairs at birth; the participation rate has been >90% among eligible mothers approached by the research staff. Details of the recruitment of the BBC were published previously.[50,51] Eligible mothers were those who delivered a single live birth at Boston Medical Center (BMC). Pregnancies resulting from in vitro fertilization, multiple-gestation pregnancies, deliveries induced by maternal trauma,

or newborns with substantial congenital disabilities were not eligible for enrollment. The Institutional Review Board (IRB) of the Boston University Medical Center and Johns Hopkins Bloomberg School of Public Health approved the BBC study. Informed consent was obtained from each participant under the IRB approved protocol (IRB No. 00003966).

Of enrolled mother-infant pairs at birth in the BBC, 3098 who continued to receive pediatric primary care at BMC were enrolled in a postnatal follow-up study.[33,50,52] Our study sample excluded participants who had missing maternal cholesterol measurements and key covariates. We further excluded children with physician-diagnosed neurodevelopmental disorders other than ADHD (Table S1). Our final analyses consisted of 1479 mother-infant pairs, including 303 children with ADHD and 1176 neurotypical children (Figure 4-1). The maternal and child characteristics for participants excluded and included are compared in Table S2.

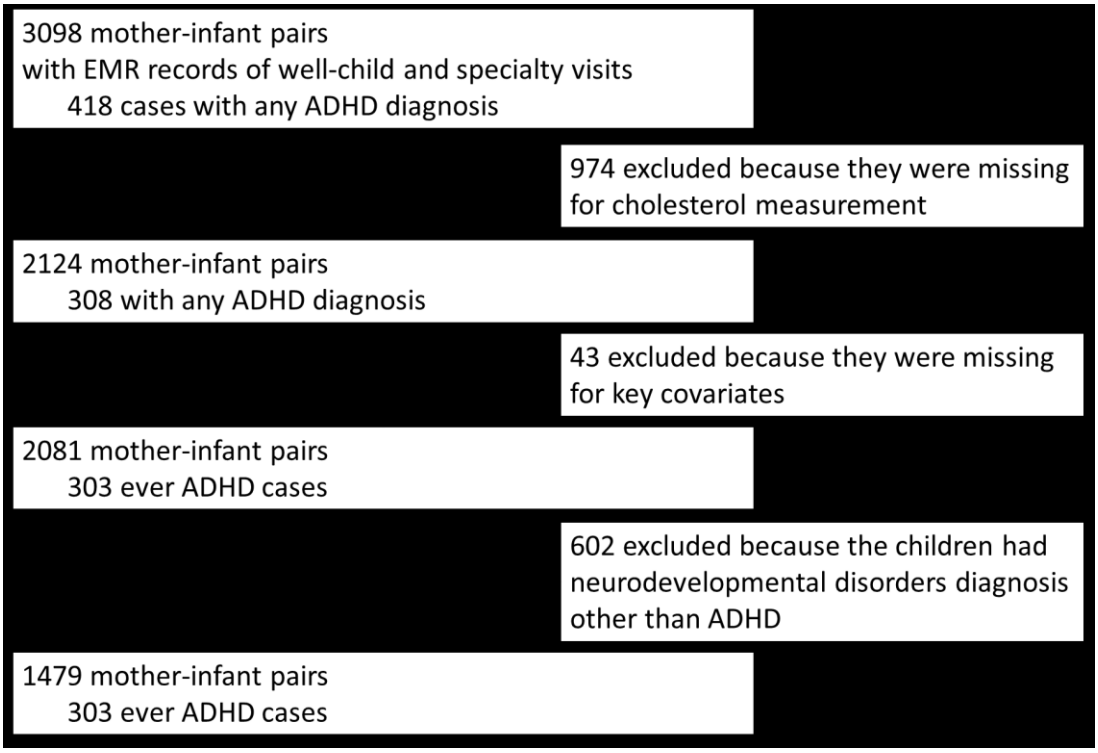


Figure 4-1 Flowchart of the sample included in the analyses.

4.3.2 Data Collection Procedures and Measures of Key Variables

Mother-infant pairs were enrolled 24 to 72 hours after birth. After obtaining informed consent, face-to-face interviews using a standardized questionnaire were conducted to collect mothers' reports on family socio-demographics, substance use, and other prenatal exposure information. The maternal and newborn medical records were extracted using a standardized abstraction form. Since 2003, electronic medical records (EMRs) became part of routine clinical data collection for the BBC, including both well-child and specialty medical visits at BMC. For each primary care visit, the EMRs contain the primary and secondary diagnoses from the International Classification of Diseases, Ninth Revision (ICD-9) (before October 1, 2015) and ICD-10 (after October 1, 2015).

Maternal serum total cholesterol (TC), triglycerides (TG), and high-density lipoprotein (HDL) levels were measured using nonfasting blood samples obtained between 24 to 72 hours after

delivery. Serum low-density lipoprotein (LDL) levels were calculated using the Friedwald equation. The detailed measurement and calculation methods are described in our previous publication.[53] Of note, nonfasting samples primarily impact TC and TG levels, which may be higher than in a fasting state.

The "ADHD group" was defined as having any of the following clinician-diagnosed ICD-9 codes: [314.0 (Attention deficit disorder of childhood), 314.00 (Attention deficit disorder without mention of hyperactivity), 314.01 (Attention deficit disorder with hyperactivity), 314.1 (Hyperkinesis with developmental delay), 314.2 (Hyperkinetic conduct disorder), 314.8 (Other specified manifestations of hyperkinetic syndrome), and 314.9 (Unspecified hyperkinetic syndrome)], or any of the following ICD-10 codes: [F90.0 (ADHD, predominantly inattentive type), F90.1 (ADHD, predominantly hyperactive type), F90.2 (ADHD, combined type), F90.8 (ADHD, other type), and F90.9 (ADHD, unspecified type)] as documented in the child's EMRs.

The "neurotypical (NT) group" was defined as not having any clinician diagnosis of autism spectrum disorder, ADHD, conduct disorders, developmental delays, intellectual disabilities, failure to thrive, or congenital anomalies. This definition was established by clinical experts and has been applied by multiple published papers. [54,55] The ICD-9 and ICD-10 codes for the diagnoses of these developmental disorders are listed in Table S1.

4.3.3 Statistical Analysis

The characteristics of the study sample between the "ADHD" and the "NT" groups were examined by t-test for continuous variables and χ^2 test for categorical variables. TC, HDL, LDL, and TG were further analyzed as categorical variables based on clinically-established cut-off points,[56,57] in addition to quartiles and the linear trend test. The clinical cut-off point for low HDL for women is <50 mg/dL.[57] The clinical cut-off point for non-fasting high TG is ≥200

mg/dL.[56] The quartile cut-off points were: TC (<176 mg/dL, 176-214 mg/dL, 215-254 mg/dL, >254 mg/dL), TG (<135 mg/dL, 135-176 mg/dL, 177-232 mg/dL, >232 mg/dL), HDL (<50 mg/dL, 50-60 mg/dL, 61-73 mg/dL, >73 mg/dL), and LDL (<96 mg/dL, 96-121 mg/dL, 122-150 mg/dL, >150 mg/dL). Next, we conducted multiple logistic regression (MLR) to examine the association between TC, HDL, LDL, and TG and the risk of ADHD diagnosis, both categorically and continuously, adjusting for maternal age at delivery, maternal race/ethnicity, maternal education, smoking during pregnancy, intrauterine infection, parity, child's sex, mode of delivery, preterm birth, and birthweight. The effect of the interaction between child's sex and each type of lipid or lipoprotein level on the risk of ADHD was tested using MLR and adjusted for the same set of covariates. Similarly, the joint effect of the child's sex with each type of lipid or lipoprotein on the risk of ADHD was tested using MLR and adjusted for the same set of covariates except for child's sex. In the sensitivity analyses, stratified analysis by each major covariate was conducted for the association between maternal HDL and ADHD. Furthermore, we repeated the above analyses within two subsets. One subset only included specialist-diagnosed ADHD as cases, while the other subset only included the ADHD cases whose age of last ADHD diagnosis was 6 years or older. All analyses were performed using STATA® version 14.0 software (Stata Corporation, College Station, TX, USA).

4.4 RESULTS

There were 303 children with a clinician diagnosis of ADHD. Of these, 214 were diagnosed by a developmental specialist and 89 only by a general pediatrician. The median age at the first ADHD diagnosis was seven years. Table 4-1 presents the bivariate comparisons of maternal and child characteristics between the "ADHD" and "NT" groups. The mothers of children with an ADHD diagnosis were more likely to have below college degree education, ever smoke before or

during pregnancy, C-section delivery, lower TC, lower HDL, and lower LDL, compared with the neurotypical group. The children with any ADHD diagnosis were more likely to be male, born prematurely and have had low birthweight, compared with the neurotypical group. The comparison results of major characteristics between excluded and included samples indicate that the included sample had less exposure to multiple risk factors, such as smoking during pregnancy, C-section delivery, lower gestational age, and lower birthweight (Table S2).

Table 4-1 Maternal and child characteristics for children with any ADHD diagnosis and neurotypical children (NT).

Variable	Total, No. (%)	NT, No. (%)	ADHD, No. (%)	P-value‡
Total	1479 (100)	1176 (79.5)	303 (20.5)	
Maternal Age				0.317
<20	148 (10.0)	111 (9.4)	37 (12.2)	
20-34	1080 (73.0)	867 (73.8)	213 (70.3)	
>=35	251 (17.0)	198 (16.8)	53 (17.5)	
Education level				0.022
Below college degree	1278 (86.4)	1004 (85.4)	274 (90.4)	
College degree or above	201 (13.6)	172 (14.6)	29 (9.6)	
Race-ethnicity				0.230
Black	968 (65.5)	759 (64.5)	209 (69.0)	
White	74 (5.0)	56 (4.8)	18 (5.9)	
Hispanic	357 (24.1)	293 (24.9)	64 (21.1)	
Others	80 (5.4)	68 (5.8)	12 (4.0)	
Parity				0.901
Nulliparous	625 (42.3)	496 (42.2)	129 (42.6)	
Multiparous	854 (57.7)	680 (57.8)	174 (57.4)	
Smoking during pregnancy				<0.001
Never	1229 (83.1)	998 (84.9)	231 (76.2)	
Quitter	111 (7.5)	72 (6.1)	39 (12.9)	
Continuous	139 (9.4)	106 (9.0)	33 (10.9)	
Intrauterine infection				0.060
No	1292 (87.4)	1037 (88.2)	255 (84.2)	
Yes	187 (12.6)	139 (11.8)	48 (15.8)	
Child's sex				<0.001
Female	749 (50.6)	664 (56.5)	85 (28.1)	
Male	730 (49.4)	512 (43.5)	218 (71.9)	
Delivery type				0.008
C-section	500 (33.8)	378 (32.1)	122 (40.3)	
Vaginal	979 (66.2)	798 (67.9)	181 (59.7)	
Season of child's birth				0.797

Jan to March	333 (22.5)	264 (22.5)	69 (22.8)	
April to June	350 (23.7)	279 (23.7)	71 (23.4)	
July to September	402 (27.2)	314 (26.7)	88 (29.0)	
October to December	394 (26.6)	319 (27.1)	75 (24.8)	
Preterm birth (<37 weeks)				0.005
No	1125 (76.1)	913 (77.6)	212 (70.0)	
Yes	354 (23.9)	263 (22.4)	91 (30.0)	
Low birthweight (<2500 g)				0.028
No	1148 (77.6)	927 (78.8)	221 (72.9)	
Yes	331 (22.4)	249 (21.2)	82 (27.1)	
Gestational age, week				<0.001
Mean (SD)	38.1 (3.1)	38.2 (2.9)	37.5 (3.8)	
Birthweight, g				0.007
Mean (SD)	2996.7 (754.0)	3023.3 (716.4)	2893.5 (878.9)	
Maternal TC, mg/dL				0.018
Mean (SD)	219.6 (60.9)	221.5 (61.3)	212.2 (58.9)	
Maternal TG, mg/dL				0.838
Mean (SD)	191.9 (80.6)	192.2 (80.1)	191.1 (83.0)	
Maternal HDL, mg/dL				<0.001
Mean (SD)	62.0 (17.6)	62.8 (17.9)	58.8 (15.8)	
Maternal LDL, mg/dL				0.011
Mean (SD)	126.6 (41.8)	128.0 (42.1)	121.2 (39.9)	

NT was defined as without any mental disorder diagnosis; ADHD was defined as any ADHD diagnosis; [†]The p-values were obtained from chi-square tests or t-tests between children with and without any ADHD diagnosis.

Table 4-2 shows the MLR results for the effects of TC, HDL, LDL, and TG on the risk of any ADHD diagnosis, after adjusting for pertinent covariates. HDL <50 mg/dL, indicating a moderate risk of heart disease, was not associated with an increased risk of ADHD diagnosis (OR=1.30, 95% CI (0.96, 1.74)). When HDL levels were analyzed as quartiles, mothers with first or second quartile HDL levels showed a similarly increased odds of having a child with any ADHD diagnosis compared to those with fourth quartile HDL levels (Q2 vs. Q4: OR=1.42, 95% CI (0.96, 2.09); Q1 vs. Q4: OR=1.54, 95% CI (1.04, 2.28)). Mothers with ≤ median HDL levels had a 39% increased odd of having a child with any ADHD diagnosis as compared to mothers with > median HDL levels (OR=1.39, 95% CI (1.06, 1.82)). When HDL was analyzed as a continuous variable, the average odds of having a child with any ADHD diagnosis dropped 19% for every 20 mg/dL increase in maternal HDL levels (OR=0.81, 95% CI (0.69, 0.95)).

Table 4-2 The association between maternal cholesterol and the risk of ADHD in offspring.

Maternal cholesterols		ADHD, No. (%)	NT, No. (%)	Crude OR	95% CI		P-value	Adjusted OR	95% CI		P-value
HDL clinical cut-off	≥ 50 mg/dL	213 (19.2)	898 (80.8)	1.00				1.00			
	< 50 mg/dL	90 (24.5)	278 (75.5)	1.36	1.03	1.81	0.030	1.30	0.96	1.74	0.085
HDL quartiles	Q4 (>73 mg/dL)	55 (15.3)	304 (84.7)	1.00				1.00			
	Q3 (61-73 mg/dL)	67 (18.1)	304 (81.9)	1.22	0.82	1.80	0.322	1.11	0.74	1.67	0.606
	Q2 (50-60 mg/dL)	91 (23.9)	290 (76.1)	1.73	1.20	2.51	0.004	1.42	0.96	2.09	0.079
	Q1 (< 50 mg/dL)	90 (24.5)	278 (75.5)	1.79	1.23	2.60	0.002	1.54	1.04	2.28	0.031
HDL binary	> median (60 mg/dL)	122 (16.7)	608 (83.3)	1.00				1.00			
	≤ median (60 mg/dL)	181 (24.2)	568 (75.8)	1.59	1.23	2.05	<0.001	1.39	1.06	1.82	0.016
HDL linear trend (every 20 mg/dL increase)		303 (20.5)	1176 (79.5)	0.76	0.65	0.88	<0.001	0.81	0.69	0.95	0.011
TG clinical cut-off	< 200 mg/dL	184 (19.8)	744 (80.2)	1.00				1.00			
	≥ 200 mg/dL	119 (21.6)	432 (78.4)	1.11	0.86	1.44	0.415	1.26	0.94	1.68	0.118
TG quartiles	Q1 (<135 mg/dL)	90 (23.9)	287 (76.1)	1.00				1.00			
	Q2 (135-176 mg/dL)	58 (16.3)	297 (83.7)	0.62	0.43	0.90	0.012	0.63	0.43	0.93	0.020
	Q3 (177-232 mg/dL)	76 (20.7)	291 (79.3)	0.83	0.59	1.18	0.300	0.88	0.61	1.27	0.495
	Q4 (>232 mg/dL)	79 (20.8)	301 (79.2)	0.84	0.59	1.18	0.309	0.98	0.66	1.44	0.909
TG binary	Q2	58 (16.3)	297 (83.7)	1.00				1.00			
	Q1, Q3, Q4	245 (21.8)	879 (78.2)	1.43	1.04	1.96	0.027	1.51	1.08	2.10	0.015
TG linear trend (every 20 mg/dL increase)		303 (20.5)	1176 (79.5)	1.00	0.97	1.03	0.838	1.02	0.98	1.06	0.348
LDL quartiles	Q1 (<96 mg/dL)	87 (23.6)	282 (76.4)	1.00				1.00			
	Q2 (96-121 mg/dL)	80 (21.8)	287 (78.2)	0.90	0.64	1.28	0.565	0.91	0.63	1.31	0.603
	Q3 (122-150 mg/dL)	67 (18.2)	301 (81.8)	0.72	0.50	1.03	0.074	0.82	0.57	1.20	0.316
	Q4 (>150 mg/dL)	69 (18.4)	306 (81.6)	0.73	0.51	1.04	0.083	0.76	0.52	1.11	0.153
LDL linear trend (every 20 mg/dL increase)		303 (20.5)	1176 (79.5)	0.92	0.87	0.98	0.011	0.93	0.87	0.99	0.033
TC quartiles	Q1 (<176 mg/dL)	92 (24.6)	282 (75.4)	1.00				1.00			
	Q2 (176-214 mg/dL)	73 (20.3)	287 (79.7)	0.78	0.55	1.10	0.161	0.82	0.57	1.18	0.289
	Q3 215-254 mg/dL)	72 (19.9)	290 (80.1)	0.76	0.54	1.08	0.125	0.86	0.59	1.25	0.424
	Q4 (>254 mg/dL)	66 (17.2)	317 (82.8)	0.64	0.45	0.91	0.013	0.73	0.50	1.08	0.111
TC linear trend (every 20 mg/dL increase)		303 (20.5)	1176 (79.5)	0.95	0.91	0.99	0.018	0.96	0.92	1.01	0.099

NT was defined as without any mental disorder diagnosis; ADHD was defined as any ADHD diagnosis; the multiple logistic regression model was adjusted for maternal age at delivery, maternal race/ethnicity, maternal education, smoking during pregnancy, intrauterine infection, parity, child's sex, mode of delivery, preterm birth, and birthweight.

For TG, the risk of ADHD diagnosis for the children whose maternal TG levels were ≥200 mg/dL (indicating marginal risk of heart disease) was not statistically significantly different to those children whose mothers with <200 mg/dL TG levels (OR=1.26, 95% CI (0.94, 1.68)). Compared to mothers with second quartile TG levels, the mothers with first, third or fourth quartile TG levels had a 51% increased odds of having a child with any ADHD diagnosis (OR=1.51, 95% CI (1.08, 2.10)), suggesting a "J" shaped association.

When LDL was analyzed as a continuous variable, the average odds of having a child with any ADHD diagnosis dropped 7% for every 20 mg/dL increase in maternal LDL levels (OR=0.93, 95% CI (0.87, 0.99)). The MLR results for maternal TC levels did not show any significant association with the child's ADHD diagnosis.

Table 4-3 shows the associations between maternal HDL levels and the risk of any ADHD diagnosis stratified by the child's sex and the joint effect of maternal HDL levels and the child's sex on ADHD risk. As expected, compared to girls, boys had 3 times higher risk of ADHD (OR=3.25, 95% CI (2.45, 4.30)). The joint effects of maternal HDL and sex showed that boys whose mothers had ≤ median HDL levels had increased odds of having any ADHD diagnosis (OR=4.25, 95% CI (2.88, 6.26)), compared to girls whose mothers had > median HDL levels. The interaction term between sex and HDL was not statistically significant (OR=1.35, 95% CI (0.77, 2.37)). Table S3 shows the stratified analysis results for the association between maternal HDL and ADHD. The results indicate that, besides child's sex, smoking during pregnancy, intrauterine infection, parity, mode of delivery, gestational age, and birthweight also influence the association between maternal HDL and ADHD. Higher maternal HDL was more likely

associated with a reduced risk of ADHD in the following stratum: boy, none smoker during pregnancy, no intrauterine infection during pregnancy, multiparous, vaginal delivery, full term and normal birth weight.

Table S4 shows the sensitivity analysis results on the joint effect of maternal HDL and sex by comparing children with specialist ADHD diagnosis and neurotypical children; and the findings were similar. Table S6 shows the results of the sensitivity analyses on the joint effect of maternal HDL and sex by excluding the children whose age of last ADHD diagnosis is under 6 years old; and the findings were also similar.

Table 4-3 The joint association of maternal HDL levels and child's sex with the risk of ADHD in offspring.

Sex	Maternal HDL	ADHD, No. (%)	NT, No. (%)	Adjusted OR	95% CI		P-value
Female		85 (11.4)	664 (88.6)	1.00			
Male		218 (29.9)	512 (70.1)	3.25	2.45	4.30	<0.001
Joint effects of maternal HDL and sex							
Female	> median	42 (10.5)	359 (89.5)	1.00			
	≤ median	43 (12.4)	305 (87.6)	1.14	0.72	1.81	0.564
Male	> median	80 (24.3)	249 (75.7)	2.75	1.82	4.16	<0.001
	≤ median	138 (34.4)	263 (65.6)	4.25	2.88	6.26	<0.001

NT was defined as without any mental disorder diagnosis; ADHD was defined as any ADHD diagnosis; covariates included maternal age at delivery, maternal race/ethnicity, maternal education, smoking during pregnancy, intrauterine infection, parity, child's sex, mode of delivery, preterm birth, and birthweight.

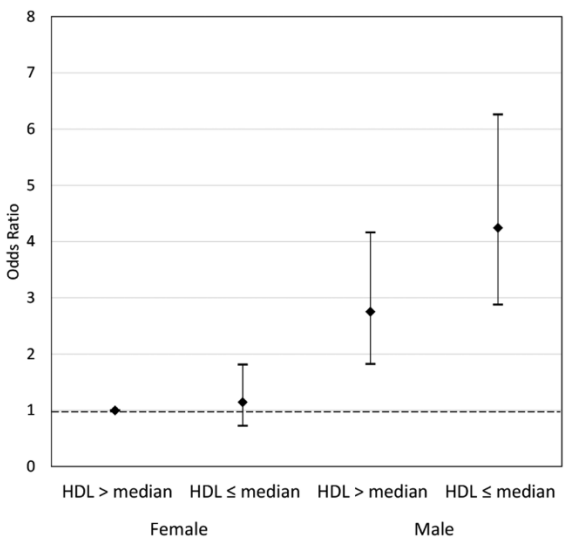
Table 4-4 shows the association between maternal TG levels and the risk of any ADHD diagnosis, stratified by the child's sex and the joint effect of maternal TG levels and the child's sex. The joint effects results showed that boys whose mothers had first, third or fourth quartile TG levels had a 394% increased odd of having any ADHD diagnosis (OR=4.94, 95% CI (2.84, 8.58)), as compared to girls whose mothers had second quartile TG levels. The interaction term between sex and TG was not statistically significant (OR=1.03, 95% CI (0.51, 2.07)). Table S5 shows the results of the sensitivity analyses on the joint effect of maternal TG and sex by comparing children with specialist ADHD diagnosis and neurotypical children; and the findings

were similar. Table S7 shows the results of the sensitivity analyses on the joint effect of maternal TG and sex by excluding the children whose age at the last ADHD diagnosis was under 6 years old; and the findings were also similar. These joint effects across HDL, TG, and sex are further illustrated in Figure 4-2 using MLR estimation and adjusting for the same covariates.

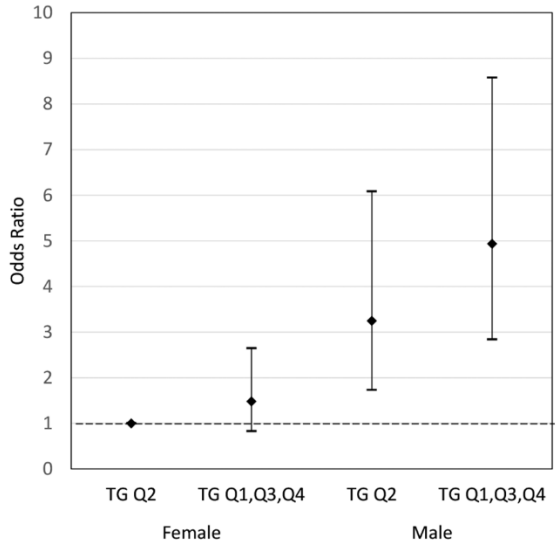
Table 4-4 The joint association of maternal TG levels and child's sex with the risk of ADHD in offspring.

Sex	Maternal TG	ADHD, No. (%)	NT, No. (%)	Adjusted OR	95% CI		P-value
Female		85 (11.4)	664 (88.6)	1.00			
Male		218 (29.9)	512 (70.1)	3.31	2.50	4.39	<0.001
Joint effects of maternal TG and sex							
Female	Q2	16 (8.8)	166 (91.2)	1.00			
	Q1, Q3, Q4	69 (12.2)	498 (87.8)	1.48	0.83	2.65	0.184
Male	Q2	42 (24.3)	131 (75.7)	3.25	1.73	6.09	<0.001
	Q1, Q3, Q4	176 (31.6)	381 (68.4)	4.94	2.84	8.58	<0.001

NT was defined as without any mental disorder diagnosis; ADHD was defined as any ADHD diagnosis; covariates included maternal age at delivery, maternal race/ethnicity, maternal education, smoking during pregnancy, intrauterine infection, parity, child's sex, mode of delivery, preterm birth, and birthweight.



(**a**)



(**b**)

Figure 4-2 (a) The odds ratio of any ADHD diagnosis across maternal HDL and child's sex groups using multiple logistic regression estimation; (b) the odds ratio of any ADHD diagnosis across maternal TG and child's sex groups using multiple logistic regression estimation.

4.5 DISCUSSION

Despite the notion that cholesterol is essential for brain health, few prospective birth cohort studies have examined the effect of maternal cholesterol on offspring's neurodevelopment. In the Boston Birth Cohort, we found a significant association between maternal cholesterol levels, particularly HDL and TG measured 24-72 hours after delivery (a proxy of peripartum maternal cholesterol levels), and ADHD risk in offspring. Furthermore, our study sheds new light on the ADHD sex difference by demonstrating that boys are more vulnerable than girls to suboptimal maternal cholesterol levels.

Our study findings were further strengthened by several aspects of our study design. We used clinician diagnosis extracted from the EMRs to define ADHD cases. More than half of the children in the ADHD group had over 3 ADHD clinician diagnoses in their EMRs. Additionally, over 80% of ADHD cases in the BBC were diagnosed by a neurodevelopmental specialist, thus, with much higher specificity and less probability of case misclassification. The results of our sensitivity analyses, which restricted ADHD cases to those with a neurobehavioral specialist diagnosis and excluded those with a diagnosis at an age younger than 6 years old, showed similar effect sizes and levels of significance as for our major findings.

While we cannot make a causality inference, and although biological mechanisms underlying the maternal HDL and child ADHD association remain to be determined, our findings are biologically plausible and in alignment with previous research. The central nervous system (CNS) is insulated from the systemic circulation by the blood-brain barrier (BBB). Cholesterol and its carrying lipoproteins in the CNS are mainly synthesized locally within the brain[58,59] while

cholesterol carried by plasma lipoproteins cannot move freely across the BBB.[37,60] Most lipoproteins found in the brain are synthesized by glial cells and astrocytes.[59] Additionally, the apolipoprotein B-containing lipoproteins, such as LDL, VLDL, and chylomicron cannot enter the brain via the BBB.[59] Nevertheless, studies have suggested that plasma-based cholesterol may still affect the integrity and function of neurons and myelin.[36,59] For instance, the discoidal apolipoprotein A-I-containing HDL particles may enter the brain through scavenger receptor class B type I (SR-BI)-mediated uptake and transcytosis.[59,61] Notably, apolipoprotein A-I, which is the major component of plasma HDL, cannot be synthesized in the CNS.[62,63] After entering the CNS, it can further collect phospholipids and unesterified cholesterol and undergo maturation into HDL-like lipoproteins in the brain.[59] In addition to small plasma HDL particles, the side-chain oxidized oxysterols, such as 27-hydroxycholesterol, can also cross the BBB.[64] Moreover, peripheral HDL, even without crossing the BBB, may still influence fetal brain development due to its potential protective effect on cerebrovascular endothelial cell function.[65] In sum, the available evidence supports our findings regarding the protective effect of higher maternal HDL levels against ADHD risk in offspring.

The mechanism underlying the actions of maternal TG appears to be different from that underlying HDL. TG cannot cross the BBB but can influence multiple hormonal transportations across the BBB. For example, TG can effectively inhibit leptin transport across the BBB.[66] Besides the beneficial role in reducing obesity risk, leptin is also a multifunctional hormone that influences many brain functions including appetite, motivation, learning, memory, and cognition.[67]

If further confirmed by future investigation, our findings may have important research, clinical and public health implications. First, our data suggest that pregnant women should maintain a

relatively higher level of HDL to meet the need for rapid fetal brain development during pregnancy and to reduce ADHD risk; this is particularly important for male fetuses. Our data indicate that the current clinical cut-off point for HDL (>50 mg/dL) for nonpregnant women, as recommended by the American Heart Association for reducing the risk of heart disease[56,57] may not be adequate for protecting against ADHD in offspring; thus, a higher cut-off point (>60 mg/dL) may be needed for identifying the fetus at risk for future ADHD. Lipid screening is not currently part of prenatal care guidelines, but it is relatively inexpensive and easily measured. Low HDL is modifiable by dietary and lifestyle changes and is treatable with pharmaceuticals.

The long-observed and striking sex difference in ADHD risk continues to be poorly understood. Our study revealed that the maternal HDL and TG effects on ADHD are most pronounced among boys. This sex differences in response to suboptimal nutritional status are also found in other chronic diseases. For example, both human and animal studies showed that male fetuses are more likely to develop hypertension in response to the mother's unfavorable nutrition and metabolic status during pregnancy.[68-72] One potential explanation is that male fetus is more vulnerable to suboptimal maternal nutrition due to their more rapid in-utero growth compared to females.[68-72]

Our study had the following limitations. First, our study only included a single measurement of maternal cholesterol, taken 24-72 hours after delivery. Ideally, a serial collection of lipid levels throughout pregnancy would best inform our hypotheses. At best, our one-time measurement reflects maternal cholesterol levels during peripartum. Second, our study used non-fasting blood samples. The values for TC and TG levels may have been inflated in non-fasting blood samples, and thus may have biased our study results towards the null. Further studies using fasting blood samples should be conducted to provide a more precise assessment of optimal TG levels during

pregnancy. Third, our study was conducted in a U.S. urban, low-income primarily minority setting; thus, this was a population at higher risk of exposure to other risk factors for ADHD. Our analyses adjusted for known risk factors of ADHD, but could not adjust for multiple parent-related factors identified in previous studies such as poor parenting,[13,14] maltreatment,[15] conflict/parent-child hostility,[23] and severe early deprivation.[24,25] Although our study sample is not representative of the general U.S. population, research in urban minority populations is limited, and our study findings help to fill in this important data gap. Finally, our adjustment for known risk factors did not include some post-natal factors that could be related to both maternal cholesterol levels and ADHD risk, such as the child's lipid levels. Although it is beyond the scope of this report, a study of the joint effects of cholesterol with other components of metabolic syndrome such as obesity, diabetes, and hypertension, may help to provide greater understanding about the associations between the maternal metabolic constellation and child neurodevelopmental outcomes. A previously published study did show that diabetes could cause a low production of brain cholesterol and its precursors, which in turn could lead to disruptions in synaptic formation and function.[73] Although our study occurred during the transition of the American Psychiatric Association's Diagnostic and Statistical Manual (DSM) from the IV to the V edition, the diagnosis of ADHD in children did not change appreciably.[74] Moreover, the DSM-V lists both ICD-9 and ICD-10 codes for transition purposes.[75]

4.6 CONCLUSIONS

In this large, prospective, predominantly U.S. urban, low income, minority birth cohort, we found that suboptimal maternal cholesterol levels, in particular, low HDL, may increase the risk of ADHD in offspring. The male fetus appears to be particularly vulnerable to suboptimal maternal cholesterol levels. Our findings raise new hypothesis for understanding of origins of

ADHD, gender differences and future targets in the prevention of ADHD, and warrant additional investigation.

4.7 SUPPLEMENTARY MATERIALS

Table S1. ICD-9 and ICD-10 codes for the diagnosis of each neurodevelopmental disorder.

Neurodevelopmental disorder	ICD-9 codes	ICD-10 codes
ASD	299.0, 299.00, 299.01, 299.8, 299.80, 299.81, 299.9, 299.90, 299.91	F84.0, F84.8, F84.9
ADHD	314.0, 314.00, 314.01, 314.1, 314.2, 314.8, 314.9	F90, F90.0, F90.1, F90.2, F90.8, F90.9
Disturbance of conduct	312.0-312.9	F91, F91.0, F91.2, F91.3, F91.8, F91.9
Delays in development	315.0-315.9	F81.0, R48.0, F81.81, F81.2, F81.89, F80.1, F80.2, H93.25, F80.4, F80.81, F80.0, F80.82, F80.89, F82, F88, F81.9, F89
Intellectual disabilities	317-317	F70, F71, F72, F73, F78, F79
Failure to thrive	783.4, 783.40, 783.41, 783.42, 783.43	R62.50, R62.51, R62.0, R62.52
Congenital anomalies	740-759.9	Q00-Q99

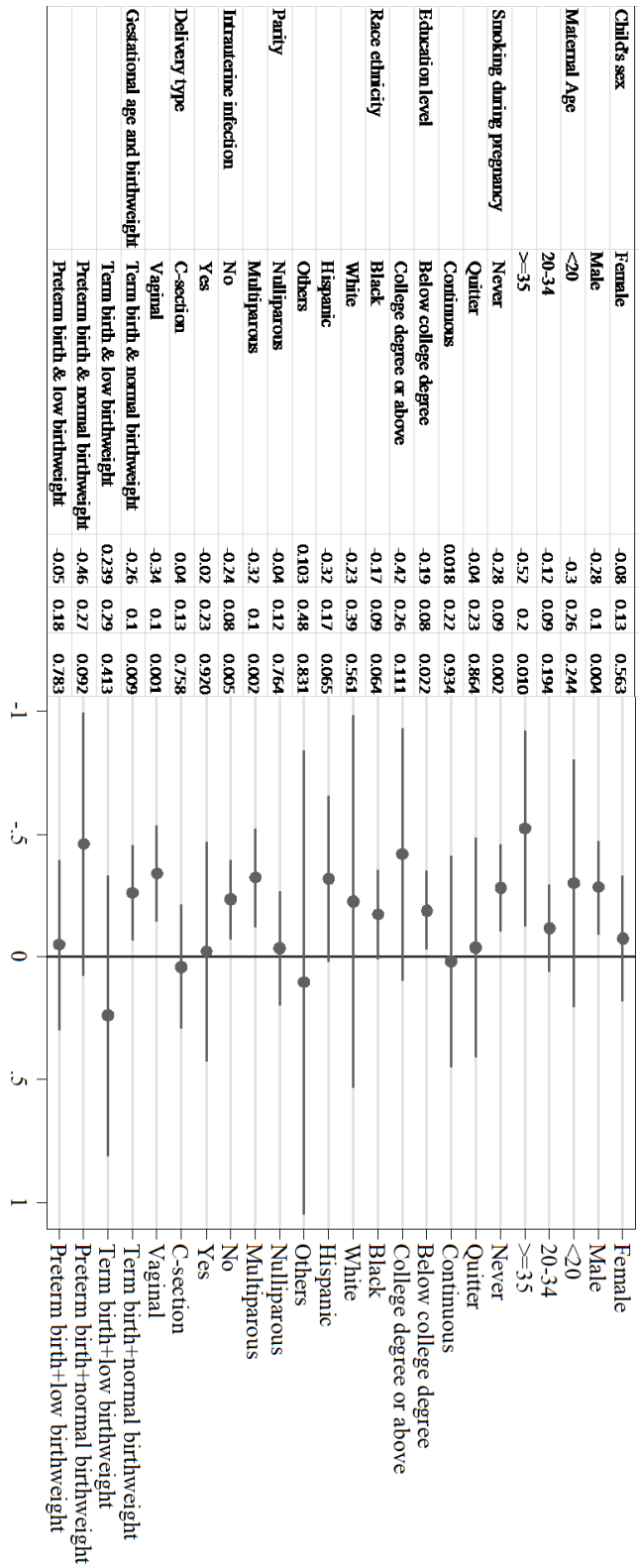
Table S2. Maternal and child characteristics for participants excluded and included in the analysis.

Variable	Total, No. (%)	Excluded, No. (%)	Included, No. (%)	P-value‡
Total	3098 (100)	1619 (52.26)	1479 (47.74)	
Maternal Age				0.209
<20	288 (9.30)	140 (8.65)	148 (10.01)	
20-34	2246 (72.50)	1166 (72.02)	1080 (73.02)	
>=35	556 (17.95)	305 (18.84)	251 (16.97)	
Education level				0.844
Below college degree	2642 (85.28)	1364 (84.25)	1278 (86.41)	
College degree or above	420 (13.56)	219 (13.53)	201 (13.59)	
Race ethnicity				<0.001
Black	1965 (63.43)	997 (61.58)	968 (65.45)	
White	227 (7.33)	153 (9.45)	74 (5.00)	
Hispanic	682 (22.01)	325 (20.07)	357 (24.14)	
Others	209 (6.75)	129 (7.97)	80 (5.41)	
Smoking during pregnancy				0.045
Never	2496 (80.57)	1267 (78.26)	1229 (83.10)	
Quitter	238 (7.68)	127 (7.84)	111 (7.51)	
Continuous	330 (10.65)	191 (11.80)	139 (9.40)	
Child's sex				0.181
Female	1529 (49.35)	780 (48.18)	749 (50.64)	
Male	1567 (50.58)	837 (51.70)	730 (49.36)	
Delivery type				0.008
C-section	1116 (36.02)	616 (38.05)	500 (33.81)	
Vaginal	1967 (63.49)	988 (61.03)	979 (66.19)	
Season of child's birth				0.697
Jan to March	721 (23.27)	388 (23.97)	333 (22.52)	
April to June	725 (23.40)	375 (23.16)	350 (23.66)	
July to September	848 (27.37)	446 (27.55)	402 (27.18)	
October to December	802 (25.89)	408 (25.20)	394 (26.64)	
Gestational age, week				<0.001
Mean (SD)	37.6(3.5)	37.2(3.8)	38.1(3.1)	
Birthweight, g				<0.001
Mean (SD)	2898.3(819.7)	2808.3(865.9)	2996.7(754.0)	

‡The p-values were obtained from chi-square test or t-test between children with and without any ADHD diagnosis.

Table S3. The stratified analysis results on the association between maternal HDL levels (every 20 mg/dL increase) and the risk of ADHD in offspring.

Variable		Beta	SE	P-value
Child's sex	Female	-0.08	0.13	0.563
	Male	-0.28	0.1	0.004
Maternal Age	<20	-0.3	0.26	0.244
	20-34	-0.12	0.09	0.194
	>=35	-0.52	0.2	0.010
Smoking during pregnancy	Never	-0.28	0.09	0.002
	Quitter	-0.04	0.23	0.864
	Continuous	0.018	0.22	0.934
Education level	Below college degree	-0.19	0.08	0.022
	College degree or above	-0.42	0.26	0.111
Race ethnicity	Black	-0.17	0.09	0.064
	White	-0.23	0.39	0.561
	Hispanic	-0.32	0.17	0.065
	Others	0.103	0.48	0.831
Parity	Nulliparous	-0.04	0.12	0.764
	Multiparous	-0.32	0.1	0.002
Intrauterine infection	No	-0.24	0.08	0.005
	Yes	-0.02	0.23	0.920
Delivery type	C-section	0.04	0.13	0.758
	Vaginal	-0.34	0.1	0.001
Gestational age and birthweight	Term birth & normal birthweight	-0.26	0.1	0.009
	Term birth & low birthweight	0.239	0.29	0.413
	Preterm birth & normal birthweight	-0.46	0.27	0.092
	Preterm birth & low birthweight	-0.05	0.18	0.783



Covariates included maternal age at delivery, maternal race/ethnicity, maternal education, smoking during pregnancy, intrauterine infection, parity, baby's gender, mode of delivery, preterm birth, birthweight.

Table S4. The Joint association of child's gender and maternal HDL levels with the risk of any specialist ADHD diagnosis.

Gender	Maternal HDL Level	ADHD, No. (%)	NT, No. (%)	OR	95% CI		P-value
Female		59 (8.16)	664 (91.84)	1.00			
Male		155 (23.24)	512 (76.76)	3.26	2.35	4.53	<0.001
	Maternal HDL effects within gender						
Female	Q4	9 (4.59)	187 (95.41)	1.00			
	Q3	19 (9.95)	172 (90.05)	2.24	0.98	5.16	0.057
	Q2	17 (10.83)	140 (89.17)	2.59	1.10	6.09	0.029
	Q1	14 (7.82)	165 (92.18)	1.65	0.68	3.99	0.266
Male	Q4	29 (19.86)	117 (80.14)	1.00			
	Q3	28 (17.50)	132 (82.50)	0.80	0.44	1.46	0.474
	Q2	48 (24.24)	150 (75.76)	1.21	0.70	2.07	0.494
	Q1	50 (30.67)	113 (69.33)	1.65	0.95	2.86	0.073
	Joint effects of maternal HDL and gender						
Female	> median	28 (7.24)	359 (92.76)	1.00			
	≤ median	31 (9.23)	305 (90.77)	1.24	0.72	2.12	0.440
Male	> median	57 (18.63)	249 (81.37)	2.87	1.77	4.67	<0.001
	≤ median	98 (27.15)	263 (72.85)	4.44	2.81	7.02	<0.001

NT was defined as free of any mental disorder diagnosis; ADHD was defined as any specialist ADHD diagnosis; covariates included maternal age at delivery, maternal race/ethnicity, maternal education, smoking during pregnancy, intrauterine infection, parity, baby's gender, mode of delivery, preterm birth, birthweight.

Table S5. The Joint association of child's gender and maternal TG levels with the risk of any specialist ADHD diagnosis.

Gender	Maternal TG Level	ADHD, No.(%)	NT, No.(%)	OR	95% CI		P-value
Female		59 (8.16)	664 (91.84)	1.00			
Male		155 (23.24)	512 (76.76)	3.31	2.39	4.59	<0.001
	Maternal TG effects within gender						
Female	Q1	14 (8.05)	160 (91.95)	1.24	0.55	2.77	0.605
	Q2	13 (7.26)	166 (92.74)	1.00			
	Q3	13 (7.30)	165 (92.70)	1.01	0.45	2.27	0.984
	Q4	19 (9.90)	173 (90.10)	1.50	0.68	3.31	0.310
Male	Q1	45 (26.16)	127 (73.84)	1.54	0.90	2.65	0.116
	Q2	30 (18.63)	131 (81.37)	1.00			
	Q3	40 (24.10)	126 (75.90)	1.39	0.80	2.42	0.242
	Q4	40 (23.81)	128 (76.19)	1.40	0.79	2.49	0.245
	Joint effects of maternal TG and gender						
Female	Q2	13 (7.26)	166 (92.74)	1.00			
	Q1, Q3, Q4	46 (8.46)	498 (91.54)	1.17	0.61	2.24	0.631
Male	Q2	30 (18.63)	131 (81.37)	2.87	1.43	5.76	0.003
	Q1, Q3, Q4	125 (24.70)	381 (75.30)	4.04	2.20	7.41	<0.001

NT was defined as free of any mental disorder diagnosis; ADHD was defined as any specialist ADHD diagnosis; covariates included maternal age at delivery, maternal race/ethnicity, maternal education, smoking during pregnancy, intrauterine infection, parity, baby's gender, mode of delivery, preterm birth, birthweight.

Table S6. The Joint association of child's gender and maternal HDL levels with the risk of any ADHD diagnosis (last diagnosis older than 6 years old).

Gender	Maternal HDL Level	ADHD, No.(%)	NT, No.(%)	OR	95% CI		P-value
Female		51 (7.13)	664 (92.87)	1.00			
Male		132 (20.50)	512 (79.50)	3.22	2.27	4.57	<0.001
	Maternal HDL effects within gender						
Female	Q4	8 (4.10)	187 (95.90)	1.00			
	Q3	17 (8.99)	172 (91.01)	2.19	0.91	5.27	0.081
	Q2	14 (9.09)	140 (90.91)	2.30	0.92	5.76	0.075
	Q1	12 (6.78)	165 (93.22)	1.49	0.58	3.83	0.411
Male	Q4	23 (16.43)	117 (83.57)	1.00			
	Q3	24 (15.38)	132 (84.62)	0.85	0.44	1.61	0.612
	Q2	40 (21.05)	150 (78.95)	1.23	0.68	2.20	0.492
	Q1	45 (28.48)	113 (71.52)	1.83	1.02	3.31	0.043
	Joint effects of maternal HDL and gender						
Female	> median	25 (6.51)	359 (93.49)	1.00			
	≤ median	26 (7.85)	305 (92.15)	1.14	0.64	2.03	0.662
Male	> median	47 (15.88)	249 (84.12)	2.63	1.57	4.41	<0.001
	≤ median	85 (24.43)	263 (75.57)	4.26	2.63	6.90	<0.001

NT was defined as free of any mental disorder diagnosis; ADHD was defined as any ADHD diagnosis; covariates included maternal age at delivery, maternal race/ethnicity, maternal education, smoking during pregnancy, intrauterine infection, parity, baby's gender, mode of delivery, preterm birth, birthweight.

Table S7. The Joint association of child's gender and maternal TG levels with the risk of any ADHD diagnosis (last diagnosis older than 6 years old).

Gender	Maternal TG Level	ADHD, No.(%)	NT, No.(%)	OR	95% CI		P-value
Female		51 (7.13)	664 (92.87)	1.00			
Male		132 (20.50)	512 (79.50)	3.25	2.30	4.61	<0.001
	Maternal TG effects within gender						
Female	Q1	12 (6.98)	160 (93.02)	1.16	0.49	2.74	0.727
	Q2	12 (6.74)	166 (93.26)	1.00			
	Q3	11 (6.25)	165 (93.75)	0.90	0.38	2.14	0.817
	Q4	16 (8.47)	173 (91.53)	1.29	0.56	2.98	0.546
Male	Q1	40 (23.95)	127 (76.05)	1.71	0.96	3.05	0.070
	Q2	24 (15.48)	131 (84.52)	1.00			
	Q3	31 (19.75)	126 (80.25)	1.32	0.72	2.43	0.371
	Q4	37 (22.42)	128 (77.58)	1.58	0.85	2.91	0.146
	Joint effects of maternal TG and gender						
Female	Q2	12 (6.74)	166 (93.26)	1.00			
	Q1, Q3, Q4	39 (7.26)	498 (92.74)	1.05	0.53	2.07	0.892
Male	Q2	24 (15.48)	131 (84.52)	2.48	1.18	5.20	0.016
	Q1, Q3, Q4	108 (22.09)	381 (77.91)	3.68	1.95	6.93	<0.001

NT was defined as free of any mental disorder diagnosis; ADHD was defined as any ADHD diagnosis; covariates included maternal age at delivery, maternal race/ethnicity, maternal education, smoking during pregnancy, intrauterine infection, parity, baby's gender, mode of delivery, preterm birth, birthweight.

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Chapter 5 AIM 2: A PROSPECTIVE BIRTH COHORT STUDY ON EARLY CHILDHOOD LEAD LEVELS AND ATTENTION DEFICIT HYPERACTIVITY DISORDER: NEW INSIGHT ON SEX DIFFERENCES

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5.1 ABSTRACT

Objective: To investigate the prospective associations between early childhood lead exposure and subsequent risk of ADHD in childhood and its potential effect modifiers.

Study design: We analyzed data from 1479 mother-infant pairs (299 ADHD, 1180 neurotypical) in the Boston Birth Cohort (BBC). The child's first blood lead measurement and physician-diagnosed ADHD was obtained from electronic medical records. Graphic plots and multiple logistic regression were employed to examine dose-response association between lead exposure and ADHD and potential effect modifiers, adjusting for pertinent covariables.

Results: Our findings show that 8.9% of BBC children had elevated lead levels (5-10μg/dL) in early childhood, which was associated with a 66% increased risk of ADHD (OR=1.66, 95%CI:1.08, 2.56). Among boys, the association was significantly stronger (OR: 2.49, 95%CI:1.46, 4.26); in girls, the association was largely attenuated (p-value for sex-lead interaction: 0.017). The odds ratio of ADHD associated with elevated lead levels among boys was reduced by half if mothers had adequate high-density lipoprotein (HDL) levels compared to low HDL, or if mothers had low stress compared to high stress during pregnancy.

Conclusions: Elevated early childhood blood lead levels increased the risk of ADHD. Boys were more vulnerable than girls at a given lead level. This risk of ADHD in boys was reduced by half if the mother had adequate HDL levels or low stress. These findings shed new light on the sex difference in ADHD and point to opportunities for early risk assessment and primary prevention of ADHD.

5.2 INTRODUCTION

Lead is a recognized environmental toxin.[1-5] Since the removal of lead from paints and gasoline and the adoption of other environmental safety measures, environmental lead exposure has declined significantly over the past decades.[6] However, exposure to low lead levels continues to be widespread in the general U.S. population, particularly in urban low-income populations.[7]

To date, critical questions remain regarding the role of early life lead exposure in the development of ADHD.[8-10] There are multiple drawbacks in the existing studies. First, they used cross-sectional designs.[2,3,5] Lead measurement occurred either simultaneous to or after the ADHD diagnosis, thus the temporal relationship between lead exposure and ADHD could not be established. Second, despite the well-observed higher likelihood of ADHD in males compared to females, few studies have investigated if lead affects boys and girls differently.[2] Additionally, most studies on ADHD only have examined lead exposure late in childhood (mean age of measurement ranging from 7 to 14 years).[10] Lastly, prior studies did not consider potential modifiers of the lead-ADHD association, which is necessary both in terms of understanding the etiology of ADHD and informing intervention strategies. [11]

In this study, we sought to examine the association between early childhood lead exposure and development of ADHD using a prospective birth cohort design. We were particularly interested in identifying early life factors that could modify lead-ADHD associations in a predominantly urban low-income minority population in the U.S. We hypothesized that there is a significant association between early childhood blood lead levels and the risk of developing ADHD. Motivated by findings from our previous work, we further hypothesized that this association might be modified by prenatal factors, including child sex, maternal high-density lipoprotein (HDL) levels[12] and degree of stress during pregnancy.

5.3 METHODS

This study used data from the Boston Birth Cohort (BBC), which recruited mother-infant pairs at birth from Boston Medical Center (BMC) since 1998, using a rolling enrollment. Details of the BBC recruitment have been published previously.[13,14] Eligible mothers delivered a single live birth at Boston Medical Center (BMC). Pregnancies resulting from in vitro fertilization, multiple-gestation pregnancies, deliveries induced by maternal trauma, or newborns with substantial congenital disabilities were not eligible for enrollment. BBC mother-infant pairs who continued to receive pediatric primary care at BMC were enrolled in a postnatal follow-up study.[13,15,16] The Institutional Review Board (IRB) of Boston University Medical Center and the IRB of Johns Hopkins Bloomberg School of Public Health approved the BBC study. Informed consent was obtained from each participant under the IRB approved protocol.

There were 3098 mother-infant pairs enrolled in the postnatal follow-up study at BMC at the time of the study. Our study sample excluded participants who had missing data for lead measurements and key covariates. We further excluded those with lead measurement after ADHD diagnosis, incorrect lead measurement dates, lead measurement age older than 4 years, and a lead level higher than 10 μg/dL (to focus on the effects in the low lead exposure range). Since many neurodevelopmental disorders may have common risk factors, we excluded those with neurodevelopmental disorders diagnoses other than ADHD (Figure 5-1). Our final analyses consisted of 1479 mother-infant pairs, who were enrolled at birth from 1998 to 2013 and followed-up prospectively until the end of 2016 (Figure 5-1). Additionally, these mother-infant pairs consisted of 299 children with ADHD and 1176 neurotypical children (Figure 5-1).

After recruiting mothers within 24 to 72 hours after delivery, a standard questionnaire interview was used to collect data on maternal demographic characteristics, smoking status, and stress

during pregnancy. Stress during pregnancy was defined according to the response to the following question: “How would you characterize the amount of stress in your life during pregnancy?” The responses to the question included: “not stressful,” “average stressful,” and “very stressful.” A medical abstraction form was used to review participants’ medical records and collect clinical-related data including parity, pregnancy-related complications, intrauterine infection, and birth outcomes such as gestational age and birthweight. Since 2003, electronic medical records (EMRs) were implemented for routine clinical data collection at BMC, including both well-child and specialty medical visits. For each primary care visit, the EMRs contain the primary and secondary diagnoses from the International Classification of Diseases, Ninth Revision (ICD-9) (before October 1, 2015) and ICD-10 (after October 1, 2015). In this study, we extracted EMR data until the end of 2016.

Maternal plasma HDL levels and lead levels in red blood cells were measured using non-fasting blood samples obtained between 24 to 72 hours after delivery. The child postnatal screening records of blood lead levels were obtained from the EMRs. The low detection limit of lead was 2 μg/dL; 659 children had blood lead levels below this threshold. The below threshold lead level was coded as 1 μg/dL when lead was analyzed as a continuous variable. For each child with repeated measurements of lead levels, the level measured at the earliest age was selected for analysis in this study.

In our study, the “ADHD” was defined as having any of the following ICD-9 codes: [314.0, 314.00, 314.01, 314.1, 314.2, 314.8, and 314.9], or any of the following ICD-10 codes: [F90.0, F90.1, F90.2, F90.8, and F90.9] as documented in the child’s EMRs. The “neurotypical (NT)” was defined as not having any diagnosis of ASD, ADHD, conduct disorders, developmental delays, intellectual disabilities, failure to thrive, and/or congenital anomalies. The ICD-9 and

ICD-10 codes for these neurodevelopmental disorders diagnosis are listed in

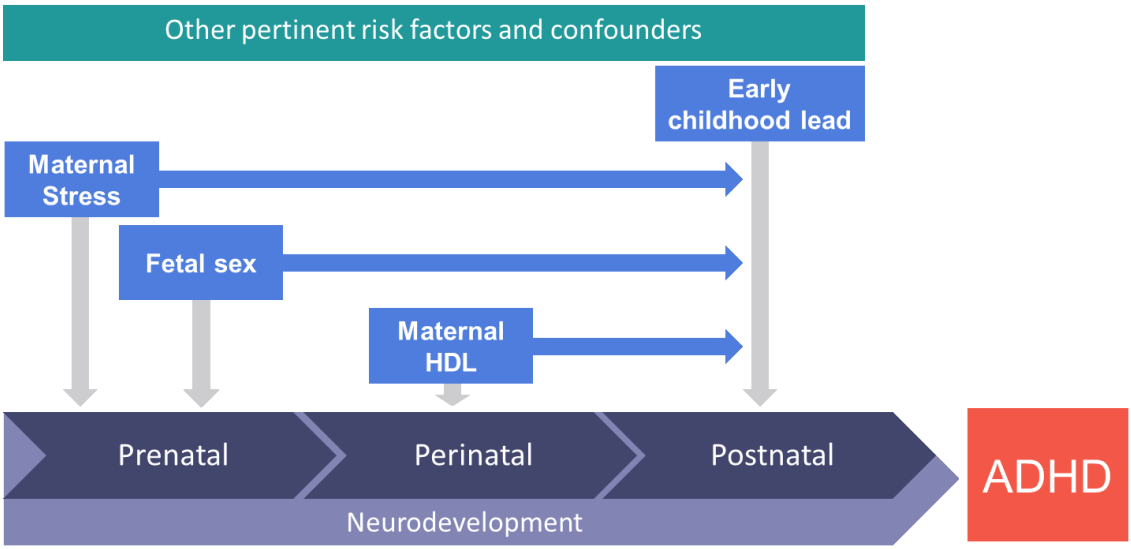

Figure 5-6 online. Conceptual framework for the prospective association of early childhood lead exposure and development of ADHD in childhood from a life course perspective.

Table 5-1.

The characteristics of the study sample for the ADHD and the NT groups were compared using t-tests for continuous variables and χ^2 tests for categorical variables. Variables with a $p < 0.05$ were included in the subsequent multivariate logistic regression (MLR) analyses as covariates. The key predictor analyzed in this study was the child's lead level, which was natural log-transformed to approximate the normal distribution. Lead level was also analyzed as a binary (5 μg/dL as the cutoff) and categorical variable (<2 μg/dL, 2-4 μg/dL, and 5-10 μg/dL) based on cut points used in previous studies and CDC guidelines.[2,8] Maternal HDL levels were analyzed as a binary variable cut at the median (60.7 mg/dL).[12] Maternal stress during pregnancy was converted from a three-category variable (not stressful, average, very stressful) into a binary variable (not stressful vs. stressful) for analysis.[17]

We conducted multiple logistic regression (MLR) to examine the association between early childhood lead level and the risk of having ADHD, both categorically and continuously, adjusting for maternal age at delivery, maternal race/ethnicity, maternal education, smoking during pregnancy, intrauterine infection, parity, child's sex, mode of delivery, preterm birth, and birthweight. Gender-stratified analyses and the joint effect of the child's sex with lead levels on the risk of ADHD diagnosis were tested using MLR, and adjusted for the same covariates except for child's sex. The interaction between child's sex and lead level on the risk of ADHD was then tested using MLR and adding the interaction term into the model while adjusting for the same covariates. We further tested the joint associations among maternal HDL level, maternal stress during pregnancy, and early childhood lead level with the risk of ADHD diagnosis both across and within the child's sex groups, adjusting for the same covariates except for child's sex. For the sensitivity analyses, we repeated the above analyses within three subsets. One analysis did

not exclude the samples with other neurodevelopmental disorders. One analysis included the samples with early childhood lead levels measured at age ≤ 2 years. The other analysis further adjusted for maternal lead levels (treated as a binary variable cut at 5 μg/dL) measured right after delivery, using the samples that had measurements for both maternal and early childhood lead levels. All analyses were performed using STATA® version 14.0 software (Stata Corporation, College Station, TX, USA).

5.4 RESULTS

Data from 1,479 mother-child pairs were analyzed in this study. The median age of the study children by our latest EMR extraction date (31 December 2016) was 9.6 years (inter-quartile range 7.4 to 12.5 years). Among them, 299 children (13.9% (before removing children with neurodevelopmental disorders diagnoses other than ADHD)) had a physician diagnosis of ADHD, and 131 children (8.9%) had lead levels of 5-10 μg/dL; 9.4% in boys and 8.3% in girls. The median age of the first diagnosis of ADHD was six years. The median age of the first lead measurement was 0.84 years (inter-quartile range 0.77 to 1.03 years). The comparison results of major characteristics between excluded and included samples indicate that the included sample had less exposure to multiple risk factors, such as less educational attainment, shorter gestational age, and lower birthweight (Table 5-2). Table 5-3 presents the bivariate comparisons of maternal and child characteristics between the "ADHD" and "NT" groups. Mothers of children with any ADHD diagnosis were more likely to have less than a college degree, ever smoked before or during pregnancy, delivered with a C-section, had high stress during pregnancy, and low HDL compared with the NT group. Children with an ADHD diagnosis were more likely to be male, born prematurely, and had low birthweight, compared with the NT group.

Table 5-4 shows the results for both the crude and adjusted associations of early childhood lead levels with the risk of ADHD diagnosis. When lead levels were analyzed as three categories, compared to those with < 2 μg/dL lead levels, the adjusted OR for children with 2-4 μg/dL and 5-10 μg/dL lead levels was 1.08, 95% CI (0.81, 1.44)) and 1.73, 95% CI (1.09, 2.73), respectively. The natural log-transformed linear trend of lead levels was significantly associated with an increased risk of ADHD diagnosis (adjusted OR=1.25, 95% CI (1.01, 1.56)). When lead was analyzed as a binary variable, children with 5-10 μg/dL lead levels had 66% increased odds of having any ADHD diagnosis as compared to children with < 5 μg/dL lead levels (adjusted OR=1.66, 95% CI (1.08, 2.56)). A test of interaction between sex and lead level (binary) was statistically significant (p-value for interaction was 0.017), which was further explored as described below.

Figure 5-2 shows that the crude percentage of ADHD diagnosis was higher among boys compared to girls within each lead exposure level. Figure 5-3 (Lowess plot) shows a positive linear trend with the risk of ADHD diagnosis among boys, while the trend was flat among girls. Table 5-5 shows the adjusted associations between child's lead levels and the risk of any ADHD diagnosis stratified by the child's sex and the joint effect of child's lead levels and the child's sex on ADHD risk. When simultaneously considering child sex and lead levels, boys with 5-10 μg/dL lead levels had 648% increased odds of having any ADHD diagnosis (adjusted OR=7.48, 95% CI (4.29, 13.02)), compared to girls with < 5 μg/dL lead levels. Table 5-6 shows the results of the sensitivity analyses on the joint effects of early childhood lead exposure on ADHD diagnosis comparing to no ADHD group; and the findings were similar to those shown in Table 5-5. Table 5-7 shows the results of the sensitivity analyses on the joint effects of early childhood lead exposure by restricting the analysis to children with lead measurements at age ≤ 2 years; and

the findings were similar. Table 5-8 shows the results of the sensitivity analyses on the joint effects of early childhood lead exposure by further adjusting for maternal lead measurements right after delivery; and the findings were also similar. Figure 5-4 displays the crude relationship between maternal lead and early childhood lead levels, which do not show correlation.

Table 5-9 shows the joint association of child's sex, maternal HDL level, maternal stress during pregnancy, and early childhood lead measurement with the risk of ADHD diagnosis. Compared to girls with low lead levels (< 5 μg/dL) and adequate maternal HDL levels (> 60.7 mg/dL), boys with high lead levels and lower maternal HDL levels had 903% increased odds of having any ADHD diagnosis (OR=10.03, 95% CI (4.38, 22.97)). This increased risk was reduced by more than half if the mother had adequate maternal HDL levels (OR=4.77, 95% CI (1.76, 12.90)).

Similarly, boys with high lead levels and high maternal stress during pregnancy had 1394% increased odds of having any ADHD diagnosis, compared to girls with low lead levels and low maternal stress (OR=14.94, 95% CI (6.88, 32.41)). This increased risk was reduced by more than half if the mother had low maternal stress during pregnancy (OR=6.10, 95% CI (2.18, 17.08)). Figure 5-5 shows the percentage of ADHD diagnosis by maternal HDL level, maternal stress during pregnancy, and early childhood lead measurement groups among boys. The within sex group comparison together with the findings shown in Figure 5-5 also indicate that the risk of ADHD diagnosis was lower for those born to mothers with adequate maternal HDL and low maternal stress during pregnancy given the same level of lead exposures for boys.

Table 5-10 and Table 5-11 show the additional sensitivity analyses to examine the independent and joint effect of gestational age and birthweight with early childhood lead exposure on ADHD. There was no indication of interaction between early childhood lead exposures and gestational age on ADHD, and the same is true for birthweight.

5.5 DISCUSSION

To our knowledge, this is the first large prospective birth cohort study to demonstrate a positive association between early childhood lead exposure and risk of developing ADHD in a U.S. predominantly urban, low-income minority cohort. Our findings are consistent with previous research regarding the effects of lead exposure on the risk of ADHD, although existing data were mostly cross-sectional in design.[10] Our study has contributed the following new information to the field.

We revealed a significant sex difference in the association between lead exposure and the risk of ADHD. When we stratified the MLR analysis by sex, we found no significant association between elevated lead levels (5-10 μg/dL) and ADHD among girls, but a strong association with ADHD among boys. This finding cannot be explained by a sex difference in lead exposure. In fact, the lead level distribution was similar between girls and boys: the percentages of elevated lead levels (5-10 μg/dL) were 8.3% and 9.4% for girls and boys, respectively, which is consistent with previous studies, indicating that there is no sex difference in blood lead levels among young children.[18,19] Moreover, a small prospective study (n=195) also found similar boy-specific lead effects, which revealed that both prenatal and childhood average lead levels (<78 months) were associated with attention factor of neuropsychological measures only within boys.[20] Taken together, the findings from previous studies and ours suggest that boys are more vulnerable than girls to the adverse effects of early life low level lead exposures.

In the context of early childhood lead exposure and ADHD in boys, we identified several potential protective factors that may attenuate the lead-ADHD association. We found that high maternal HDL levels and low maternal stress during pregnancy could partially counteract the increased odds of ADHD associated with early life lead exposure in boys. Except for the

subgroups with sample sizes that were too small, our findings showed that high maternal HDL levels and low maternal stress during pregnancy could reduce the odds of ADHD by more than half compared to their counterparts. Conversely, boys born to mothers with low HDL and high stress during pregnancy were more vulnerable to the adverse effects of lead exposure on the risk of ADHD, given their level of lead exposure.

Within a subset of samples with both early childhood and maternal blood lead levels measured at delivery, we further explored if the association we identified could be altered if we further adjusted for maternal lead levels. We found that the sex-specific relationship between the early childhood lead level and the risk of ADHD remained even after adjusting for maternal lead levels. Furthermore, we found no significant correlation between maternal lead levels and early childhood lead levels. This finding is consistent with the correlation results reported in a previous study, which measured the lead levels of nearly 100 mother-child pairs from Montevideo, Uruguay.[21]

Although lead exposure in children has declined in the U.S. since the ban of leaded gasoline,[9] lead exposure has remained a significant risk factor for certain segments of children for two major reasons. First, accumulating evidence has revealed that even low-level lead exposures still have adverse effects on neurodevelopment. As a result, the identified blood lead level of concern for the fetus or young child has been revised many times from 40 μg/dL to the current CDC guidelines which specify that there is no safe level of exposure.[6,9,22-24] Indeed, more recent research lends even further support for the CDC guidelines that there is no threshold for the adverse health effects of lead exposure.[8,9]

Second, while any exposure is considered unsafe, there still are profound disparities in who is more exposed to lead in the U.S. A study conducted in South Carolina showed that the soil lead

concentration was much higher in urban areas because of more potential lead sources based there, such as road networks and industries.[25] Related to this, low-income and racial/ethnic minority individuals, including children aged younger than 6 years, have a much higher risk of lead exposure because they are the ones who tend to live in these urban areas and in neighborhoods that are closer to these lead sources.[25] Additionally, children are more biologically susceptible to the toxic effects of lead compared to adults due to their much higher gastrointestinal bioaccessibility to lead.[26] In the BBC, about 9% of children had blood lead levels above 5 μg/dL. Consistently, many other studies have also found that low-income minority populations are more likely to live in highly lead-contaminated areas and have higher median blood lead concentrations, particularly among children.[25,27-29] Thirdly, our data suggest that urban low-income populations may be more vulnerable to lead toxicity due to other risk factors including maternal dyslipidemia and high stress, as we demonstrated in this study. As such, lead remains a significant public health concern, especially for poor pregnant mothers and their children living in lead-contaminated areas.

The exact neurotoxicological pathways by which lead exposure affects ADHD risk remain unclear. Current biological studies suggest that lead disrupts the hippocampal region of the brain through interaction with the N-methyl-D-aspartate receptor both synaptically and extra-synaptically.[30] The lead-induced damage in the developing brain preferentially occurs in the prefrontal cortex (PFC), hippocampus, and cerebellum,[31-33] and not surprisingly the brains of individuals with ADHD also show a reduction in the volume and activity of the PFC and cerebellum.[34] The neurotoxicity induced by lead depends on both age[35] and lead exposure level.[36] Compared to adults, children not only absorb more lead, but they do so directly into the brain due to a higher potential intake from the environment and an underdeveloped blood-brain

barrier.[37,38] Moreover, the fetus is also at a high risk of lead exposure via transplacental transfer during pregnancy, which is a particularly sensitive period for fetal central nervous system development.[39,40]

The striking sex difference in ADHD is well-observed but poorly understood. An animal study showed that lead toxicity behaves differently in males and females.[41] After treating with lead acetate, female rabbits showed an earlier and higher increase in Zn protoporphyrin (a screening marker for lead poisoning[42]) than males.[41] However, to date, no study has explained the biological mechanism behind male dominance in ADHD and sex differences in lead neurotoxicity in humans. Some have postulated that the sex difference in the lead-ADHD association might be explained by the sex difference in lead metabolism.[43,44] Over 90% of lead is stored in bone with an average of a 10 year half-life.[43] A study on occupational lead exposure showed that the rate of bone lead release for women is slower than it is for men.[44] Moreover, a study of Swedish twins showed that the genetic factors related to lead uptake and storage explain nearly 60% of the blood lead levels among nonsmoking women. In contrast, nonsmoking men's blood lead levels mainly reflect environmental exposures. One study identified three polymorphic genes that influence lead accumulation and toxicokinetics.[45] These genes are responsible for encoding enzyme delta-aminolevulinic acid dehydratase (ALAD), the vitamin D receptor (VDR), and the human hemochromatosis (HFE) protein.[45] It is possible that the weak association between blood lead levels and risk of ADHD among girls might be due to a sex difference in the frequency and expression of these genes.[46]

Our study had several limitations. First, the early childhood blood lead measurements obtained during routine pediatric screening could not precisely assess the very lowest lead exposures due to a detection limit of 2 μg/dL. As a result, we could not examine the dose-response relationship

between lead levels below 2 μg/dL and ADHD.[2] Second, the EMR data used in our study (2003-2016) spanned the transition of the diagnostic and statistical manual (DSM) from the DSM-IV-TR to the DSM-V edition.[47] However, this transition did not affect our ADHD determination[48] in children, since the main changes from DSM-IV-TR to DSM-V were more relevant to the adult diagnostic criteria. Third, missing information on lead levels and major covariates can cause selection bias if the missing is not at random. However, we compared children with and without lead measurements and did not find a systematic difference among major covariates. Lastly, although we adjusted for multiple major risk factors identified in previous studies, data related to multiple family-related factors such as poor parenting,[49,50] maltreatment,[51] conflict/parent-child hostility,[52] and severe early deprivation[53,54] were not available in BBC. Thus, we could not wholly eliminate potential residual confounding in this study. However, those factors are less likely to share the same biological mechanism in their effect on neurodevelopment. Thus, would not anticipate any impact on the association between early childhood lead and risk of ADHD even if it was possible to adjust for those factors.

5.6 CONCLUSIONS

In this urban, low income, high-risk minority birth cohort, we found that about 9% of children had elevated early childhood blood lead levels (5-10 μg/dL), and that these moderately elevated levels were associated with an increased risk of ADHD in childhood, in particular, among boys. As illustrated in Figure 6, while early childhood lead levels are our primary exposure of interest, multiple pre-, peri- and post-natal risk factors of ADHD, such as child's sex, maternal HDL, and maternal stress during pregnancy could also affect the risk of ADHD either in the context of risk factors, effect modifiers or confounders (Figure 6; online). In our study population, boys were at much higher risk of ADHD than girls, given the same levels of lead exposure. Furthermore, our

analysis suggested that maternal factors could alter this risk. For example, the risk could be reduced by more than half if the mother had adequate HDL levels or had low stress during pregnancy. These findings shed new light on the sex difference seen in ADHD and its effect modifiers, and, if confirmed, may offer new opportunities for early risk assessment and primary prevention of ADHD.

5.7 TABLE AND FIGURES

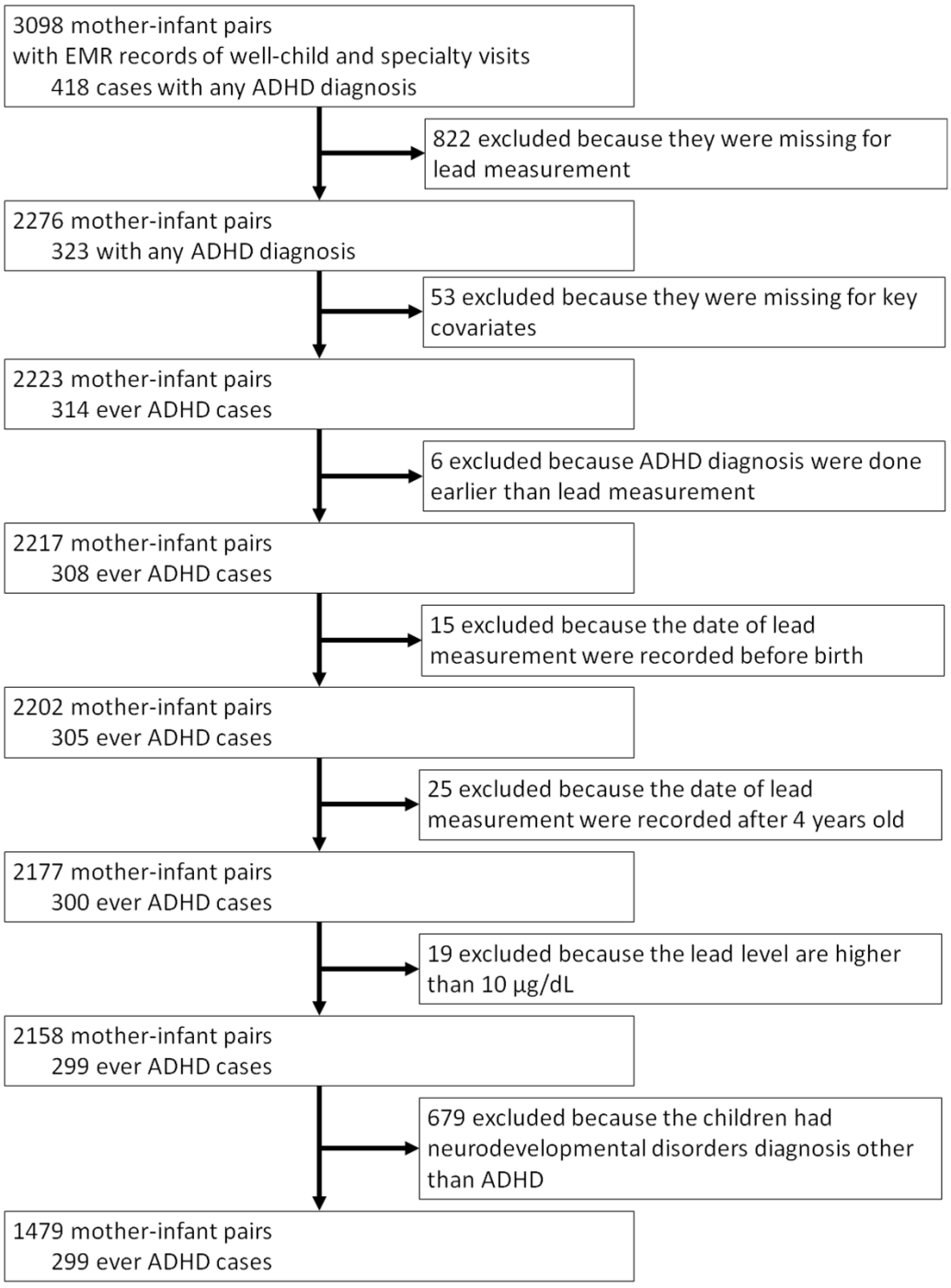

Figure 5-1 online. Flowchart of the Child Health Study and the sample included in the analysis.

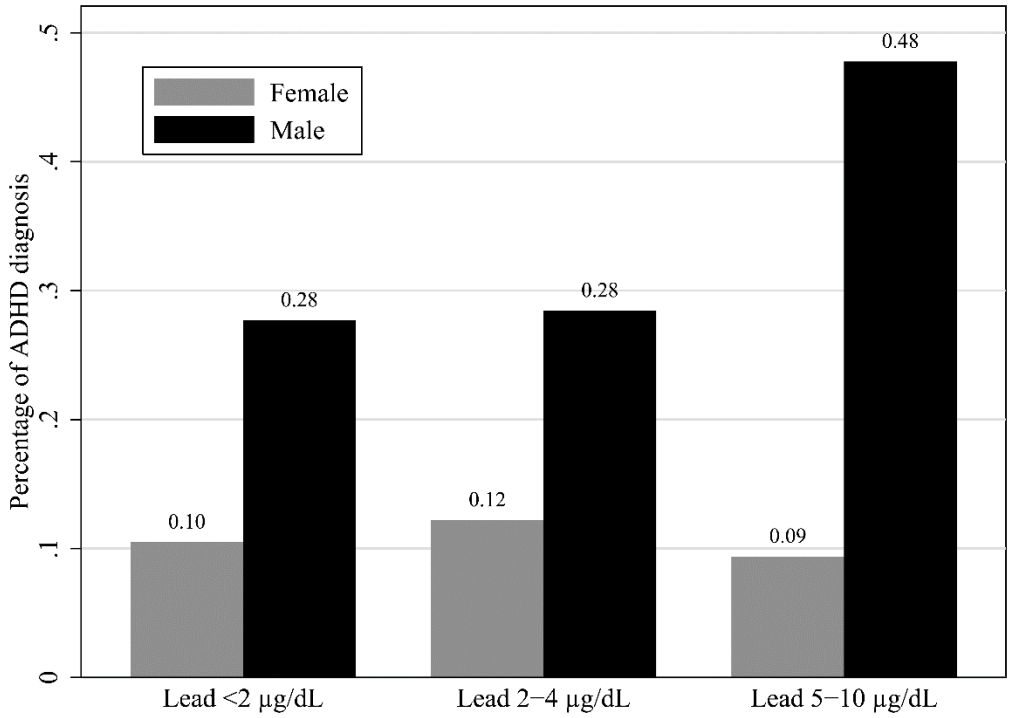

Figure 5-2 The percentage of ADHD diagnosis in the BBC children, stratified by child's sex and early childhood lead measurement groups.

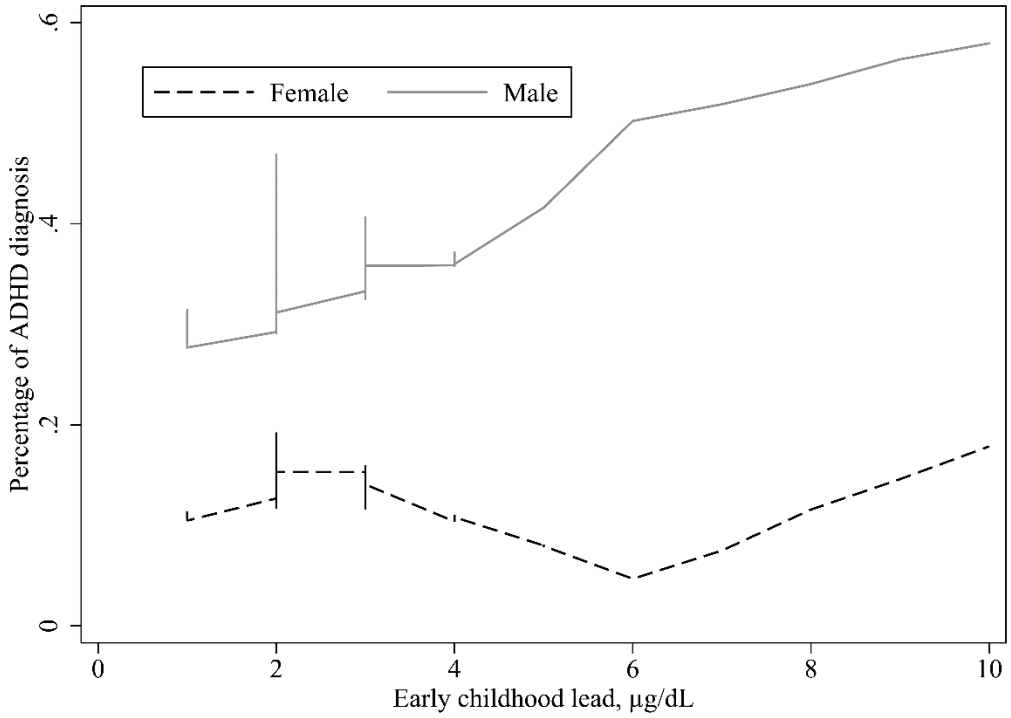

Figure 5-3 online. The crude relationship between early childhood lead exposure and ADHD diagnosis by child's sex.

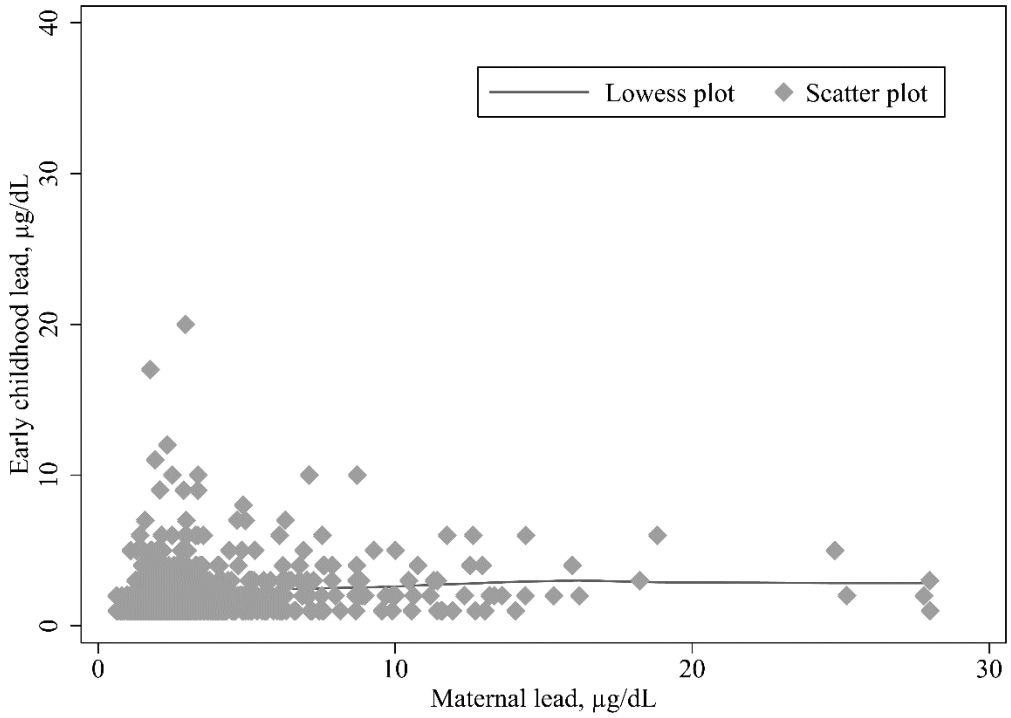

Figure 5-4 online. The crude relationship between maternal lead levels and early childhood lead levels.

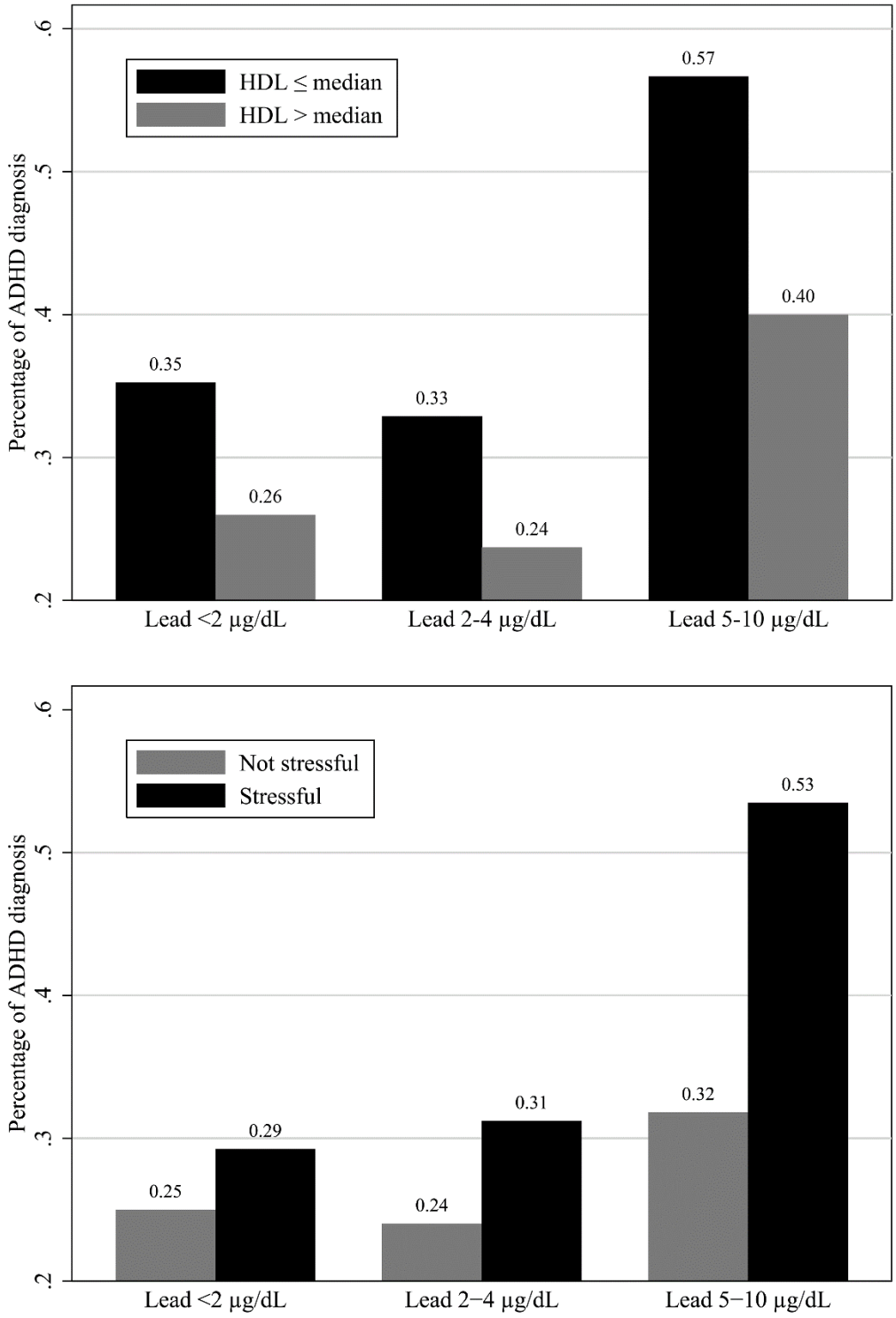

Figure 5-5 online. The percentage of ADHD diagnosis within maternal HDL level (top), maternal stress during pregnancy (bottom), and early childhood lead measurement groups among boys.

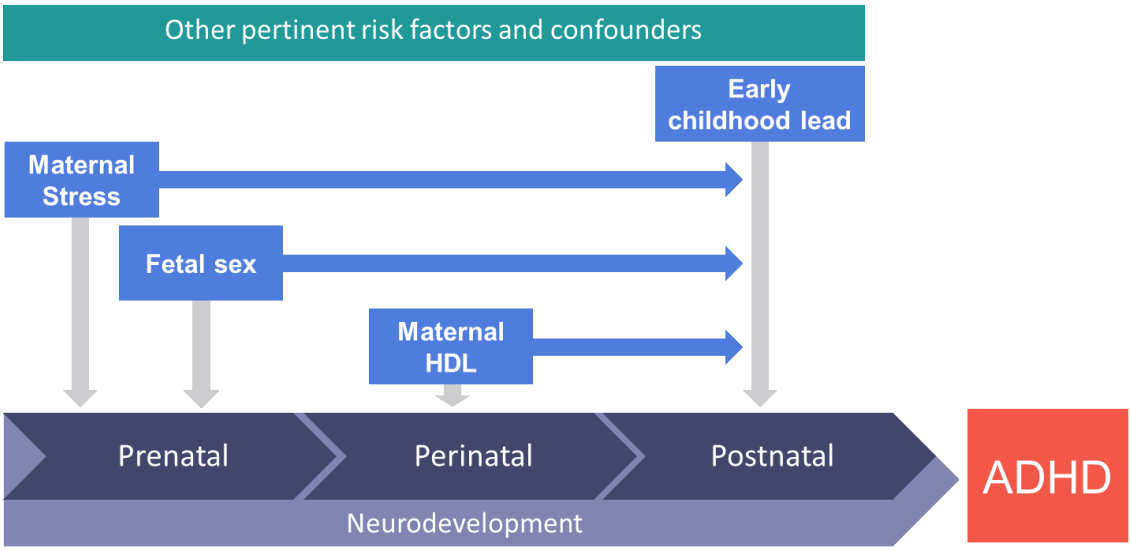

Figure 5-6 online. Conceptual framework for the prospective association of early childhood lead exposure and development of ADHD in childhood from a life course perspective.

Table 5-1 online. List of ICD-9 and ICD-10 codes for the diagnosis of each neurodevelopmental disorder.

Neurodevelopmental disorder	ICD-9 codes	ICD-10 codes
ASD	299.0, 299.00, 299.01, 299.8, 299.80, 299.81, 299.9, 299.90, 299.91	F84.0, F84.8, F84.9
ADHD	314.0, 314.00, 314.01, 314.1, 314.2, 314.8, 314.9	F90, F90.0, F90.1, F90.2, F90.8, F90.9
Disturbance of conduct	312.0-312.9	F91, F91.0, F91.2, F91.3, F91.8, F91.9
Delays in development	315.0-315.9	F81.0, R48.0, F81.81, F81.2, F81.89, F80.1, F80.2, H93.25, F80.4, F80.81, F80.0, F80.82, F80.89, F82, F88, F81.9, F89
Intellectual disabilities	317-317	F70, F71, F72, F73, F78, F79
Failure to thrive	783.4, 783.40, 783.41, 783.42, 783.43	R62.50, R62.51, R62.0, R62.52
Congenital anomalies	740-759.9	Q00-Q99

Table 5-2 online. Maternal and child characteristics for participants excluded and included in the analysis.

Variable	Total	Excluded	Included	P-value[a]
Total	3098 (100)	1619 (52.26)	1479 (47.74)	
Maternal Age (years, *n* (%))				0.106
<20	288 (9.30)	138 (8.52)	150 (10.14)	
20-34	2246 (72.50)	1165 (71.96)	1081 (73.09)	
>=35	556 (17.95)	308 (19.02)	248 (16.77)	
Education level, *n* (%)				<0.001
Below college degree	2642 (85.28)	1401 (86.53)	1241 (83.91)	
College degree or above	420 (13.56)	182 (11.24)	238 (16.09)	
Maternal race, *n* (%)				<0.001
Black	1965 (63.43)	940 (58.06)	1025 (69.30)	
White	227 (7.33)	115 (7.10)	112 (7.57)	
Hispanic	682 (22.01)	448 (27.67)	234 (15.82)	
Others	209 (6.75)	101 (6.24)	108 (7.30)	
Smoking during pregnancy, *n* (%)				0.412
Never	2496 (80.57)	1277 (78.88)	1219 (82.42)	
Quitter	238 (7.68)	128 (7.91)	110 (7.44)	
Continuous	330 (10.65)	180 (11.12)	150 (10.14)	
Child's sex, *n* (%)				0.008
Female	1529 (49.35)	762 (47.07)	767 (51.86)	
Male	1567 (50.58)	855 (52.81)	712 (48.14)	
Delivery type, *n* (%)				0.057
C-section	1116 (36.02)	606 (37.43)	510 (34.48)	
Vaginal	1967 (63.49)	998 (61.64)	969 (65.52)	
Gestational age, week				<0.001
Mean ± SD	37.6 ± 3.5	37.1 ± 3.9	38.2 ± 3.0	
Birthweight, g				<0.001
Mean ± SD	2898.3 ±819.7	2794.7 ±881.7	3011.6 ± 729.5	

Note: [a]The p-values were obtained from chi-square test or t-test between children included and excluded in the main analysis.

Table 5-3 Maternal and child characteristics for children with any ADHD diagnosis (ADHD) and neurotypical (NT) children.

Variable	Total	NT	ADHD	*P*-value[a]
Total	1,479 (100)	1,180 (79.78)	299 (20.22)	
Maternal Age (years, *n* (%))				0.094
<20	150 (10.14)	110 (9.32)	40 (13.38)	
20-34	1,081 (73.09)	874 (74.07)	207 (69.23)	
>=35	248 (16.77)	196 (16.61)	52 (17.39)	
Education level, *n* (%)				0.033
Below college degree	1,241 (83.91)	978 (82.88)	263 (87.96)	
College degree or above	238 (16.09)	202 (17.12)	36 (12.04)	
Maternal race, *n* (%)				0.137
Black	1025 (69.30)	811 (68.73)	214 (71.57)	
White	112 (7.57)	96 (8.14)	16 (5.35)	
Hispanic	234 (15.82)	181 (15.34)	53 (17.73)	
Others	108 (7.30)	92 (7.80)	16 (5.35)	
Parity, *n* (%)				0.715
Nulliparous	649 (43.88)	515 (43.64)	134 (44.82)	
Multiparous	830 (56.12)	665 (56.36)	165 (55.18)	
Smoking during pregnancy, *n* (%)				<0.001
Never	1219 (82.42)	995 (84.32)	224 (74.92)	
Quitter	110 (7.44)	71 (6.02)	39 (13.04)	
Continuous	150 (10.14)	114 (9.66)	36 (12.04)	
Intrauterine infection, *n* (%)				0.073
No	1283 (86.75)	1033 (87.54)	250 (83.61)	
Yes	196 (13.25)	147 (12.46)	49 (16.39)	
Child's sex, *n* (%)				<0.001
Female	767 (51.86)	681 (57.71)	86 (28.76)	
Male	712 (48.14)	499 (42.29)	213 (71.24)	
Delivery type, *n* (%)				0.021
C-section	510 (34.48)	390 (33.05)	120 (40.13)	
Vaginal	969 (65.52)	790 (66.95)	179 (59.87)	
Season of child's birth, *n* (%)				0.077
Jan to March	346 (23.39)	282 (23.90)	64 (21.40)	
April to June	355 (24.00)	282 (23.90)	73 (24.41)	
July to September	400 (27.05)	303 (25.68)	97 (32.44)	
October to December	378 (25.56)	313 (26.53)	65 (21.74)	
Preterm birth (<37 weeks, *n* (%))				<0.001
No	1,156 (78.16)	954 (80.85)	202 (67.56)	
Yes	323 (21.84)	226 (19.15)	97 (32.44)	

Low birthweight (<2,500 g, *n* (%))				<0.001
No	1,164 (78.70)	963 (81.61)	201 (67.22)	
Yes	315 (21.30)	217 (18.39)	98 (32.78)	
Stress during pregnancy, *n* (%)				<0.001
Not stressful	562 (38.00)	475 (40.25)	87 (29.10)	
Stressful	909 (61.46)	700 (59.32)	209 (69.90)	
Gestational age, week				<0.001
Mean ± SD	38.2 ± 3.0	38.5 ± 2.5	37.1 ± 4.2	
Birthweight, g				<0.001
Mean ± SD	3011.6 ±729.5	3069.9 ± 665.4	2781.6 ± 906.3	
Early Childhood lead, µg/dL				0.009
Mean ± SD	2.2 ± 1.6	2.1 ± 1.5	2.4 ± 1.9	
Maternal HDL, mg/dL				0.001
Median (25^{th}-75^{th} quantile)	60.7 (50.3-72.3)	61.5 (51.0-73.9)	57.4 (49.3-67.5)	

Note: NT was defined as without any neurodevelopmental disorder diagnosis; ADHD was defined as any ADHD diagnosis; [a]The p-values were obtained from chi-square tests for categorical variables or t-tests for continuous variables, between children with ADHD and the NT controls.

Table 5-4 The association of early childhood lead levels with the risk of ADHD diagnosis.

	ADHD, *n* (%)	NT, *n* (%)	Crude OR	95% CI	*P*-value	Adjusted OR	95% CI	*P*-value
	299 (20.22)	1180 (79.78)	1.24	(1.01, 1.53)	0.037	1.25	(1.01, 1.56)	0.045
	125 (18.97)	534 (81.03)	1.00			1.00		
L	136 (19.74)	553 (80.26)	1.05	(0.80, 1.38)	0.720	1.08	(0.81, 1.44)	0.622
dL	38 (29.01)	93 (70.99)	1.75	(1.14, 2.67)	0.010	1.73	(1.09, 2.73)	0.019
	261 (19.36)	1087 (80.64)	1.00			1.00		
dL	38 (29.01)	93 (70.99)	1.70	(1.14, 2.54)	0.009	1.66	(1.08, 2.56)	0.020

without any neurodevelopmental disorder diagnosis; ADHD was defined as any ADHD
ogistic regression model was adjusted for maternal age at delivery, maternal race/ethnicity,
king during pregnancy, intrauterine infection, parity, child's sex, mode of delivery, preterm birth,

Table 5-5 The joint association of child's sex and early childhood lead levels with the risk of ADHD diagnosis.

ead Level	ADHD, *n* (%)	NT, *n* (%)	Crude OR	95% CI	P-value	Adjusted OR	95% CI	P-value
	86 (11.21)	681 (88.79)	1.00			1.00		
	213 (29.92)	499 (70.08)	3.38	(2.57, 4.45)	<0.001	3.42	(2.57, 4.55)	<0.001
-based lead (binary) interaction						3.58	(1.25, 10.20)	0.017
s within child's sex								
<5 µg/dL	80 (11.38)	623 (88.62)	1.00			1.00		
5-10 µg/dL	6 (9.38)	58 (90.63)	0.81	(0.34, 1.93)	0.627	0.68	(0.27, 1.69)	0.401
<5 µg/dL	181 (28.06)	464 (71.94)	1.00			1.00		
5-10 µg/dL	32 (47.76)	35 (52.24)	2.34	(1.41, 3.90)	0.001	2.49	(1.46, 4.26)	0.001
s of child's sex and lead								
<5 µg/dL	80 (11.38)	623 (88.62)	1.00			1.00		
5-10 µg/dL	6 (9.38)	58 (90.63)	0.81	(0.34, 1.93)	0.627	0.69	(0.28, 1.71)	0.426
<5 µg/dL	181 (28.06)	464 (71.94)	3.04	(2.27, 4.06)	<0.001	3.02	(2.24, 4.06)	<0.001
5-10 µg/dL	32 (47.76)	35 (52.24)	7.12	(4.18, 12.13)	<0.001	7.48	(4.29, 13.02)	<0.001

s defined as without any neurodevelopmental disorder diagnosis; ADHD was defined as any ADHD
e multiple logistic regression model was adjusted for maternal age at delivery, maternal race/ethnicity,
cation, smoking during pregnancy, intrauterine infection, parity, child's sex, mode of delivery, preterm birth,
ght.

Table 5-6 online. The joint association of child's sex and early childhood lead levels with the risk of ADHD diagnosis comparing to no ADHD group.

Level	ADHD, *n* (%)	No ADHD, *n* (%)	Crude OR	95% CI	P-Value	Adjusted OR	95% CI	P-Value
	86 (8.05)	982 (91.95)	1.00			1.00		
	213 (19.54)	877 (80.46)	2.77	(2.12, 3.62)	<0.001	2.84	(2.16, 3.72)	<0.001
ed lead (binary) interaction						3.13	(1.15, 8.48)	0.025
hin child's sex								
g/dL	80 (8.06)	912 (91.94)	1.00			1.00		
ug/dL	6 (7.89)	70 (92.11)	0.98	(0.42, 2.32)	0.958	0.82	(0.34, 2.01)	0.668
g/dL	181 (18.03)	823 (81.97)	1.00			1.00		
ug/dL	32 (37.21)	54 (62.79)	2.69	(1.69, 4.29)	<0.001	2.80	(1.74, 4.52)	<0.001
child's sex and lead								
g/dL	80 (8.06)	912 (91.94)	1.00			1.00		
ug/dL	6 (7.89)	70 (92.11)	0.98	(0.41, 2.32)	0.958	0.89	(0.37, 2.15)	0.802
g/dL	181 (18.03)	823 (81.97)	2.51	(1.90, 3.32)	<0.001	2.54	(1.91, 3.37)	<0.001
ug/dL	32 (37.21)	54 (62.79)	6.76	(4.12, 11.06)	<0.001	7.10	(4.29, 11.75)	<0.001

was defined as without any ADHD diagnosis; ADHD was defined as any ADHD diagnosis; the multiple
n model was adjusted for maternal age at delivery, maternal race/ethnicity, maternal education, smoking
, intrauterine infection, parity, child's sex, mode of delivery, preterm birth, and birthweight.

Table 5-7 online. The joint association of child's sex and early childhood lead levels (age ≤ 2 years) with the risk of ADHD diagnosis.

Sex	Lead Level	ADHD, *n* (%)	NT, *n* (%)	Crude OR	95% CI	P-Value	Adjusted OR	95% CI	P-Value
Female		81 (11.11)	648 (88.89)	1.00			1.00		
Male		203 (29.90)	476 (70.10)	3.41	(2.57, 4.53)	<0.001	3.51	(2.62, 4.70)	<0.001
Test for sex-based lead (binary) interaction							8.60	(2.24, 33.00)	0.002
Lead effects within child's sex									
Female	<5 µg/dL	78 (11.61)	594 (88.39)	1.00			1.00		
	5-10 µg/dL	3 (5.26)	54 (94.74)	0.42	(0.13, 1.39)	0.155	0.34	(0.10, 1.16)	0.085
Male	<5 µg/dL	173 (27.86)	448 (72.14)	1.00			1.00		
	5-10 µg/dL	30 (51.72)	28 (48.28)	2.77	(1.61, 4.78)	<0.001	3.02	(1.70, 5.34)	<0.001
Joint effects of child's sex and lead									
Female	<5 µg/dL	78 (11.61)	594 (88.39)	1.00			1.00		
	5-10 µg/dL	3 (5.26)	54 (94.74)	0.42	(0.13, 1.39)	0.155	0.35	(0.10, 1.18)	0.091
Male	<5 µg/dL	173 (27.86)	448 (72.14)	2.94	(2.19, 3.95)	<0.001	2.95	(2.18, 3.99)	<0.001
	5-10 µg/dL	30 (51.72)	28 (48.28)	8.16	(4.63, 14.38)	<0.001	8.88	(4.92, 16.03)	<0.001

Note: NT was defined as without any neurodevelopmental disorder diagnosis; ADHD was defined as any ADHD diagnosis; the multiple logistic regression model was adjusted for maternal age at delivery, maternal race/ethnicity, maternal education, smoking during pregnancy, intrauterine infection, parity, child's sex, mode of delivery, preterm birth, and birthweight.

Table 5-8 online. The joint association of child's sex and early childhood lead levels with the risk of ADHD diagnosis (further adjusted for maternal lead levels immediately after delivery).

Sex	Lead Level	ADHD, *n* (%)	NT, *n* (%)	Crude OR	95% CI	P-Value	Adjusted OR	95% CI	P-Value
Female		22 (11.06)	177 (88.94)	1.00			1.00		
Male		60 (31.91)	128 (68.09)	3.77	(2.20, 6.46)	<0.001	3.66	(2.07, 6.46)	<0.001
Test for sex-based lead (binary) interaction							2.79	(0.23, 33.98)	0.421
Lead effects within child's sex									
Female	<5 µg/dL	21 (11.35)	164 (88.65)	1.00			1.00		
	5-10 µg/dL	1 (7.14)	13 (92.86)	0.60	(0.07, 4.83)	0.632	0.53	(0.06, 5.02)	0.578
Male	<5 µg/dL	54 (31.40)	118 (68.60)	1.00			1.00		
	5-10 µg/dL	6 (37.50)	10 (62.50)	1.31	(0.45, 3.79)	0.617	1.58	(0.50, 5.03)	0.436
Joint effects of child's sex and lead									
Female	<5 µg/dL	21 (11.35)	164 (88.65)	1.00			1.00		
	5-10 µg/dL	1 (7.14)	13 (92.86)	0.60	(0.07, 4.83)	0.632	0.54	(0.06, 5.01)	0.586
Male	<5 µg/dL	54 (31.40)	118 (68.60)	3.57	(2.05, 6.24)	<0.001	3.42	(1.91, 6.15)	0.001
	5-10 µg/dL	6 (37.50)	10 (62.50)	4.69	(1.55, 14.21)	0.006	5.14	(1.57, 16.84)	0.007

Note: NT was defined as without any neurodevelopmental disorder diagnosis; ADHD was defined as any ADHD diagnosis; the multiple logistic regression model was adjusted for maternal lead levels right after delivery, maternal lead levels, maternal age at delivery, maternal race/ethnicity, maternal education, smoking during pregnancy, intrauterine infection, parity, child's sex, mode of delivery, preterm birth, and birthweight.

Table 5-9 online. The joint association among child's sex, maternal HDL level, maternal stress during pregnancy, and early childhood lead levels with the risk of ADHD diagnosis.

Sex	Lead Level	HDL or Stress level	ADHD, *n* (%)	NT, *n* (%)	Across sex group			Within sex group		
					Adjusted OR	95% CI	*P*-Value	Adjusted OR	95% CI	*P*-Value
Joint effects of child's sex, lead, and maternal HDL										
Female	<5 μg/dL	≤ median	30 (12.88)	203 (87.12)	1.06	(0.62, 1.82)	0.833	1.06	(0.61, 1.85)	0.827
	<5 μg/dL	> median	33 (11.46)	255 (88.54)	1.00			1.00		
	5-10 μg/dL	≤ median	2 (8.00)	23 (92.00)	0.62	(0.14, 2.84)	0.539	0.52	(0.11, 2.49)	0.414
	5-10 μg/dL	> median	3 (11.54)	23 (88.46)	0.80	(0.22, 2.97)	0.739	0.79	(0.20, 3.04)	0.730
Male	<5 μg/dL	≤ median	91 (33.96)	177 (66.04)	3.55	(2.25, 5.59)	<0.001	1.45	(0.96, 2.19)	0.075
	<5 μg/dL	> median	56 (25.00)	168 (75.00)	2.49	(1.53, 4.03)	<0.001	1.00		
	5-10 μg/dL	≤ median	17 (56.67)	13 (43.33)	10.03	(4.38, 22.97)	<0.001	4.02	(1.79, 8.99)	0.001
	5-10 μg/dL	> median	8 (40.00)	12 (60.00)	4.77	(1.76, 12.90)	0.002	1.94	(0.73, 5.18)	0.187
Joint effects of child's sex, lead, and maternal stress										
Female	<5 μg/dL	Not stressful	21 (7.50)	259 (92.50)	1.00			1.00		
	<5 μg/dL	Stressful	59 (14.05)	361 (85.95)	2.00	(1.17, 3.41)	0.011	1.89	(1.09, 3.28)	0.023
	5-10 μg/dL	Not stressful	0 (0.00)	19 (100.00)	NA			NA		
	5-10 μg/dL	Stressful	6 (13.33)	39 (86.67)	1.68	(0.62, 4.57)	0.310	1.59	(0.57, 4.42)	0.374
Male	<5 μg/dL	Not stressful	59 (24.48)	182 (75.52)	4.18	(2.43, 7.20)	<0.001	1.00		
	<5 μg/dL	Stressful	121 (30.17)	280 (69.83)	5.10	(3.08, 8.44)	<0.001	1.21	(0.83, 1.77)	0.312
	5-10 μg/dL	Not stressful	7 (31.82)	15 (68.18)	6.10	(2.18, 17.08)	0.001	1.53	(0.58, 4.05)	0.389
	5-10 μg/dL	Stressful	23 (53.49)	20 (46.51)	14.94	(6.88, 32.41)	<0.001	3.53	(1.75, 7.14)	<0.001

Note: NT was defined as without any neurodevelopmental disorder diagnosis; ADHD was defined as any ADHD diagnosis; the multiple logistic regression model was adjusted for maternal age at delivery, maternal race/ethnicity, maternal education, smoking during pregnancy, intrauterine infection, parity, child's sex, mode of delivery, preterm birth, and birthweight.

Table 5-10 online. Joint effect of gestational age and early childhood lead levels on the risk of ADHD diagnosis.

tational age 7 weeks)	Lead Level	ADHD, No. (%)	NT, No. (%)	Crude OR	95% CI	P-Value	Adjusted OR	95% CI	P-Value
m		202 (17.47)	954 (82.53)	1.00			1.00		
erm		97 (30.03)	226 (69.97)	2.03	(1.53, 2.69)	<0.001	1.94	(1.43, 2.63)	<0.001
for gestational age lead (binary) interaction									0.715
d's effect within gestational age group									
m	<5 µg/dL	177 (16.73)	881 (83.27)	1.00			1.00		
	5-10 µg/dL	25 (25.51)	73 (74.49)	1.70	(1.05, 2.76)	0.030	1.75	(1.06, 2.89)	0.030
erm	<5 µg/dL	84 (28.97)	206 (71.03)	1.00			1.00		
	5-10 µg/dL	13 (39.39)	20 (60.61)	1.59	(0.76, 3.35)	0.219	1.63	(0.71, 3.71)	0.246
t effect of gestational age and lead									
m	<5 µg/dL	177 (16.73)	881 (83.27)	1.00			1.00		
	5-10 µg/dL	25 (25.51)	73 (74.49)	1.70	(1.05, 2.76)	0.030	1.76	(1.06, 2.91)	0.028
erm	<5 µg/dL	84 (28.97)	206 (71.03)	2.03	(1.50, 2.74)	<0.001	2.61	(1.80, 3.78)	<0.001
	5-10 µg/dL	13 (39.39)	20 (60.61)	3.24	(1.58, 6.62)	0.001	3.76	(1.69, 8.36)	0.001

NT is defined as without any mental disorder diagnosis; ADHD is defined as any ADHD diagnosis; the multiple
ic regression model was adjusted for maternal age at delivery, maternal race/ethnicity, maternal education, smoking
g pregnancy, intrauterine infection, parity, child's sex, and mode of delivery.

Table 5-11 online. Joint effect of birthweight and early childhood lead levels on the risk of ADHD diagnosis.

Birthweight (<2500g)	Lead Level	ADHD, No. (%)	NT, No. (%)	Crude OR	95% CI	P-Value	Adjusted OR	95% CI	P-Value
Normal		201 (17.27)	963 (82.73)	1.00			1.00		
Low		98 (31.11)	217 (68.89)	2.16	(1.63, 2.87)	<0.001	2.10	(1.54, 2.85)	<0.001
Test for birthweight lead (binary) interaction									0.421
Lead's effect within birthweight group									
Normal	<5 µg/dL	174 (16.38)	888 (83.62)	1.00			1.00		
	5-10 µg/dL	27 (26.47)	75 (73.53)	1.84	(1.15, 2.94)	0.011	1.89	(1.16, 3.09)	0.011
Low	<5 µg/dL	87 (30.42)	199 (69.58)	1.00			1.00		
	5-10 µg/dL	11 (37.93)	18 (62.07)	1.40	(0.63, 3.08)	0.407	1.22	(0.51, 2.90)	0.658
Joint effect of birthweight and lead									
Normal	<5 µg/dL	174 (16.38)	888 (83.62)	1.00			1.00		
	5-10 µg/dL	27 (26.47)	75 (73.53)	1.84	(1.15, 2.94)	0.011	1.88	(1.15, 3.07)	0.012
Low	<5 µg/dL	87 (30.42)	199 (69.58)	2.23	(1.65, 3.01)	<0.001	2.69	(1.86, 3.90)	<0.001
	5-10 µg/dL	11 (37.93)	18 (62.07)	3.12	(1.45, 6.72)	0.004	3.16	(1.37, 7.26)	0.007

Note: NT is defined as without any mental disorder diagnosis; ADHD is defined as any ADHD diagnosis; the multiple logistic regression model was adjusted for maternal age at delivery, maternal race/ethnicity, maternal education, smoking during pregnancy, intrauterine infection, parity, child's sex, and mode of delivery.

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Chapter 6 AIM 3: MATERNAL BIOMARKERS OF ACETAMINOPHEN USE AND OFFSPRING ATTENTION DEFICIT HYPERACTIVITY DISORDER

This work is under review by Pediatrics

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6.1 TABLE OF CONTENTS SUMMARY

This birth cohort study investigated the prospective association between maternal plasma acetaminophen metabolites and attention deficit hyperactivity disorder (ADHD) diagnosis in the offspring.

6.2 WHAT'S KNOWN ON THIS SUBJECT

Multiple large prospective studies have suggested a positive association between self-reported maternal acetaminophen use during pregnancy and ADHD diagnosis in offspring. The major limitations of these studies were self-reported use, lack of dose quantification, and unmeasured confounders.

6.3 WHAT THIS STUDY ADDS

This study provides the first evidence of the association between maternal biomarkers of acetaminophen use (an objective measurement within 1-3 days postpartum) and offspring ADHD diagnosis. Such a link is specific to ADHD diagnosis and is in a dose-response manner.

6.4 ABSTRACT

Background and Objective: Previous studies have suggested a positive association between self-reported maternal acetaminophen use during pregnancy and risk of attention deficit hyperactivity disorder (ADHD) in offspring. We sought to examine the prospective association between maternal plasma biomarkers of acetaminophen intake and ADHD diagnosis in the offspring.

Method: This report analyzed 1180 children enrolled at birth and followed prospectively as part of the Boston Birth Cohort, including 188 with ADHD diagnosis based on electronic medical record review of all the study children at Boston Medical Center. Maternal biomarkers of acetaminophen intake were measured in plasma samples obtained within 1-3 days postpartum. Odds ratios (ORs) for having ADHD diagnosis or other developmental disorders were estimated using multinomial logistic regression models, adjusting for pertinent covariables.

Results: Compared to neurotypical children, we observed significant positive associations with ADHD diagnosis for each maternal acetaminophen biomarker: unchanged acetaminophen (Third tertile vs. First tertile): OR=2.05, 95% confidence interval [CI] 1.27-3.32; 3-(N-Acetyl-L-cystein-S-yl) acetaminophen (Above median vs. No detection): OR=2.03, 95% CI 1.26-3.27; and acetaminophen glucuronide (Above median vs. No detection): OR=2.00, 95% CI 1.26-3.18. The dose-response associations persisted after adjusting for pertinent covariables; and were specific to ADHD, rather than other neurodevelopmental disorders. In the stratified analyses, differential point estimates of the associations were observed across some strata of covariates. However, these differences were not statistically significant.

Conclusions: Maternal acetaminophen biomarkers were explicitly associated with increased risk of ADHD diagnosis in offspring. Additional clinical and mechanistic investigations are warranted.

6.5 INTRODUCTION

Attention deficit hyperactivity disorder (ADHD) is one of the most common lifelong neurodevelopmental disorders in the world. Its prevalence among children ages 4-17 years in the U.S. increased significantly from 7.0% to 10.2% during the past two decades.[1] The rapid rise of ADHD cannot be attributed to genetic mutations. Indeed, multiple social and environmental risk factors have been associated with the development of ADHD, including family-related factors,[2-14] maternal obesity,[15] maternal smoking,[7,16,17], maternal drinking,[7] low birthweight and preterm birth,[18] exposure to organophosphates,[19] polychlorinated biphenyls,[20,21] and lead exposure.[20,22-24] These findings underscore the role of environmental factors in the etiology of ADHD, and the need to explore other important yet unknown risk factors for ADHD.[25] Acetaminophen is widely used and recommended over-the-counter medication for fever and pain relief during pregnancy. The extent of acetaminophen use during pregnancy is over 65% in the U.S. and over 50% in Europe.[26,27] The inhibition of prostaglandin synthesis is part of the therapeutic effect of acetaminophen.[28] Prostaglandins not only act as fever determinants but also play essential roles in brain function, including long-term potentiation,[29] learning,[30] and cerebellar development.[28] Because of its widespread use and role in brain function, the potential unknown adverse effects of acetaminophen use on developing fetal brain need to be clarified.[31]

Since 2013, research studies analyzing five prospective cohorts from Europe and New Zealand have consistently shown a positive association between maternal intake of acetaminophen during pregnancy and increased risk of ADHD and its related symptoms in offspring.[32-36] The Society for Maternal-Fetal Medicine and the Food and Drug Administration expressed concern that the data from these recent studies are still too inconclusive to draw any causal inference between prenatal acetaminophen use and ADHD development in offspring.[37,38] Their primary criticisms

included the use of self-reported exposure, lack of dose quantification, and unmeasured confounders.[37] To address the concerns and criticisms related to previous studies and improve our understanding of acetaminophen's effect during pregnancy, there is need for a well-designed prospective birth cohort study with blood samples available to measure maternal acetaminophen levels. Currently, no such study exists. In this study, using the data from the Boston Birth Cohort, we sought to examine the prospective association between maternal plasma acetaminophen metabolites levels measured within a few days after delivery and ADHD diagnosis in the offspring. We hypothesized that maternal levels of acetaminophen biomarkers are positively associated with risk of offspring ADHD diagnosis.

6.6 METHODS

6.6.1 Sample

Since 1998, mother/infant pairs were recruited at birth from the Boston Medical Center (BMC) for participation in the Boston Birth Cohort (BBC).[39,40] The BMC serves a predominately low income, urban, minority population and is also the largest safety net hospital in New England. Eligible mothers were those who delivered a singleton live birth at BMC. They were approached for consent and enrollment within 24 to 72 hours after delivery. Infants who continued to receive primary or specialty care at BMC were invited (beginning at age 6 months) to participate in the follow-up study in which they are prospectively followed from birth onwards.[39,41,42] After obtaining informed consent, a standardized questionnaire was administered by trained research staff and a maternal venous blood sample was obtained. Mothers who conceived via in vitro fertilization, multiple-gestation pregnancies, deliveries induced by maternal trauma, and/or newborns with substantial congenital disabilities were not eligible for participation. Both the

baseline and the follow-up study have been approved by the Institutional Review Boards (IRB) of Boston University Medical Center and Johns Hopkins Bloomberg School of Public Health.

As illustrated in the study flowchart, of the 3098 children followed in the BBC, we excluded 1686 participants who had missing data for maternal acetaminophen metabolites measurements and 232 participants who had missing data for key covariates. Our final sample comprised 1180 mother-infant pairs with pertinent data (Supplemental Figure 1). This sample was similar to the excluded sample in terms of baseline maternal and newborn characteristics (Supplemental Table 2), except for having a slightly higher percentage of black children, longer gestation and higher birthweight.

6.6.2 Definitions for ADHD, ASD, other DD, and Neurotypical Children

We extracted information regarding each child's neuro-developmental diagnoses as documented in their EMRs. Beginning in 2003, BMC implemented EMR as part of routine data collection for both well-child and specialty clinical visits. The primary and secondary diagnoses for each clinical visit were coded in the EMR using the International Classification of Diseases, Ninth Revision (ICD-9) (before October 1, 2015) and ICD-10 (after October 1, 2015). Thus, all children in the BBC postnatal follow-up study with a related ICD-9 (314.0, 314.00, 314.01, 314.1, 314.2, 314.8, or 314.9) or ICD-10 (F90.0, F90.1, F90.2, F90.8, or F90.9) code included in their EMR between 2003 and 2016 were classified as having ADHD. Similarly, children with an ICD-9 (299.0, 299.00, 299.01, 299.8, 299.80, 299.81, 299.9, 299.90, or 299.91) or ICD-10 (F84.0, F84.8, or F84.9) code were classified as having an autism spectrum disorder (ASD). Furthermore, children with any of following developmental disorder diagnoses noted in their EMR were classified as having other developmental disorders (other DD): conduct disorders, developmental delays, intellectual disabilities, failure to thrive, or congenital anomalies. Children

without any diagnosis of ASD, ADHD, conduct disorders, developmental delays, intellectual disabilities, failure to thrive, or congenital anomalies were classified as neurotypical (NT). Supplemental Table 1 lists the ICD-9 and ICD-10 codes for each developmental disorder diagnosis.

6.6.3 Maternal biomarkers of acetaminophen use

Maternal plasma biomarkers of acetaminophen use were measured using nonfasting blood samples obtained within 1-3 days postpartum. As illustrated in Figure 6-1, the main metabolites (and proportion) of acetaminophen include unchanged acetaminophen (~5%), acetaminophen glucuronide (52-57%), acetaminophen sulfate (30-44%), and hepatotoxic N-acetyl-p-benzoquinone imine (NAPQI) (5-10%). NAPQI can be further detoxified as 3-(N-Acetyl-L-cystein-S-yl) acetaminophen.[43] The peak intensity of unchanged acetaminophen, acetaminophen glucuronide, and 3-(N-Acetyl-L-cystein-S-yl) acetaminophen in maternal blood was measured using liquid chromatography tandem-mass spectrometry (LC-MS) techniques at the MIT Broad Institute Metabolite Profiling Laboratory. All the intensity levels were inverse normal transformed for the subsequent statistical analyses.

6.6.4 Covariates

Based on previous literature,[32-36] the following covariates were included as potential confounders: maternal age at delivery, maternal race/ethnicity, maternal education, smoking from 6 months before pregnancy to birth (never smoked, quit during this period, continued to smoke during this period), ever drank alcohol from 6 months before pregnancy to birth, maternal pre-pregnancy BMI, parity, maternal fever during pregnancy, intrauterine infection/inflammation, baby's sex, delivery type, gestational age, birthweight, breastfeeding, and early childhood lead levels. . Maternal demographic covariates were collected using a standard

questionnaire interview. Maternal and child clinically-related covariates were abstracted from their medical records, respectively. The lead levels of the children were collected as part of the pediatric routine lead screening and extracted from their EMRs. The first lead levels measured were chosen for the analysis.

6.6.5 Statistical analyses

The characteristics of the study sample for the ADHD, ASD (excluding participants with ADHD diagnosis), other DD, and NT groups were compared using one-way ANOVA for continuous variables and χ^2 tests for categorical variables. The main exposures analyzed in this study were maternal acetaminophen metabolite levels, which were inverse normal transformed to approximate the normal distribution. The inverse normal transformed unchanged acetaminophen levels were also categorized into tertiles. Due to the high rate of non-detection, the inverse normal transformed acetaminophen glucuronide and 3-(N-Acetyl-L-cystein-S-yl) acetaminophen levels were categorized into three groups: no detection, below the median, and above the median of detected values. Based on previous findings regarding the proportions of acetaminophen metabolites typically found in blood samples,[43] we further calculated a variable to reflect overall "acetaminophen burden" by combining all of the acetaminophen metabolites levels with a weighting of their proportions in the acetaminophen metabolic pathway [acetaminophen burden=(unchanged acetaminophen/5%+ acetaminophen glucuronide/50%+ 3-(N-Acetyl-L-cystein-S-yl) acetaminophen/5%)/60%].[43] The acetaminophen burden levels were then also categorized into three groups: no detection, below median, above median. Each child's early life lead level was converted into a binary variable (5 μg/dL as the cutoff) for analysis based on CDC guidelines.[44]

We conducted sequential multinomial logistic regression models to examine the association between maternal acetaminophen metabolite levels and the risk of having ADHD diagnosis, ASD diagnosis (excluding ADHD diagnosis), or other DD diagnosis in offspring. Models included a crude model (Model 1); a model adjusted for maternal age at delivery, maternal race/ethnicity, maternal education, smoking during pregnancy, drinking during pregnancy, parity, maternal pre-pregnancy BMI, baby's sex, delivery type, gestational age, and birthweight (Model 2); and models further adjusted for maternal fever during pregnancy (Model 3), intrauterine infection/inflammation (Model 4), and breastfeeding (Model 5), respectively and combined (Model 6). We also performed stratified analyses by each stratum of covariates for binary acetaminophen burden (detected vs. no detection) using simple logistic regression comparing those with an ADHD diagnosis to the NT group. For the sensitivity analyses, we repeated the sequential models for each of the following outcomes: “ADHD only” (excluding ASD diagnosis), “ASD only” (excluding ADHD diagnosis), and “ADHD and ASD” (having both diagnoses), all compared to the NT group. STATA® version 14.0 software was used to perform all analyses (Stata Corporation, College Station, TX, USA).

6.7 RESULTS

In the final sample there were 188 children with a diagnosis of ADHD, 44 children with a diagnosis of ASD (without ADHD diagnosis), 344 children with a diagnosis of other DD, and 604 NT children (without any diagnoses of developmental disorders). The median age at first ADHD diagnosis was 7 years. Figure 6-2 shows the distribution of each acetaminophen metabolite and acetaminophen burden across diagnosis groups. Both the ADHD diagnosis and ASD diagnosis (without ADHD diagnosis) groups had more mothers with higher levels of acetaminophen metabolites compared to the NT and other DD diagnosis groups. Table 6-1

presents the crude comparisons of maternal and child characteristics among the ADHD diagnosis, ASD diagnosis (without ADHD diagnosis), other DD diagnosis, and NT groups. The ADHD and ASD groups had the highest percentage of detectable unchanged acetaminophen and its metabolites. Mothers of children with any ADHD diagnosis were also more likely to have below college degree education, ever smoked before or during pregnancy, and C-section delivery, compared with the NT group. Children with any ADHD, ASD, or any other DD diagnosis were more likely to be male, born prematurely and have had low birthweight, compared with the NT group.

Table 6-2 shows the sequential multinomial logistic regression model results for the relationship between acetaminophen metabolites and the risk of ADHD diagnosis, ASD diagnosis (excluding ADHD), or other DD diagnosis, before and after adjusting for pertinent covariates. The group with the highest plasma level of each acetaminophen metabolite was significantly associated with the risk of ADHD diagnosis, and the effect size was similar across all models. Moreover, we identified dose-responsive patterns across all acetaminophen metabolites and burden. Compared to levels in the non- detection category, below median and above median levels of maternal acetaminophen burden were associated with a 58% and 88% increase in the odds of ADHD diagnosis respectively (Model 6: OR for below median =1.58, 95% CI (1.02, 2.46); OR for above median =1.88, 95% CI (1.18, 3.00)). In contrast, the risks of ASD diagnosis and other DD diagnoses were not significantly associated with maternal plasma levels of acetaminophen metabolites across all models. Supplemental Table 3 further confirms that in our sensitivity analyses the acetaminophen metabolite levels were explicitly associated with the risk of having an ADHD diagnosis (without ASD diagnosis).

We also explored if the associations between acetaminophen metabolites and ADHD varied by strata of covariables. Figure 6-3 presents the forest plot of the stratified analyses for binary acetaminophen burden (detected vs. non-detection) by each stratum of covariates using simple logistic regression comparing ADHD diagnosis to NT. The point estimates of the acetaminophen burden-ADHD associations were similar among strata of maternal age, smoking before or during pregnancy, and maternal obesity. On the other hand, larger difference in the point estimate of the odds ratios was observed across strata of child's sex, alcohol drinking before or during pregnancy, intrauterine infection/inflammation, delivery type, birthweight, gestational age, and breastfeeding. However, tests of interaction between each covariate and binary acetaminophen burden (detected vs. non-detection) were not significant.

I further did stratified analysis and interaction test to investigate the potential protective effects of optimal maternal cholesterol levels in reducing the risk of ADHD caused by maternal acetaminophen exposure. Supplemental Table 4 presents the association between maternal acetaminophen burden and the risk of ADHD diagnosis in offspring by maternal HDL levels groups. The maternal acetaminophen levels only significantly associate with the risk of ADHD diagnosis when maternal HDL ≥60 mg/dL (OR=1.57, 95% CI (1.12, 2.22)). There is no indication of interaction between maternal HDL levels and maternal acetaminophen levels on the risk of ADHD diagnosis. This result is not presented in the manuscript for *Pediatric*.

6.8 DISCUSSION

In this prospective birth cohort study, we found a significant positive association between maternal blood acetaminophen metabolite levels measured within 1-3 days postpartum and ADHD diagnosis in offspring; such an association was not observed for other developmental

disorders. This association remained even after adjusting for potential confounders including indications of acetaminophen use such as maternal fever and intrauterine infection/inflammation during pregnancy. This study has contributed the following new information to the field.

Even though positive associations between maternal reported intake of acetaminophen during pregnancy and risk of ADHD diagnosis in their offspring have been reported by multiple independent large cohort studies, [32-36] there has been a dearth of prospective birth cohort studies to examine the biomarkers of acetaminophen use to address specific concerns about self-reported exposure and lack of dose quantification in those studies.

To our knowledge, this is the first prospective birth cohort study to examine the association between maternal plasma biomarkers of acetaminophen and offspring ADHD diagnosis, and to take into account a large number of potential covariables. Our study was further strengthened by the diagnosis of ADHD by both general pediatricians and developmental specialists. By demonstrating a prospective and dose-response relationship using biomarkers specific to acetaminophen intake, our study findings lend further support to the previous studies that found a positive association between self-report of acetaminophen and ADHD.

Although the causality and biological mechanisms underlying the maternal acetaminophen and child ADHD association remain to be determined, the potential for neurotoxicity is plausible according to previous findings. First, acetaminophen can be transferred through the placenta and remains in fetal/infant circulation much longer than it does in adults.[45] The prolonged detection of acetaminophen among children is due to their undeveloped liver, which slowly metabolizes the acetaminophen.[46] On the one hand, the low metabolic capacity in early life makes it safer for children to use acetaminophen because of slower production of toxic NAPQI, but on the other hand, it makes the fetus more vulnerable to maternal metabolized toxic NAPQI during

pregnancy. Second, the therapeutic effect of acetaminophen inhibits prostaglandin production.[28] Prostaglandin synthesis involves multiple essential biological processes underlying the function and development of the brain, such as long-term potentiation,[29] learning,[30] and cerebellar development.[28] Third, accumulating studies have shown that acetaminophen not only rapidly enters the cerebrospinal fluid but also shows a profound influence on adult brain function. [47-50] Thus, the long-term exposure of the fetus to maternal acetaminophen metabolites during pregnancy in addition to limited metabolic capacity might lead to both direct toxic damage from maternal NAPQI and potential disruption in neurodevelopment function due to prostaglandin inhibition.

While tests of interaction were not significant (likely due to lack of power), our stratified analyses identified multiple maternal and fetal factors that may enhance the association between maternal acetaminophen metabolites and ADHD diagnosis in offspring. There is biological plausibility for their influence on the association, which is worthwhile for future studies to investigate. For instance, we found that the effect size of acetaminophen use on the risk of ADHD diagnosis is more pronounced among women who drank alcohol 6 months before or during pregnancy. Effect modification by alcohol is supported by biological studies.[51,52] A mechanistic study showed that ethanol could cause induction of cytochrome P450 2E1 and selective depletion of mitochondrial glutathione, which could lead to limited clearance capacity of the toxic NAPQI.[52] Additionally, the stronger and more significant acetaminophen-ADHD association among male children indicates the need to investigate further the potential sex-specific biological mechanism underlying the acetaminophen exposure.

Our study also had some limitations. First, this study only included a one-time measurement of maternal acetaminophen metabolite levels within 1-3 days postpartum. The findings would be

strengthened if we could have included maternal acetaminophen metabolite measures taken at least once for each trimester. Given the fact that the rate of prenatal acetaminophen use during pregnancy is over 65% in the U.S.,[27] the one-time measurement in our study at best reflects maternal acetaminophen use around the time of delivery. Second, although we adjusted for major known risk factors of ADHD, we could not adjust for multiple familial factors identified in previous studies such as maternal personality,[53] poor parenting,[2,3] maltreatment,[4] conflict/parent-child hostility,[12] and severe early deprivation.[13,14] We also cannot rule out the possibility of unmeasured or unknown residual confounding. Lastly, our study sample consists of the predominantly urban low-income minority population. This characteristic may limit the generalization of our results to all pregnant women living in the U.S. However, longitudinal research on this topic using biomarker data did not exist in the past. Our study findings help to fill in this critical data gap, with particular relevance to an urban low-income minority population, a population known at high risk of ADHD.

6.9 CONCLUSION

Maternal plasma biomarkers of acetaminophen use measured within a few days of delivery were associated with higher risk of ADHD diagnosis in offspring, but they were not associated with other developmental disorders. This association remained after adjusting for multiple previously identified potential confounders. While our study provides the first biomarker evidence of the relationship between prenatal acetaminophen use and ADHD diagnosis in offspring, we could not provide definitive support for a causal inference of this relationship, given the observational nature of this study and the limitations outlined above. However, by specifically addressing concerns raised by the Society for Maternal-Fetal Medicine and the Food and Drug Administration,[37,38] our findings lent further support for the association between acetaminophen

and ADHD. Taking past findings together with the novel findings from this study, the potential adverse effect of maternal acetaminophen use on ADHD risk in offspring warrants additional investigations.

6.10 TABLES AND FIGURES

Table 6-1 Maternal and child characteristics for children with ADHD diagnosis, ASD diagnosis (excluding ADHD), other developmental disorder diagnosis (other DD), and neurotypical children (NT).

Variable	Total, N (%)	NT, N (%)	ADHD, N (%)	ASD, N (%)	Other DD, N (%)	P-value‡
Total	1180(100)	604(51.19)	188(15.93)	44(3.73)	344(29.15)	
Maternal Age						0.101
<35	965 (81.78)	510(84.44)	151 (80.32)	34 (77.27)	270 (78.49)	
≥35	215 (18.22)	94(15.56)	37 (19.68)	10 (22.73)	74 (21.51)	
Maternal race/ethnicity						0.073
Black	809 (68.56)	425(70.36)	126 (67.02)	26 (59.09)	232 (67.44)	
White	48 (4.07)	24(3.97)	11 (5.85)	2 (4.55)	11 (3.20)	
Hispanic	256 (21.69)	112(18.54)	44 (23.40)	15 (34.09)	85 (24.71)	
Others	67 (5.68)	43(7.12)	7 (3.72)	1 (2.27)	16 (4.65)	
Education level						0.208
Below college degree	1033 (87.54)	520(86.09)	172 (91.49)	37 (84.09)	304 (88.37)	
College degree or above	147 (12.46)	84(13.91)	16 (8.51)	7 (15.91)	40 (11.63)	
Smoking before or during pregnancy						0.018
Never	977 (82.80)	520(86.09)	141 (75.00)	38 (86.36)	278 (80.81)	
Quitter	90 (7.63)	38(6.29)	18 (9.57)	3 (6.82)	31 (9.01)	
Continuous	113 (9.58)	46(7.62)	29 (15.43)	3 (6.82)	35 (10.17)	
Drinking before or during pregnancy						0.491
No	1086 (92.03)	560(92.72)	173 (92.02)	38 (86.36)	315 (91.57)	
Yes	94 (7.97)	44(7.28)	15 (7.98)	6 (13.64)	29 (8.43)	
Parity						0.484
Nulliparous	527 (44.66)	281(46.52)	85 (45.21)	18 (40.91)	143 (41.57)	
Multiparous	653 (55.34)	323(53.48)	103 (54.79)	26 (59.09)	201 (58.43)	
Child's sex						<0.001
Female	576 (48.81)	351(58.11)	49 (26.06)	14 (31.82)	162 (47.09)	
Male	604 (51.19)	253(41.89)	139 (73.94)	30 (68.18)	182 (52.91)	
Delivery type						0.008
C-section	426 (36.10)	192(31.79)	75 (39.89)	22 (50.00)	137 (39.83)	
Vaginal	754 (63.90)	412(68.21)	113 (60.11)	22 (50.00)	207 (60.17)	
Maternal fever						0.594
No	1108 (93.90)	570(94.37)	178 (94.68)	42 (95.45)	318 (92.44)	
Yes	72 (6.10)	34(5.63)	10 (5.32)	2 (4.55)	26 (7.56)	
Intrauterine infection/inflammation						0.136
No	1023 (86.69)	537(88.91)	157 (83.51)	38 (86.36)	291 (84.59)	
Yes	157 (13.31)	67(11.09)	31 (16.49)	6 (13.64)	53 (15.41)	
Maternal BMI						0.304
<18.50	41 (3.47)	20(3.31)	9 (4.79)	2 (4.55)	10 (2.91)	
18.50-24.99	514 (43.56)	284(47.02)	72 (38.30)	15 (34.09)	143 (41.57)	

25-29.99	337 (28.56)	164(27.15)	58 (30.85)	11 (25.00)	104 (30.23)	
>30	288 (24.41)	136(22.52)	49 (26.06)	16 (36.36)	87 (25.29)	
Breastfeeding						0.351
Bottle only	286 (24.24)	142(23.51)	55 (29.26)	9 (20.45)	80 (23.26)	
Both or breastfed only	894 (75.76)	462(76.49)	133 (70.74)	35 (79.55)	264 (76.74)	
Unchanged acetaminophen*						0.027
First tertile	411 (34.83)	227(37.58)	46 (24.47)	13 (29.55)	125 (36.34)	
Second tertile	375 (31.78)	192(31.79)	66 (35.11)	12 (27.27)	105 (30.52)	
Third tertile	394 (33.39)	185(30.63)	76 (40.43)	19 (43.18)	114 (33.14)	
3-(N-Acetyl-L-cystein-S-yl) acetaminophen*						0.013
No detection	441 (37.37)	248(41.06)	51 (27.13)	15 (34.09)	127 (36.92)	
Below median	361 (30.59)	182(30.13)	62 (32.98)	10 (22.73)	107 (31.10)	
Above median	378 (32.03)	174(28.81)	75 (39.89)	19 (43.18)	110 (31.98)	
Acetaminophen glucuronide*						0.018
No detection	531 (45.00)	299(49.50)	68 (36.17)	15 (34.09)	149 (43.31)	
Below median	315 (26.69)	152(25.17)	52 (27.66)	15 (34.09)	96 (27.91)	
Above median	334 (28.31)	153(25.33)	68 (36.17)	14 (31.82)	99 (28.78)	
Acetaminophen burden**						0.027
No detection	531 (45.00)	299(49.50)	68 (36.17)	15 (34.09)	149 (43.31)	
Below median	315 (26.69)	151(25.00)	54 (28.72)	14 (31.82)	96 (27.91)	
Above median	334 (28.31)	154(25.50)	66 (35.11)	15 (34.09)	99 (28.78)	
Gestational age, week						<0.001
Mean (SD)	37.9(3.3)	38.5(2.5)	37.3(3.6)	37.0(4.6)	37.2(4.0)	
Birthweight, g						<0.001
Mean (SD)	2966.2(789.9)	3085.5(669.7)	2865.0(819.4)	2860.9(1026.2)	2825.5(898.0)	

Note: NT was defined as free of any developmental disorder diagnosis; ADHD was defined as any ADHD diagnosis; ASD was defined as any ASD diagnosis without having an ADHD diagnosis; other DD was defined as any developmental disorder diagnosis other than ASD and ADHD; ǂThe p-values were obtained from χ^2 tests or one-way ANOVA among the four diagnosis groups.

* Inverse normal transformed intensity

** Sum of all the acetaminophen metabolites

Table 6-2 The association between maternal acetaminophen metabolites and the risk of ADHD diagnosis, ASD diagnosis (excluding ADHD), and other DD diagnosis in offspring.

Model		ADHD, 188(15.9%)			ASD, 44(3.7%)			Other DD, 344(29.2%)		
		Odds Ratio	95% CI	P-value	Odds Ratio	95% CI	P-value	Odds Ratio	95% CI	P-value
Unchanged acetaminophen*										
Model 1	Second tertile	1.70	(1.11,2.59)	0.014	1.09	(0.49,2.45)	0.832	0.99	(0.72,1.37)	0.967
	Third tertile	2.03	(1.34,3.07)	0.001	1.79	(0.86,3.73)	0.118	1.12	(0.81,1.54)	0.490
Model 2	Second tertile	1.72	(1.10,2.70)	0.018	0.98	(0.43,2.27)	0.970	0.99	(0.71,1.40)	0.977
	Third tertile	2.08	(1.29,3.35)	0.003	1.38	(0.60,3.18)	0.451	0.94	(0.65,1.35)	0.732
Model 3	Second tertile	1.73	(1.10,2.72)	0.017	0.99	(0.43,2.30)	0.989	0.97	(0.69,1.37)	0.883
	Third tertile	2.08	(1.29,3.35)	0.003	1.39	(0.60,3.20)	0.443	0.93	(0.65,1.35)	0.706
Model 4	Second tertile	1.71	(1.09,2.68)	0.020	0.98	(0.42,2.27)	0.968	0.98	(0.70,1.39)	0.931
	Third tertile	2.06	(1.28,3.33)	0.003	1.38	(0.60,3.18)	0.453	0.93	(0.65,1.35)	0.705
Model 5	Second tertile	1.72	(1.10,2.70)	0.018	0.98	(0.42,2.26)	0.961	0.99	(0.70,1.39)	0.958
	Third tertile	2.06	(1.28,3.32)	0.003	1.40	(0.61,3.23)	0.432	0.94	(0.65,1.36)	0.749
Model 6	Second tertile	1.74	(1.10,2.73)	0.017	0.99	(0.43,2.29)	0.979	0.97	(0.69,1.37)	0.869
	Third tertile	2.05	(1.27,3.32)	0.003	1.40	(0.60,3.24)	0.433	0.93	(0.65,1.35)	0.718
3-(N-Acetyl-L-cystein-S-yl) acetaminophen*										
Model 1	Below median	1.66	(1.09,2.51)	0.018	0.91	(0.40,2.07)	0.819	1.15	(0.83,1.58)	0.399
	Above median	2.10	(1.40,3.14)	<0.001	1.81	(0.89,3.65)	0.100	1.23	(0.90,1.70)	0.198
Model 2	Below median	1.68	(1.08,2.61)	0.021	0.73	(0.31,1.72)	0.474	1.08	(0.77,1.52)	0.644
	Above median	2.06	(1.28,3.31)	0.003	1.21	(0.53,2.75)	0.653	0.96	(0.66,1.40)	0.835
Model 3	Below median	1.70	(1.09,2.65)	0.020	0.74	(0.31,1.75)	0.494	1.05	(0.75,1.48)	0.763
	Above median	2.06	(1.28,3.31)	0.003	1.22	(0.53,2.78)	0.640	0.95	(0.65,1.38)	0.789
Model 4	Below median	1.66	(1.06,2.58)	0.025	0.73	(0.31,1.72)	0.468	1.06	(0.76,1.49)	0.716
	Above median	2.04	(1.27,3.28)	0.003	1.20	(0.53,2.75)	0.661	0.95	(0.65,1.38)	0.785
Model 5	Below median	1.67	(1.07,2.60)	0.024	0.74	(0.31,1.75)	0.497	1.09	(0.78,1.53)	0.619
	Above median	2.04	(1.27,3.28)	0.003	1.23	(0.54,2.82)	0.621	0.97	(0.67,1.41)	0.868
Model 6	Below median	1.68	(1.08,2.63)	0.022	0.75	(0.32,1.78)	0.513	1.06	(0.75,1.49)	0.734
	Above median	2.03	(1.26,3.27)	0.004	1.23	(0.54,2.82)	0.626	0.96	(0.66,1.39)	0.811
Acetaminophen glucuronide*										
Model 1	Below median	1.50	(1.00,2.27)	0.051	1.97	(0.94,4.13)	0.074	1.27	(0.92,1.75)	0.150
	Above median	1.95	(1.33,2.88)	0.001	1.82	(0.86,3.88)	0.118	1.30	(0.94,1.79)	0.110
Model 2	Below median	1.49	(0.96,2.31)	0.074	1.47	(0.68,3.19)	0.332	1.14	(0.81,1.60)	0.465
	Above median	2.03	(1.28,3.22)	0.003	1.26	(0.53,2.99)	0.602	1.07	(0.73,1.55)	0.738
Model 3	Below median	1.51	(0.97,2.34)	0.068	1.50	(0.69,3.29)	0.306	1.11	(0.78,1.56)	0.569
	Above median	2.03	(1.28,3.23)	0.003	1.28	(0.54,3.04)	0.579	1.05	(0.72,1.53)	0.787
Model 4	Below median	1.47	(0.95,2.29)	0.085	1.47	(0.67,3.21)	0.333	1.12	(0.80,1.58)	0.516
	Above median	2.01	(1.26,3.18)	0.003	1.26	(0.53,3.00)	0.599	1.05	(0.73,1.53)	0.784
Model 5	Below median	1.49	(0.96,2.31)	0.075	1.47	(0.68,3.20)	0.330	1.13	(0.81,1.59)	0.466
	Above median	2.01	(1.27,3.19)	0.003	1.27	(0.54,3.02)	0.584	1.07	(0.74,1.55)	0.726
Model 6	Below median	1.51	(0.97,2.35)	0.068	1.50	(0.69,3.28)	0.308	1.11	(0.78,1.56)	0.564
	Above median	2.00	(1.26,3.18)	0.003	1.28	(0.54,3.05)	0.578	1.05	(0.73,1.53)	0.780
Acetaminophen burden**										

Model 1	Below median	1.57	(1.05,2.36)	0.029	1.85	(0.87,3.93)	0.110	1.28	(0.92,1.76)	0.139
	Above median	1.88	(1.28,2.78)	0.001	1.94	(0.92,4.08)	0.080	1.29	(0.94,1.78)	0.119
Model 2	Below median	1.56	(1.01,2.42)	0.045	1.39	(0.63,3.06)	0.410	1.14	(0.81,1.61)	0.439
	Above median	1.91	(1.21,3.04)	0.006	1.36	(0.58,3.20)	0.477	1.05	(0.73,1.53)	0.779
Model 3	Below median	1.58	(1.02,2.45)	0.041	1.43	(0.64,3.15)	0.381	1.11	(0.79,1.57)	0.543
	Above median	1.92	(1.21,3.05)	0.006	1.38	(0.59,3.25)	0.457	1.04	(0.72,1.52)	0.824
Model 4	Below median	1.54	(1.00,2.39)	0.052	1.39	(0.63,3.08)	0.411	1.13	(0.80,1.59)	0.488
	Above median	1.89	(1.19,3.01)	0.007	1.37	(0.58,3.22)	0.475	1.04	(0.72,1.51)	0.826
Model 5	Below median	1.56	(1.01,2.41)	0.045	1.39	(0.63,3.07)	0.409	1.14	(0.81,1.61)	0.442
	Above median	1.90	(1.20,3.02)	0.007	1.38	(0.59,3.25)	0.459	1.06	(0.73,1.54)	0.763
Model 6	Below median	1.58	(1.02,2.46)	0.040	1.43	(0.64,3.15)	0.382	1.11	(0.79,1.57)	0.539
	Above median	1.88	(1.18,3.00)	0.008	1.39	(0.59,3.27)	0.456	1.05	(0.72,1.52)	0.815

Note: NT was defined as free of any developmental disorder diagnosis; ADHD was defined as any ADHD diagnosis; ASD was defined as any ASD diagnosis without having an ADHD diagnosis; other DD was defined as any developmental disorder diagnosis other than ASD and ADHD;
Model 1: Multinomial logistic regression without adjustment;
Model 2: Model 1 further adjusted for maternal age at delivery, maternal race/ethnicity, maternal education, smoking before or during pregnancy, drinking before or during pregnancy, maternal BMI, parity, child's sex, delivery type, preterm birth, and birthweight;
Model 3: Model 2 further adjusted for maternal fever during pregnancy;
Model 4: Model 2 further adjusted for maternal intrauterine infection/inflammation during pregnancy;
Model 5: Model 2 further adjusted for breastfeeding;
Model 6: Model 2 further adjusted for maternal fever, maternal intrauterine infection/inflammation during pregnancy, and breastfeeding.
* Inverse normal transformed intensity ** Sum of all the acetaminophen metabolites.
Unchanged acetaminophen: first tertile as reference; For other exposures: no detection as reference

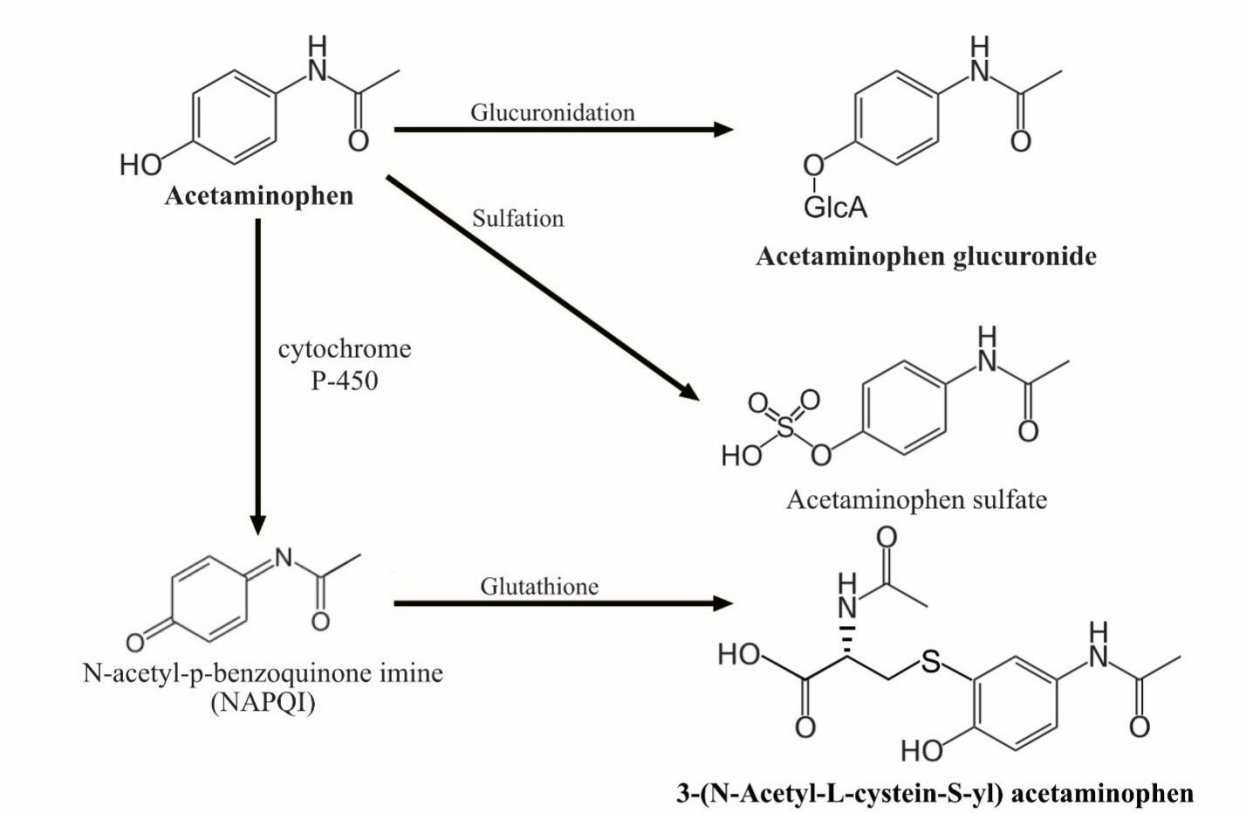


Figure 6-1 Pathways of acetaminophen metabolism.

Note: Bolded metabolites were measured in this study.

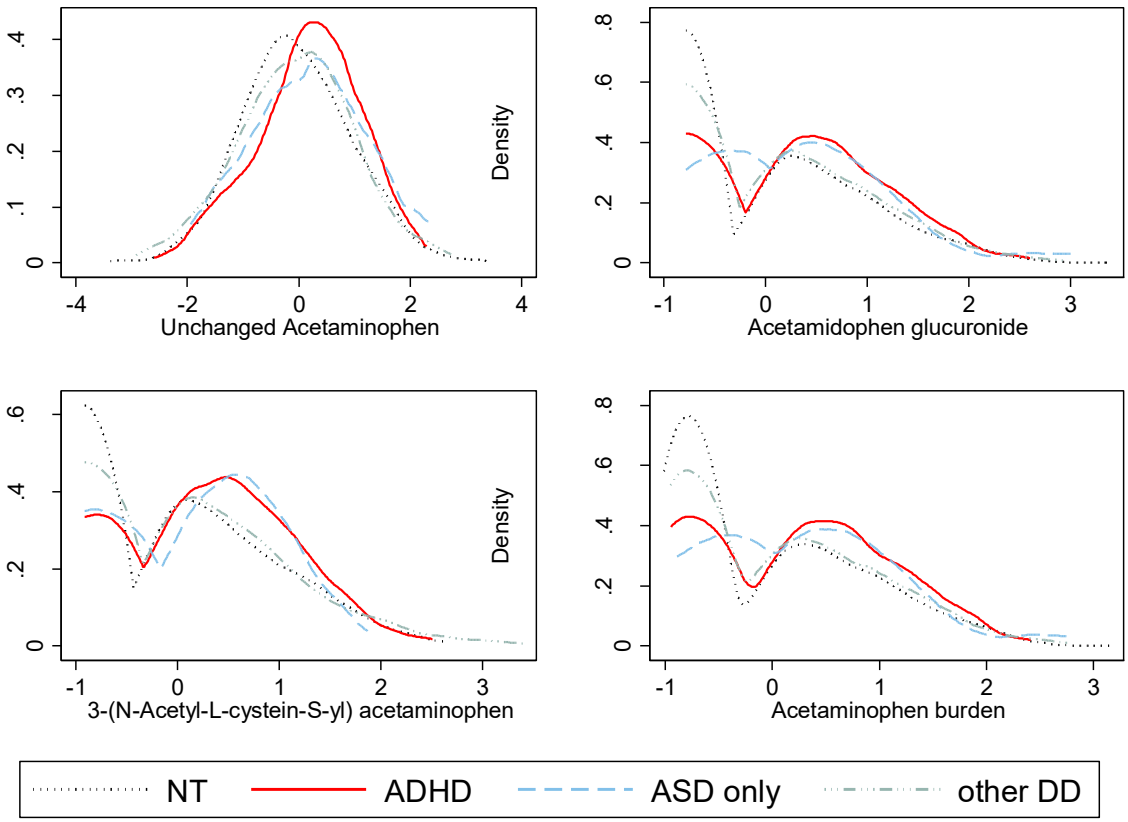


Figure 6-2 Comparison of the distributions of acetaminophen metabolites and acetaminophen burden by specific diagnosis groups.

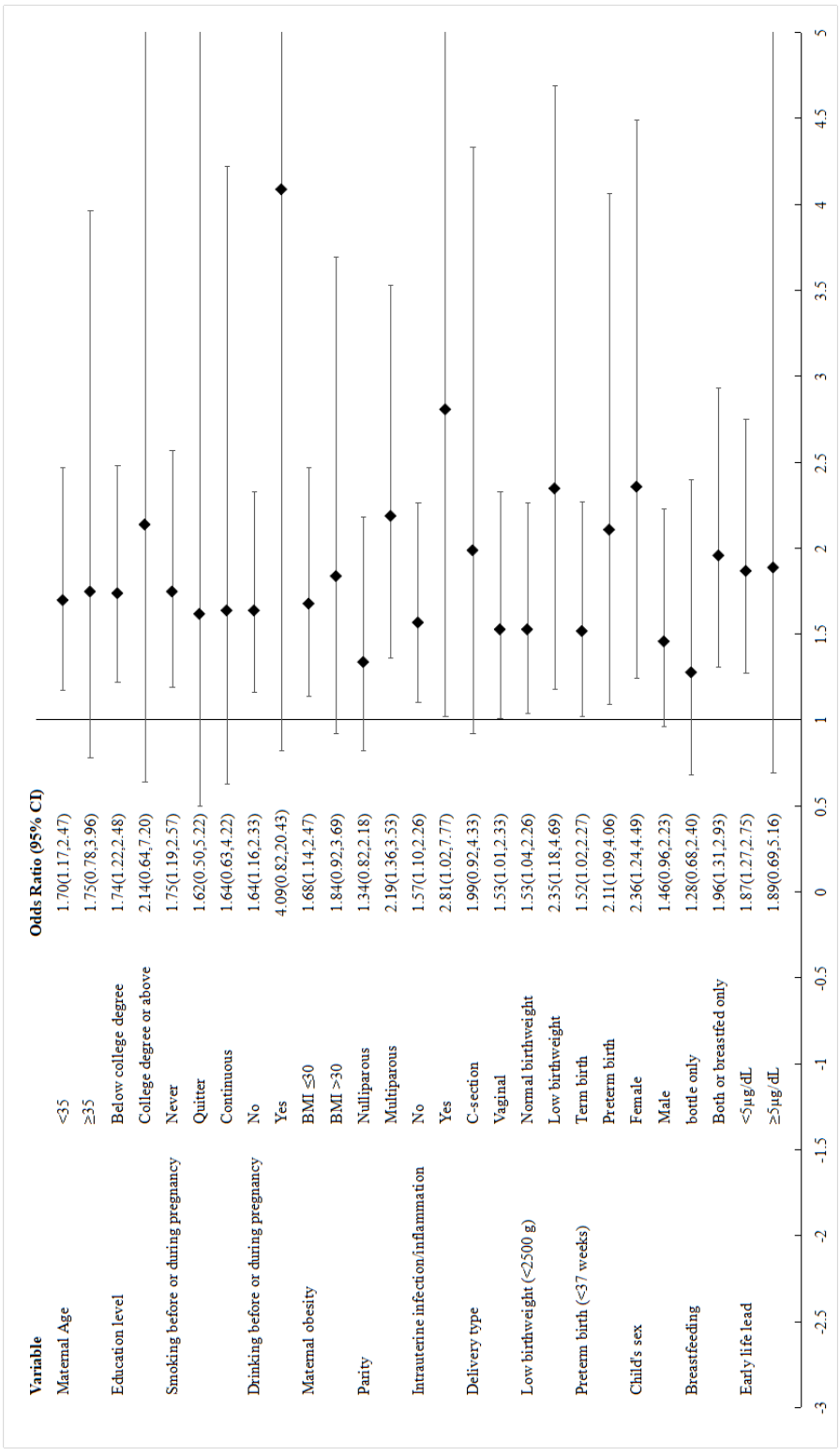


Figure 6-3 The forest plot for the crude association between maternal binary acetaminophen burden (detected vs. no detection) and the risk of ADHD diagnosis in offspring across each stratum of pertinent covariables.

6.11 SUPPLEMENTARY MATERIALS

Supplemental Table 1. List of ICD-9 and ICD-10 codes for the diagnosis of each developmental disorder.

Developmental disorder	ICD-9 codes	ICD-10 codes
ASD	299.0, 299.00, 299.01, 299.8, 299.80, 299.81, 299.9, 299.90, 299.91	F84.0, F84.8, F84.9
ADHD	314.0, 314.00, 314.01, 314.1, 314.2, 314.8, 314.9	F90, F90.0, F90.1, F90.2, F90.8, F90.9
Disturbance of conduct	312.0-312.9	F91, F91.0, F91.2, F91.3, F91.8, F91.9
Delays in development	315.0-315.9	F81.0, R48.0, F81.81, F81.2, F81.89, F80.1, F80.2, H93.25, F80.4, F80.81, F80.0, F80.82, F80.89, F82, F88, F81.9, F89
Intellectual disabilities	317-317	F70, F71, F72, F73, F78, F79
Failure to thrive	783.4, 783.40, 783.41, 783.42, 783.43	R62.50, R62.51, R62.0, R62.52
Congenital anomalies	740-759.9	Q00-Q99

Supplemental Table 2. Maternal and child characteristics for participants excluded and included in the analysis.

Variable	Total, N (%)	Excluded, N (%)	Included, N (%)	P-value‡
Total	3098 (100)	1918 (61.91)	1180 (38.09)	
Maternal Age				0.796
<35	2534 (81.79)	1569 (81.80)	965 (81.78)	
≥35	556 (17.95)	341 (17.78)	215 (18.22)	
Education level				0.109
Below college degree	2642 (85.28)	1609 (83.89)	1033 (87.54)	
College degree or above	420 (13.56)	273 (14.23)	147 (12.46)	
Maternal race/ethnicity				<0.001
Black	1965 (63.43)	1156 (60.27)	809 (68.56)	
White	227 (7.33)	179 (9.33)	48 (4.07)	
Hispanic	682 (22.01)	426 (22.21)	256 (21.69)	
Other	209 (6.75)	142 (7.40)	67 (5.68)	
Smoking before or during pregnancy				0.222
Never	2496 (80.57)	1519 (79.20)	977 (82.80)	
Quitter	238 (7.68)	148 (7.72)	90 (7.63)	
Continuous	330 (10.65)	217 (11.31)	113 (9.58)	
Drinking before or during pregnancy				0.627
No	2740 (88.44)	1654 (86.24)	1086 (92.03)	
Yes	247 (7.97)	153 (7.98)	94 (7.97)	
Child's sex				0.617
Female	1529 (49.35)	953 (49.69)	576 (48.81)	
Male	1567 (50.58)	963 (50.21)	604 (51.19)	
Delivery type				0.930
C-section	1116 (36.02)	690 (35.97)	426 (36.10)	
Vaginal	1967 (63.49)	1213 (63.24)	754 (63.90)	
Gestational age, week				0.003
Mean (SD)	37.6(3.5)	37.5(3.6)	37.9(3.3)	
Birthweight, g				<0.001
Mean (SD)	2898.3(819.7)	2856.5(834.9)	2966.2(789.9)	

Note: ‡The p-values were obtained from χ^2 tests or t-tests between children included in and excluded from the main analysis.

Supplemental Table 3. The association between maternal acetaminophen metabolites and the risk of ADHD diagnosis only, ASD diagnosis only, and diagnoses of both ADHD and ASD in offspring.

Model	ADHD only, N=166			ASD only, N=44			ADHD and ASD, N=22		
	Odds Ratio	95% CI	P-value	Odds Ratio	95% CI	P-value	Odds Ratio	95% CI	P-value
Unchanged acetaminophen*									
Model 1 Second tertile	1.92	(1.22,3.01)	0.005	1.09	(0.49,2.45)	0.832	0.79	(0.28,2.25)	0.657
Third tertile	2.29	(1.47,3.57)	<0.001	1.79	(0.86,3.73)	0.118	0.95	(0.35,2.61)	0.928
Model 2 Second tertile	1.96	(1.20,3.20)	0.007	0.96	(0.41,2.25)	0.932	0.85	(0.28,2.64)	0.785
Third tertile	2.32	(1.38,3.89)	0.001	1.32	(0.57,3.07)	0.523	0.92	(0.27,3.20)	0.901
Model 3 Second tertile	1.96	(1.20,3.20)	0.007	0.97	(0.41,2.28)	0.947	0.91	(0.29,2.83)	0.868
Third tertile	2.32	(1.38,3.89)	0.001	1.32	(0.57,3.08)	0.516	0.92	(0.26,3.21)	0.891
Model 4 Second tertile	1.95	(1.19,3.18)	0.008	0.96	(0.41,2.25)	0.926	0.85	(0.27,2.63)	0.778
Third tertile	2.30	(1.37,3.86)	0.002	1.31	(0.56,3.05)	0.534	0.91	(0.26,3.14)	0.884
Model 5 Second tertile	1.97	(1.21,3.22)	0.007	0.96	(0.41,2.24)	0.920	0.85	(0.28,2.64)	0.783
Third tertile	2.29	(1.37,3.85)	0.002	1.33	(0.57,3.10)	0.509	0.93	(0.27,3.24)	0.912
Model 6 Second tertile	2.01	(1.22,3.29)	0.006	0.98	(0.42,2.29)	0.958	0.91	(0.29,2.85)	0.872
Third tertile	2.28	(1.36,3.84)	0.002	1.33	(0.57,3.10)	0.516	0.92	(0.26,3.22)	0.892
3-(N-Acetyl-L-cystein-S-yl) acetaminophen*									
Model 1 Below median	1.82	(1.17,2.83)	0.008	0.91	(0.40,2.07)	0.819	0.91	(0.32,2.60)	0.858
Above median	2.31	(1.50,3.55)	<0.001	1.81	(0.89,3.65)	0.100	1.11	(0.41,3.03)	0.841
Model 2 Below median	1.90	(1.18,3.05)	0.008	0.77	(0.33,1.82)	0.557	0.98	(0.32,2.99)	0.974
Above median	2.28	(1.37,3.80)	0.002	1.19	(0.52,2.73)	0.688	0.90	(0.26,3.13)	0.868
Model 3 Below median	1.91	(1.18,3.08)	0.008	0.78	(0.33,1.86)	0.576	1.12	(0.37,3.43)	0.843
Above median	2.28	(1.37,3.81)	0.002	1.19	(0.52,2.75)	0.680	0.91	(0.26,3.18)	0.888
Model 4 Below median	1.87	(1.16,3.01)	0.010	0.76	(0.32,1.80)	0.537	1.00	(0.33,3.07)	0.996
Above median	2.25	(1.34,3.76)	0.002	1.17	(0.51,2.70)	0.717	0.89	(0.26,3.08)	0.860
Model 5 Below median	1.88	(1.17,3.03)	0.009	0.78	(0.33,1.85)	0.576	1.00	(0.33,3.05)	0.994
Above median	2.25	(1.35,3.75)	0.002	1.21	(0.52,2.78)	0.660	0.91	(0.26,3.18)	0.882
Model 6 Below median	1.93	(1.19,3.11)	0.008	0.80	(0.33,1.89)	0.604	1.15	(0.37,3.53)	0.812
Above median	2.24	(1.34,3.76)	0.002	1.20	(0.52,2.78)	0.676	0.91	(0.26,3.20)	0.889
Acetaminophen glucuronide*									
Model 1 Below median	1.69	(1.09,2.60)	0.018	1.97	(0.94,4.13)	0.074	0.66	(0.21,2.07)	0.471
Above median	2.16	(1.44,3.26)	<0.001	1.82	(0.86,3.88)	0.118	0.98	(0.36,2.65)	0.964
Model 2 Below median	1.67	(1.04,2.68)	0.033	1.39	(0.63,3.07)	0.411	0.51	(0.15,1.79)	0.293
Above median	2.30	(1.40,3.78)	0.001	1.20	(0.50,2.88)	0.680	0.88	(0.24,3.20)	0.845
Model 3 Below median	1.67	(1.04,2.69)	0.034	1.42	(0.64,3.15)	0.390	0.56	(0.16,2.00)	0.373
Above median	2.30	(1.40,3.78)	0.001	1.22	(0.51,2.92)	0.663	0.88	(0.24,3.22)	0.851
Model 4 Below median	1.66	(1.04,2.66)	0.035	1.38	(0.63,3.05)	0.422	0.51	(0.14,1.79)	0.293
Above median	2.26	(1.37,3.72)	0.001	1.19	(0.49,2.86)	0.702	0.88	(0.24,3.17)	0.845
Model 5 Below median	1.68	(1.05,2.69)	0.032	1.39	(0.63,3.06)	0.418	0.51	(0.15,1.78)	0.290
Above median	2.28	(1.38,3.75)	0.001	1.21	(0.50,2.91)	0.668	0.89	(0.24,3.25)	0.858
Model 6 Below median	1.74	(1.08,2.81)	0.023	1.44	(0.65,3.20)	0.373	0.56	(0.16,2.02)	0.378
Above median	2.26	(1.37,3.73)	0.001	1.21	(0.50,2.92)	0.675	0.88	(0.24,3.24)	0.852
Acetaminophen burden**									

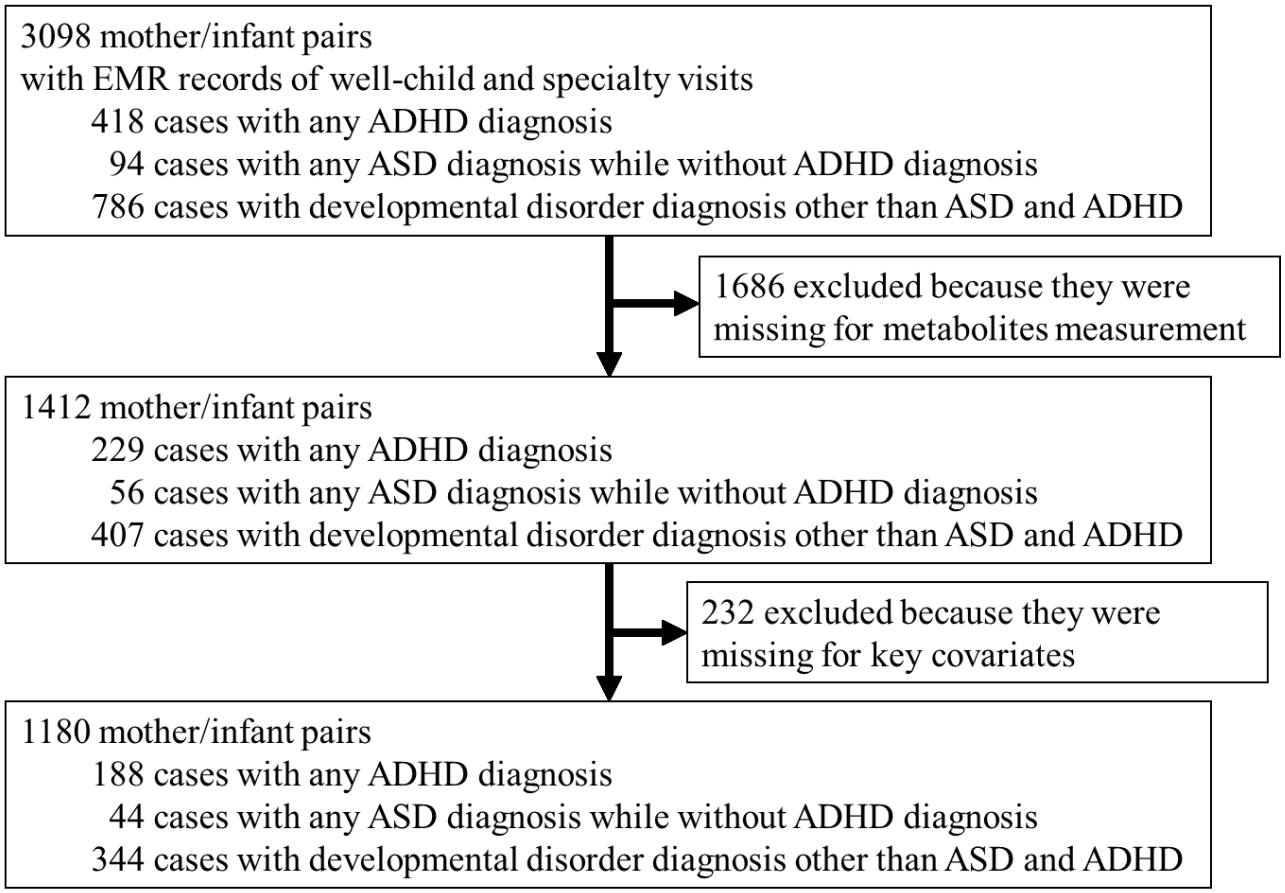
Model 1	Below median	1.77	(1.15,2.71)	0.009	1.85	(0.87,3.93)	0.110	0.66	(0.21,2.08)	0.478
	Above median	2.08	(1.38,3.14)	0.001	1.94	(0.92,4.08)	0.080	0.97	(0.36,2.64)	0.954
Model 2	Below median	1.76	(1.10,2.80)	0.018	1.32	(0.59,2.95)	0.495	0.52	(0.15,1.81)	0.303
	Above median	2.16	(1.31,3.55)	0.003	1.30	(0.55,3.08)	0.553	0.86	(0.24,3.12)	0.819
Model 3	Below median	1.76	(1.10,2.82)	0.019	1.35	(0.60,3.02)	0.471	0.57	(0.16,2.03)	0.387
	Above median	2.16	(1.31,3.55)	0.003	1.31	(0.55,3.12)	0.539	0.86	(0.24,3.14)	0.822
Model 4	Below median	1.74	(1.09,2.79)	0.020	1.31	(0.59,2.93)	0.506	0.52	(0.15,1.82)	0.303
	Above median	2.12	(1.28,3.50)	0.003	1.28	(0.54,3.06)	0.572	0.86	(0.24,3.09)	0.819
Model 5	Below median	1.77	(1.11,2.82)	0.017	1.31	(0.59,2.93)	0.505	0.52	(0.15,1.80)	0.299
	Above median	2.13	(1.29,3.52)	0.003	1.31	(0.55,3.12)	0.539	0.87	(0.24,3.17)	0.832
Model 6	Below median	1.84	(1.14,2.96)	0.012	1.37	(0.61,3.07)	0.452	0.57	(0.16,2.05)	0.392
	Above median	2.11	(1.27,3.49)	0.004	1.30	(0.55,3.12)	0.550	0.86	(0.24,3.15)	0.823

Note: NT was defined as free of any developmental disorder diagnosis; ADHD only was defined as any ADHD diagnosis without having an ASD diagnosis; ASD only was defined as any ASD diagnosis without having an ADHD diagnosis; ADHD and ASD was defined as having both ADHD and ASD diagnosis;
Model 1: Multinomial logistic regression without adjustment;
Model 2: Model 1 further adjusted for maternal age at delivery, maternal race/ethnicity, maternal education, smoking before or during pregnancy, drinking before or during pregnancy, maternal BMI, parity, child's sex, delivery type, preterm birth, and birthweight;
Model 3: Model 2 further adjusted for maternal fever during pregnancy;
Model 4: Model 2 further adjusted for maternal intrauterine infection/inflammation during pregnancy;
Model 5: Model 2 further adjusted for breastfeeding;
Model 6: Model 2 further adjusted for maternal fever, maternal intrauterine infection/inflammation during pregnancy, and breastfeeding.
* Inverse normal transformed intensity ** Sum of all the acetaminophen metabolites.
Unchanged acetaminophen: first tertile as reference; For other exposures: no detection as reference

Supplemental Table 4. The stratified analysis results on the association between maternal acetaminophen burden and the risk of ADHD in offspring by maternal HDL groups

		ADHD			otherDD		
Variable		OR	95% CI	P-value	OR	95% CI	P-value
Maternal HDL	≥60 mg/dL	1.57	(1.12,2.22)	0.010	1.17	(0.90,1.52)	0.231
	<60 mg/dL	1.10	(0.82,1.48)	0.532	0.85	(-0.67,1.07)	0.170
Interaction				0.074			0.111

Supplemental Figure 1. Flowchart of the sample included in the analysis.



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Chapter 7 CONCLUSIONS

7.1 OVERVIEW

This chapter begins with a summary of my key findings, followed by a discussion of the strengths and limitations of the research approach, then a summary of the research, policy, and , clinical and public health implications of this work, and ends with the conclusions reached based on the research findings.

7.2 KEY FINDINGS

7.2.1 Aim 1: Prospective association between maternal cholesterol levels and ADHD in the offspring

The multiple logistic regression results showed that a low maternal high-density lipoprotein level (HDL) (≤60 mg/dL) was associated with an increased risk of ADHD, compared to a higher maternal HDL level, after adjusting for pertinent covariables. A "J" shaped relationship was observed between triglycerides (TG) and ADHD risk. The associations with ADHD for maternal HDL and TG were more pronounced among boys.

7.2.2 Aim 2: Prospective association between early childhood lead levels and ADHD in the offspring

Nearly one-tenth of BBC children had elevated lead levels (5-10µg/dL) in early childhood, which was associated with a 66% increased risk of ADHD. Among boys, the association was significantly stronger (p-value for sex-lead interaction: 0.017). The odds ratio of ADHD associated with elevated lead levels among boys was reduced by about half if mothers had adequate HDL levels compared to low HDL, or if mothers had low stress compared to high stress during pregnancy.

7.2.3 Aim 3: Prospective association between maternal blood acetaminophen metabolites levels and ADHD in the offspring

Compared to neurotypical children, significant positive associations with ADHD diagnosis were identified for each maternal acetaminophen biomarker: unchanged acetaminophen (Third tertile vs. First tertile): OR=2.05, 95% CI: 1.27-3.32; 3-(N-Acetyl-L-cystein-S-yl) acetaminophen (Above median vs. No detection): OR=2.03, 95% CI: 1.26-3.27; and acetaminophen glucuronide (Above median vs. No detection): OR=2.00, 95% CI: 1.26-3.18. The dose-response associations persisted after adjusting for pertinent covariables; these associations were specific to ADHD rather than other neurodevelopmental disorders.

7.3 STRENGTHS AND LIMITATIONS

7.3.1 Strengths

Study design: This study used a prospective longitudinal birth cohort established in the U.S. to investigate the development of ADHD. The birth cohort design makes it feasible to investigate early life factors on ADHD during the critical neurodevelopmental window. Moreover, the temporal nature between the exposure and outcome measurements help us to understand better the temporal and causal pathways underlying the development of ADHD. Additionally, the large sample size of male and female ADHD cases in this study made it possible to investigate sex interactions with the risk factors of interest.

ADHD diagnosis: This study used physician diagnosis extracted from the EMR to define ADHD cases. More than half of the children with ADHD had over three ADHD diagnoses in their EMRs. Additionally, over 80% of ADHD cases in the BBC were diagnosed by a

neurobehavioral specialist, thus, with much higher specificity and lesser probability of case misclassification.

High-risk population: The BBC's study population is mainly comprised of a low-income, urban, minority population from the Boson area. The mothers from this population have much higher rates of obesity[1] and lead exposure[2] than the national average. As such, higher rates for these adverse conditions provided greater power to investigate their potential effects on ADHD in offspring.

Cholesterol biomarkers: This is the first study to investigate the inter-generational effect of maternal lipid profiles on the risk of ADHD in offspring. Moreover, this is the first study to illustrate the potential protective effects of maternal cholesterols against lead toxicity.

Early lead measurement: Most previous studies examined the consequences of postnatal lead exposure either at the time of ADHD diagnosis (cross-sectional) or after the diagnosis (retrospective), with a mean age of measurement ranged from age 7-14 years.[3] This is the first large longitudinal study to investigate ADHD with lead levels measured before the age of 2 years.

Sex-specific effects: This is the first study to show that boys are more vulnerable than girls to suboptimal maternal cholesterol levels and early childhood lead exposure in terms of ADHD development.

Acetaminophen metabolites: This is the first prospective birth cohort study to examine the association between maternal plasma biomarkers of acetaminophen and offspring ADHD diagnosis, and to take into account a large number of potential confounders including indications

of acetaminophen use such as maternal fever and intrauterine infection/inflammation during pregnancy.

7.3.2 Limitations

Transition from DSM-IV to DSM-V and from ICD-9 to ICD-10: this study occurred during the transition of the American Psychiatric Association's Diagnostic and Statistical Manual (DSM) from the 4th to the 5th edition. The transition to DSM-5 and ICD-10 raises concerns about the consistency of the diagnoses over time. Fortunately, this transition did not affect the ADHD determination in children, since the main changes from DSM-IV-TR to DSM-5 were more relevant to the adult diagnostic criteria. [4]

Generalizability of the study findings: The study population mainly consisted of urban, low-income, minority women who live in the Boston area. This population has been shown to have much higher levels of exposure to common ADHD risk factors and other risk factors of interest compared to the general U.S. population. Since this sample is not representative of the US general population, caution is needed to generalize the findings beyond US urban low-income setting.

Unmeasured confounders: Although adjustments for major known risk factors were made during the analyses, there may still have been unmeasured or unknown factors that may have influenced levels of exposure and/or outcomes. My analyses adjusted for known risk factors of ADHD, but could not adjust for multiple familial factors identified in previous studies such as maternal personality,[5] poor parenting,[6,7] maltreatment,[8] conflict/parent-child hostility,[9] and severe early deprivation.[10,11] Moreover, the adjustment for known risk factors did not include some post-natal factors that could be related to both maternal cholesterol levels and ADHD risk, such as the child's cholesterol levels.

One-time measurement of biomarkers of interest: This study only had a one-time measurement of maternal cholesterol and acetaminophen metabolite levels within 1-3 days postpartum. In the ideal situation, maternal cholesterol and acetaminophen metabolites would be collected at least once for each trimester. However, this was not feasible for my study sample. The one-time measurement for these exposures can at best reflect these two exposures around the time of delivery.

Non-fasting blood samples: This study used non-fasting blood samples. The values for total cholesterol and TG levels may have been inflated in non-fasting blood samples and thus may have biased my study results towards the null. Further studies using fasting blood samples should be conducted to provide a more precise assessment of optimal TG levels during pregnancy.

7.4 STUDY IMPLICATIONS

This research provides new insights into the effects of maternal cholesterol levels, early childhood lead and maternal acetaminophen use on the risk of ADHD diagnosis in the offspring. Additionally, these investigations have for the first time identified sex-specific effects of maternal cholesterol and early childhood lead exposure on ADHD. As detailed below, these findings, if further confirmed, would have tremendous implications for research, policy, and clinical intervention.

7.4.1 Research implications

7.4.1.1 Understanding of ADHD etiology

This dissertation research has provided multiple new insights into the role of early life factors in the development of ADHD.

Aim 1 was the first study designed to explore the prospective relationship between maternal cholesterols and childhood ADHD. The findings provided several lines of support for casual evidence, such as a strong dose-responsive HDL-ADHD association, temporal relationship between maternal HDL and offspring ADHD, and sex-specific responses to maternal HDL levels.

Aim 2 was the first birth cohort study in a US urban low-income minority population to examine the lead-ADHD relationship. The findings provided several supports for casual evidence, including a temporal relationship, sex difference, and biological plausibility.

Aim 3 was the first study to investigate prospective relationship between maternal acetaminophen biomarkers and offspring ADHD risk. The findings provided several supports for casual evidence, including temporal and dose-response associations. Moreover, these associations are ADHD-specific.

However, given that this is an observational study, the findings be regarded as hypothesis generating, rather than conclusive. To establish a causal relationship between risk factors and ADHD, it requires multiple levels of evidence, including a strong association, consistency across different situations, specificity between exposure and outcome, as well as a temporal relationship, a dose-responsive relationship, biological plausibility and coherence, outcome changes after exposure manipulation, and analogy to other comparable situation with better understanding of risk factors. [12] As such, the study findings warrant additional investigation.

7.4.1.2 Need of Repeated Cholesterol and Acetaminophen measurements

The study findings suggest that maternal HDL and acetaminophen levels may influence ADHD development in offspring. However, due to the use of one-time plasma measurements taken

within 1-3 days postpartum, these biomarkers at best reflect the exposure during the perinatal period. Under ideal conditions, maternal blood samples be collected at least once for each trimester, and this should be considered in future studies. Using such detailed data on lipid profiles and medication use specific to each trimester, future studies could identify critical period when fetal development is most vulnerable to suboptimal HDL and acetaminophen exposure. Moreover, with the help of OMICs technology, the multipoint measurements across pregnancy might ultimately help to unravel the biological pathways of ADHD development.

7.4.1.3 Study population

As mentioned above, the study population had much higher levels of exposure to common ADHD risk factors and other risk factors of interest compared to the general U.S. population. Future research could attain more generalizable findings by studying a nationally representative population.

7.4.1.4 New insights in sex difference in ADHD risk

The long-observed and striking sex difference in ADHD risk remains poorly understood. The study findings included here reveal that boys and girls respond differently to prenatal and postnatal factors. In Aim 1 and Aim 2, I found that the effects of maternal HDL, maternal TG, and early childhood lead levels on ADHD were most pronounced among boys. Sex differences in response to prenatal suboptimal nutritional status are also found in other chronic diseases such as hypertension. Both human and animal studies have shown that the male fetus is more likely to develop hypertension in response to the mother's unfavorable nutrition and metabolic status during pregnancy.[13-17] These sex differences have been explained by hypotheses that male fetuses are more vulnerable to suboptimal maternal nutrition due to their more rapid in-utero growth than females.[13-17] While only a few biological theories have been identified for prenatal

sex differences, studies on postnatal sex-specific responses are extremely rare. In the future, new studies on early life risk factors should focus more on the sex-risk interactions, by including enough female ADHD cases to make those analyses feasible.

In summary, based on the collective findings generated from this dissertation, I have the following research recommendations:

- Additional studies should be pursued to provide evidence of consistency across different populations with different socio-demographic characteristics.
- Additional studies should be conducted among a nationally representative population.
- Maternal plasma should be collected at least once for each trimester.
- Future studies should also consider potential interaction between sex and early life risk factors.

7.4.2 Policy implications

7.4.2.1 labeling change for acetaminophen and searching for alternatives

Although multiple previous large cohort studies have provided consistent self-reported evidence for potential acetaminophen neurotoxicity during pregnancy,[18-22] the Society for Maternal-Fetal Medicine (SMFM) and the Food and Drug Administration (FDA) have issued statements regarding their belief that current studies are still too inconclusive to draw any causal inference between prenatal acetaminophen use and ADHD in offspring. [23,24] Their primary criticism included self-reported exposure, lack of dose quantification, unmeasured confounders, and lack of adjustment for multiple testing. [23] In fact, the findings detailed in this dissertation provide dose-responsive evidence in support of acetaminophen's neurotoxicity, even after adjusting for several previously identified potential confounders. My research findings lent further support to the concern that prenatal acetaminophen use may increase the risk of ADHD in offspring. The impulse to reject the possible causality must be supported by stronger evidence than opinion.[25] As a widely used over-the-counter medication among pediatric and pregnant populations, current

labels for acetaminophen state that they are “safe, gentle, and effective”. [26] Given the fact that no other ‘safer’ medications are available for use during pregnancy, two steps are needed for government to minimize the impact of the potential neurotoxicity of acetaminophen. First, the FDA should request that pharmaceutical manufacturers add information to the labels of acetaminophen-containing medications, which should state that acetaminophen is not recommended for the pregnant women if their symptom or discomfort has no strong indication or presents little risk. [25] Taking it under unnecessary condition could harm the neurodevelopment of the fetus. [25] Second, the FDA should create fast approval incentives to inspire the search for safer alternative treatments.

7.4.2.2 Correcting dyslipidemia as a potential intervention target

ADHD is one of the most common and costly neurodevelopmental disorders in the U.S. Nearly one-tenth of children ages 4-17 are diagnosed with ADHD in the U.S, and most of their symptoms will be carried into adolescence and adulthood. This high prevalence and these persistent symptoms across the lifespan can have a severe impact on both the individuals themselves and on society. The most recent estimation of the annual cost of ADHD to society, including costs related to health care utilization, medication utilization, education, crime, and unemployment, is $14,500 per individual ($42.5 billion in total). [27] Currently, the most common clinical practice is still symptom control using behavioral or pharmaceutical interventions. At such a high prevalence, clearly this type of symptom control practice has and will likely continue to lead to a huge financial burden to society. To reverse the rising trend of ADHD and associted rising costs to manage ADHD, the government should allocate more resources to the primary prevention of ADHD. For instance, the study findings included herein show that maternal dyslipidemia might increase the risk of ADHD in offspring. Considering that the global obesity

and diabetes epidemics are growing, the percentage of pregnant women with dyslipidemia during pregnancy is also likely to increase. If the government invests more resources in pre- and perinatal dyslipidemia control programs, the incidence of ADHD and related financial burden are likely to decrease. For instance, the government can optimize the Women, Infants, and Children (WIC) program's food voucher selections to make it more beneficial for maintaining healthy cholesterol levels. Moreover, given the essential role of cholesterol in brain development, this change might also reduce other neurodevelopment risks.

In summary, based on the collective findings generated from the studies presented in this dissertation, I have the following policy recommendations:

- The FDA should discourage the use of acetaminophen for minor symptoms and discomforts during pregnancy and peripartum, by requesting additional warning to be added to the labels of acetaminophen-containing medications.
- The FDA should create fast approval incentives to accelerate the search for safer alternative treatments.
- If further confirmed by future studies, the government should invest more resources in pre- and perinatal dyslipidemia control programs as a primary prevention strategy to reduce the incidence of ADHD.

7.4.3 Clinical and Public Health Implications

7.4.3.1 Limiting acetaminophen use in obstetrics and gynecology practice

Acetaminophen is a widely used and recommended over-the-counter medication for fever and pain relief during pregnancy. The percentage of pregnant women who use acetaminophen during pregnancy is over 65% in the U.S. and over 50% in Europe.[28,29] The primary concern is related to the fact that the inhibition of prostaglandin synthesis is part of the therapeutic effect of acetaminophen. [30] Biological evidence suggests that prostaglandins not only act as a fever determinant but also play essential roles in brain function, including long-term potentiation,[31] learning,[32] and cerebellar development.[30] Hence, given its widespread usage and influence in

neuronal function, it is too risky to ignore any potential unknown side effects.[25] As a result, based on the findings presented here I strongly urge clinicians to provide advice to pregnant women about using this drug carefully, avoiding overdose and high-frequency use as well as unnecessary use for minor conditions.

7.4.3.2 An emphasis of primary prevention of ADHD

The hope is that the findings presented here may help to transform clinical practice from secondary- and tertiary-prevention into primary prevention of ADHD. Currently, major clinical practice is still focused on symptom control using behavioral or pharmaceutical interventions. Clearly, as ADHD prevalence continues to increase, this practice will not be sustainable due to a growing financial burden on individuals, families and society. Moreover, the ADHD medications in use today not only cannot cure the disease but also have multiple side effects. Shifting to primary prevention of ADHD by reducing the major modifiable risk factors and optimizing maternal nutritional profiles as early as the prenatal period would largely reduce the onset of ADHD in the future. For instance, the findings for Aim 1 of the research strategy indicate that maternal dyslipidemia is an important risk factor for ADHD diagnosis in offspring. Dyslipidemia is modifiable by dietary and lifestyle changes and is treatable with pharmaceuticals. Thus, adding lipid screening to the prenatal care guidelines would offer a relatively inexpensive way to move toward the primary prevention of ADHD.

7.4.3.3 Guideline changes for cholesterol levels during pregnancy

The findings for Aim 1 underscore the need to refine the cholesterol level cut-off points for pregnant women in consideration of the potential adverse impact on fetal and child neurodevelopment. For instance, my data suggest that pregnant women should maintain a relatively higher level of HDL to meet the need for rapid fetal brain development during

pregnancy and to reduce ADHD risk; this is particularly important for the male fetus. My data indicate that the current clinical cut point for HDL (>50 mg/dL) for nonpregnant women, as recommended by the American Heart Association for reducing the risk of heart disease [33,34] may not be adequate for pregnant women for protecting against ADHD in offspring; thus, a higher cut-off point (>60 mg/dL) may be needed for identifying the fetus at risk for future ADHD.

In summary, based on the collective findings generated from the studies presented in this dissertation, I have the following clinical recommendations:

- Clinicians should provide advice to pregnant women about how to use drugs containing acetaminophen carefully.
- Clinical practice should shift from secondary- and tertiary-prevention to the primary prevention of ADHD by controlling major modifiable risk factors.
- Pregnancy-specific guidelines for optimal cholesterol levels should be developed.

7.5 CONCLUSIONS

ADHD is one of the most common neurodevelopmental disorders that imposes an enormous cost on individual, family, and society in the U.S. Unfortunately, the current understanding of this highly prevalent and costly disorder is insufficient. The exact cause of ADHD is still unknown, not to mention the biological mechanisms behind the sex difference in ADHD risk. My thesis research revealed that maternal cholesterol levels,[35] early childhood lead exposure,[36-38] and maternal plasma acetaminophen metabolite levels[18-22,39] are each highly possible to influence the risk of ADHD in offspring, using the data of mother-infant pairs already enrolled and followed in the Boston Birth Cohort (BBC).

First, I evaluated the prospective association of maternal cholesterol levels measured within a few days of delivery with the risk of ADHD diagnosis among 1479 mother-infant pairs from the

BBC. I showed that suboptimal maternal cholesterol levels might increase the risk of ADHD in offspring and that the male fetus appeared to be particularly vulnerable to suboptimal levels.

Second, I investigated the prospective associations between early childhood lead exposure and ADHD diagnosis and its potential effect modifiers among 1479 BBC mother-infant pairs. Elevated early childhood blood lead levels increased the risk of ADHD, and boys were more vulnerable than girls at a given lead level. This risk of ADHD was reduced by half if the mother had adequate high-density lipoprotein (HDL) levels or low stress during pregnancy.

Third, I examined the prospective association between maternal plasma biomarkers of acetaminophen intake measured within a few days of delivery and ADHD diagnosis in the offspring among 1180 BBC mother-infant pairs. Maternal acetaminophen use was specifically associated with a higher risk of ADHD diagnosis in offspring, not with other developmental disorders.

In conclusion, maternal low HDL levels, early childhood lead exposure, and perinatal acetaminophen use were associated with a higher risk of ADHD. The male fetus was more sensitive to both low levels of HDL and lead exposure. Maternal adequate HDL levels and low-stress levels could reduce the adverse effect of lead on the risk of ADHD. Given the observational nature of these studies, the findings are regarded as hypothesis generating rather than definitive support for causal inference. As such, these novel findings warrant additional investigations.

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Chapter 8 CURRICULUM VITAE

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Education

Doctor of Philosophy in Maternal and Child Health, Johns Hopkins Bloomberg School of Public Health, U.S. 2018
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Subject of the dissertation: Early Life Exposures and Development of ADHD in Childhood.

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Pharmacy Scholarship. **Subject of the dissertation:** Targeting Nitrosative Stress for Neurovascular Protection: New Implications in Brain Diseases. **Supervisor:** Feng Han, Ph.D.

Experience

Research Assistant, Center on the Early Origins of Disease, Johns Hopkins Bloomberg School of Public Health, U.S. 2013–
Screened early life biomarkers (from pregnancy to neonatal for both mother and baby) for baby's later life metabolic syndrome risks using a large preterm birth cohort. **Oversaw and organized a** database of human blood, stool, placenta, urine. **Contributed** to the preparation of NIH grant writing and development of theoretical frameworks. **Supported** the preparation of research findings for submission to multiple peer-reviewed publications. **Lead** independent project in early life origin of neurodevelopmental outcomes using birth cohort design.

Research Assistant, Department of Pharmacology and Pharmaceutical Sciences, University of Southern California, U.S. 2011–2013
Conducted molecular and animal experiments to investigate the risk factors of Alzheimer Disease at different life stage.

Research Assistant, Institute of Pharmacology, Toxicology and Biochemical Pharmaceuticals, Zhejiang University, China 2009–2011
Lead several student research training projects in multiple fields, such as drug synthesis, drug purity test, making sustained-release tablets, pharmacokinetics, drug development,and drug evaluation.

Leadership

Student coordinator, Center on the Early Origins of Disease, Johns Hopkins Bloomberg School of Public Health, U.S. 2013–
Team leader of the data entry team, training and supervising 4 undergraduate students and 2 master students. In charge of quality control for their work. Biostatistics support for other Ph.D. students in our team.

Analytical skills

Data management, Metabolomics, Macro in STATA, four-way decomposition, GxE analysis on GWAS, Propensity score matching, Instrumental variables, Interrupted time series, Regression discontinuity, Structural equation modeling, Exploratory factor analysis, Confirmatory factor analysis, Data visualization using Tableau, R, and d3.js

Computer skills

Analytics: R, SAS, STATA, SPSS, PLINK, MetaboAnalyst

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Personal interests

Semi-professional photographer (one of 80 attendees of the first Fujifilm Festival, New York, 2017), cooking, swimming, tennis

Publications

Manuscripts under review

[1] Maternal biomarkers of acetaminophen use and offspring attention deficit hyperactivity disorder
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