FOLATE, LEPTIN, ADIPONECTIN AND DEVELOPMENT OF AUTISM SPECTRUM DISORDER: A PROSPECTIVE BIRTH COHORT STUDY

by

Ramkripa Raghavan, MPH, MSc

A dissertation submitted to Johns Hopkins University in conformity with the requirements for the degree of Doctor of Public Health.

Baltimore, Maryland

March, 2018

ABSTRACT

Autism spectrum disorder (ASD) is a complex neurodevelopmental condition characterized by impairments in social interaction and communication, and by the presence of restrictive repetitive behavior. The precise cause of ASD is largely unknown, but is thought to be due to genetic predisposition combined with environmental factors. Emerging epidemiological studies have identified several non-genetic risk factors for ASD, many of which may have their origins during prenatal and early postnatal periods. Among these, nutritional and metabolic factors during critical early life windows may be important, but they have not been well studied in a prospective birth cohort.

To address some of these knowledge gaps, this dissertation focused on the prospective associations between: 1) maternal folate and vitamin B12 status and development of ASD in children (specific aim 1); and 2) child's leptin and adiponectin levels and development of ASD (specific aims 2 and 3). Data from the Boston Birth Cohort (BBC), a large prospective birth cohort, consisting of a predominantly urban, low-income minority population, were used. The BBC recruited mother-child pairs at birth and followed them from birth onwards for pediatric outcomes including ASD. Electronic Medical Records were used to identify children with ASD and other neurodevelopmental disorders.

For specific aim 1, maternal folate status was defined using two complementary measures: maternal self-reported intake via questionnaire and maternal plasma biomarker of folate and vitamin B12 levels. Mothers' supplement intake during pregnancy was associated with a 'U-shaped' relationship in ASD risk in their children. In addition, extremely high maternal

plasma folate and vitamin B12 levels were associated with a greater risk of developing ASD in children.

For specific aims 2 and 3, the child's plasma leptin and adiponectin levels were measured at two-time points: cord blood collected at delivery and venous blood collected in early childhood. Children with highest early childhood leptin levels (quartile 4), when compared to those with lowest levels (quartile 1), showed an increased risk of developing ASD. Extremely rapid weight gain during 1st year of life was associated with a greater ASD risk, when compared to those children whose growth was on track and this association persisted after adjusting for pertinent confounders. Early childhood leptin partially mediated the association between 1st year weight gain and ASD. However, no association with ASD was found for fetal growth pattern and cord leptin levels. On the other hand, cord adiponectin levels were inversely associated with ASD risk and this was independent of preterm birth, early childhood adiponectin and other known ASD risk factors. While higher early childhood adiponectin was also associated with a lower risk of ASD, the association attenuated after adjusting for cord adiponectin.

The findings from this dissertation, if confirmed by future studies, could have important clinical and public health implications. Notably, findings from specific aim 1 underscore the need to 1) regularly monitor folate levels in women of reproductive age (both at clinical and population level); and 2) identify an optimal plasma folate levels, so that adverse effects of both low and high levels of folate can be averted. Findings from specific aims 2 and 3 provide a basis to further explore whether biomarkers such as leptin and adiponectin, in combination with

early life growth pattern can improve our ability to detect children at high risk of developing ASD at the earliest possible age, to inform targets for early prevention.

Primary Reader: Xiaobin Wang

Secondary Readers: Anne W. Riley, M. Daniele Fallin, Li-Ching Lee, Namanjeet Ahluwalia

COMMITTEE OF FINAL THESIS READERS

Committee Members	Department
Xiaobin Wang, MD, MPH, ScD Professor and Thesis Advisor	Population, Family and Reproductive Health Johns Hopkins Bloomberg School of Public Health
Anne W. Riley, PhD Professor	Population, Family and Reproductive Health Johns Hopkins Bloomberg School of Public Health
M. Daniele Fallin, PhD Professor and Committee Chair	Mental Health Johns Hopkins Bloomberg School of Public Health
Li-Ching Lee, PhD Associate Scientist	Epidemiology Johns Hopkins Bloomberg School of Public Health
Namanjeet Ahluwalia, PhD, DSc Nutrition Monitoring Advisor	Division of Health and Nutrition Examination Surveys National Center for Health Statistics Centers for Disease Control and Prevention
Alternate Committee Members	
Lawrence J. Cheskin, MD Associate Professor	Health, Behavior and Society Johns Hopkins Bloomberg School of Public Health
Guoying Wang, MD, PhD Assistant Scientist	Population, Family and Reproductive Health Johns Hopkins Bloomberg School of Public Health
Stan Becker, PhD Professor	Population, Family and Reproductive Health Johns Hopkins Bloomberg School of Public Health

ACKNOWLEDGMENTS

First, I would like to thank Dr. Xiaobin Wang, my advisor who has been a great mentor and role model. Her mentorship and guidance has been pivotal in chiseling and nurturing me into a public health professional that I'm today. The support and inspiration that she provided me through this journey is incredible! I'm thankful for how much Dr. Wang has advocated for me at every step of the process and forever grateful for the numerous hours, both on weekdays and weekends that she spent on me. I could not have asked for a better mentor.

I'm indebted to Dr. Anne Riley for giving me a chance during my early days and providing thoughtful insights and guidance over the course of my time in the department. A special thanks to Dr. Dani Fallin for opening my eyes to the world of autism and supporting me during some of the most exciting and challenging times at Hopkins. I'm so grateful to Drs. Li-Ching Lee, David Paige and Cuilin Zhang for their insightful feedback and encouragement. I'm also thankful to Dr. Naman Ahluwalia for being such a great mentor and colleague. A special thanks to Drs. Larry Cheskin and Stan Becker for being alternates on my committee.

I owe a lot to the BBC research team, especially Drs. Xiumei Hong and Guoying Wang who provided access to BBC data and patiently answered any data and lab related questions. I'm also thankful to the Boston Medical Center team including Dr. Barry Zuckerman and Ms. Colleen Pearson, as well as the BBC field team, without whom this data would never exist.

The student funding from the Wendy Klag Center was instrumental in laying the foundation for this entire dissertation. I'm also thankful to the Department of Population, Family

and Reproductive Health for providing financial support through the Bernard and Jane Guyer Scholarship and John & Alice Chenoweth-Pate Fellowship.

I want to express my sincere thanks and gratitude to my colleagues at the Center for Nutrition Policy and Promotion, USDA who were very supportive and flexible throughout this process. Their support was instrumental in helping me complete my dissertation alongside with a demanding full-time job.

Last, but not least, I would have not begun or made it through this journey without my family. My parents, Radha and Vasudevan who planted the seed of getting a doctoral degree when was a kid and wanted to see me achieve, what they couldn't. They were truly invested in my success and flew from India multiple times to support me during my crucial exams and to take care of my family. Thanks to my in-laws, Vasanthi and Srinivasa Raghavan, who were always positive and encouraged me to realize my dream. There are no words to thank my husband Srikanth Raghavan who has been a beacon of patience, support and encouragement through the troughs and tides of my course work and dissertation. To my cute kids Bhargav and Brinda who have partially let go of their mommy, sacrificing their weekend fun, vacations these 4 years – all with a smile.

Table of Contents

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

Introduction

1.1 Background

Autism Spectrum Disorder (ASD) is a heterogeneous group of neurodevelopmental conditions characterized by impairments in social interaction and communication, and by the presence of repetitive and/or restrictive behaviors and interests (1-5). Until recently, ASD prevalence in the U.S. has been increasing steadily (6, 7). According to recent estimates in the U.S., the risk of ASD is about one in 68 individuals, with one in 42 boys (23.6 per 1,000) and one in 189 girls (5.3 per 1,000) diagnosed with ASD (4, 7-9). The precise cause of ASD is largely unknown, but is thought to be due to genetic predisposition combined with environmental factors (6, 10-12). While genetic factors are implicated in the etiology of ASD, to a large extent, ASD is also likely to be attributed to pre-, peri- and postnatal environmental factors (13). Several neuropathologic changes observed in the brain hint that ASD originates in utero; however, considering the brain's plasticity, postnatal factors may still influence ASD's natural history (14).

Maternal nutrition is critical for fetal neurodevelopment (15-18); yet, there is a dearth of research on prenatal nutritional status and ASD (13). All nutrients are important for normal brain functioning with some (e.g. B vitamins such as folate, vitamin B12 and minerals such as iodine, iron) having a greater influence than others (3, 19). Emerging research has hypothesized the role of folate status in ASD, but the evidence is largely inconclusive (20, 21). Also, other nutrients involved in one-carbon metabolism (e.g. vitamin B12) have not been assessed in the context of ASD (13).

In addition to nutritional factors, maternal metabolic conditions such as overweight, obesity and diabetes mellitus have also been linked with ASD (22-24). In addition to being a fat

depot, adipose tissue also functions as an immune and endocrine organ that is involved in metabolic responses to insults and also orchestrates inflammatory responses. Adipokines are cytokines that are predominantly produced by adipose tissue and includes leptin and adiponectin, in addition to resistin, visfatin and others (25). Leptin and adiponectin are known for their ability in mediating feeding behavior and thermogenesis; in addition, recent studies have suggested that they possess a neurotrophic role during critical developmental periods (26) with elevated levels of leptin (27-31) and decreased levels of adiponectin observed in children with ASD compared to typically developing children (32). While emerging evidence may suggest a possible association, more research is needed to understand the prospective relationships between cord blood, early childhood plasma leptin and adiponectin and ASD risk (27). Leptin and adiponectin are associated with other prenatal/postnatal conditions, such as preterm birth (33-40), fetal growth and rapid growth in infancy (28, 41) and these factors are also independently associated with ASD (42-54). However, the potential role of cord and early childhood adipokines, prenatal/postnatal growth pattern, and preterm birth has not been jointly evaluated in the context of ASD.

The timing of nutritional and metabolic insults may have a profound effect on the brain development and function (55, 56). The developing brain is especially sensitive to nutritional imbalances during late fetal/ neonatal periods and early childhood, during which there is a rapid development of several neurologic processes (19, 57). Similarly, metabolic insults during critical windows of development can have an impact on subcutaneous fat mass, insulin signaling pathways and may increase leptin expression (58). Given the importance of timing,

there is an urgent need to examine the ramifications of sub-optimal nutritional and metabolic states (58) during different critical developmental windows on ASD.

In summary, although the field of early life origins of ASD is brimming with new research, only few studies have explored the relationship between maternal nutritional status, offspring metabolic status and their association with ASD (59). The timing of these insults during critical periods of development (e.g. fetal vs. early childhood period) is also unknown. Among a small number of studies that have researched nutritional and metabolic biomarkers in children, most were done after ASD diagnosis (27, 28). As such, existing studies cannot determine if sub-optimal levels reflect active determinants in the development of ASD or a secondary phenomenon to the disease onset (27, 60).

To address these knowledge gaps, this dissertation examined the relationship between: 1) maternal nutritional status (prenatal supplement intake; folate and vitamin B12 biomarkers) and risk of developing ASD; and 2) child's metabolic biomarkers (leptin and adiponectin) measured in cord blood and venous blood during early childhood and risk of developing ASD. The existing data from the Boston Birth Cohort (BBC) were used to test the study hypotheses on the nutritional and metabolic factors implicated in ASD. The BBC is an ongoing prospective birth cohort funded continuously by the National Institutes of Health over the past 16 years. Since its inception in 1998, the BBC has been recruiting mother-infant pairs at birth and following them longitudinally to study child growth, development and health.

1.2 Specific Aims

Aim 1: To evaluate the associations between maternal multivitamin supplement intake, B-vitamin biomarkers (plasma folate and vitamin B12), and risk of developing ASD in children

Sub-aim 1a: To assess the association between maternal multivitamin supplement intake during preconception and each trimester of pregnancy and risk of developing ASD

- Hypothesis: Sub-optimal intake of maternal multivitamin supplements is associated with an increased risk of ASD.

Sub-aim 1b: To assess the association between maternal B-vitamin biomarkers measured at delivery and risk of developing ASD

- Hypothesis: Sub-optimal (both deficiency and excess) levels of B-vitamin biomarkers in mothers are associated with an increased risk of developing ASD in children.

Aim 2: To evaluate the association between cord blood, early childhood plasma leptin, and risk of developing ASD in children

Sub-aim 2a: To assess the association between cord blood, early childhood plasma leptin, and subsequent ASD risk

- Hypothesis: Higher levels of leptin in cord blood and venous blood measured in early childhood are associated with an increased ASD risk.

Sub-aim 2b: To assess the association between fetal growth, first year weight gain pattern, and subsequent ASD risk

- Hypothesis: Abnormal fetal growth and rapid first year weight gain pattern are associated with an increased ASD risk.

Sub-aim 2c: To investigate whether the association between fetal growth, first year weight gain pattern, and ASD risk is mediated by cord and early childhood leptin

- Hypothesis: Cord and early childhood leptin mediates the relationship between fetal growth and first year weight gain pattern and ASD.

Aim 3: To evaluate the association between cord blood, early childhood plasma adiponectin, and risk of developing ASD in children

Sub-aim 3a: To assess the association between adiponectin in cord blood and in early childhood plasma and subsequent ASD risk

- Hypothesis: Higher levels of adiponectin in cord blood and in early childhood plasma are associated with a decreased ASD risk.

Sub-aim 3b: To investigate the joint effects of preterm birth and cord/early childhood adiponectin on ASD risk

- Hypothesis: Cord/early childhood adiponectin and preterm birth have joint effects on ASD risk.

1.3 Conceptual Framework

Figure 1-1 presents the conceptual framework that guided this dissertation. The framework utilizes a life-course perspective to illustrate the early life origins of ASD and highlights specific time periods including pregnancy, delivery/birth and infancy/early childhood. For the purposes of this dissertation, the time period spans from conception until ASD diagnosis.

The conceptual framework is organized by specific aims. Specific aim 1 (represented in orange color) illustrates the associations between 1) prenatal multivitamin supplement intake,

2) maternal plasma folate and vitamin B12, and ASD. Although the study explored the relationship between prenatal supplement intake and maternal biomarkers, that was not the primary focus of the dissertation (as signified by the dotted line).

Specific aim 2 (represented in blue color) has multiple components. This specific aim independently studied the associations between 1) cord leptin, 2) early childhood leptin, 3) fetal growth, and 4) first year weight gain, and ASD. This dissertation also assessed the mediating role of early childhood leptin in the relationship between growth during infancy and ASD (specified by the bold blue line). The interrelationship between many of the predictors is previously known (shown in dotted line), thus is not a focus of this dissertation.

Specific aim 3 (represented in green color) assesses the relationship between 1) cord blood adiponectin, 2) early childhood plasma adiponectin, and ASD risk. Preterm birth is a well-known risk factor of ASD. Yet, the joint effects of adiponectin and preterm birth in the context of ASD have not been examined and the role of adiponectin in mediating the relationship between preterm birth and ASD has not been explored (specified in bold green line) and thus will be assessed as part of this dissertation. Although, the study evaluated the relationship between cord and early adiponectin, it was not a primary focus of this dissertation (shown in dotted line).

1.4 Dissertation Overview

This dissertation is organized in a manuscript format. Chapter 1 serves as an overall introduction to the entire project and presents the research aims, hypotheses, description of the conceptual framework and an overview of the dissertation. In Chapter 2, the literature relevant to this dissertation is elaborated. Chapter 3 describes the study population, the

measures and methods used to address each specific aim. Chapter 4 addresses Specific aim 1 with a manuscript titled “Maternal Multivitamin Intake, Plasma Folate and Vitamin B12 levels and Autism Spectrum Disorder Risk in Offspring.” Chapter 5 addresses Specific aim 2, with a manuscript titled “Cord and early childhood plasma leptin, fetal and infancy growth pattern, and development of Autism Spectrum Disorder in the Boston Birth Cohort.” Chapter 6 addresses Specific aim 3, with a manuscript titled “Cord and early childhood plasma adiponectin levels and autism risk.” Chapter 7 elaborates the public health, clinical and research implications of this dissertation. Chapter 8 summarizes the key findings and the contributions of this dissertation to the literature along with its strengths and limitations.

1.5 References

1. Leitner Y. The co-occurrence of autism and attention deficit hyperactivity disorder in children - what do we know? Frontiers in human neuroscience. 2014;8:268.
2. Murray MJ. Attention-deficit/Hyperactivity Disorder in the context of Autism spectrum disorders. Current psychiatry reports. 2010;12(5):382-8.
3. Schaevitz L, Berger-Sweeney J, Ricceri L. One-carbon metabolism in neurodevelopmental disorders: using broad-based nutraceutics to treat cognitive deficits in complex spectrum disorders. Neuroscience and biobehavioral reviews. 2014;46 Pt 2:270-84.
4. Jeste SS. Neurodevelopmental behavioral and cognitive disorders. Continuum. 2015;21(3 Behavioral Neurology and Neuropsychiatry):690-714.
5. Zhubi A, Cook EH, Guidotti A, Grayson DR. Epigenetic mechanisms in autism spectrum disorder. International review of neurobiology. 2014;115:203-44.
6. Krakowiak P, Walker CK, Bremer AA, Baker AS, Ozonoff S, Hansen RL, et al. Maternal metabolic conditions and risk for autism and other neurodevelopmental disorders. Pediatrics. 2012;129(5):e1121-8.
7. Van Naarden Braun K, Christensen D, Doernberg N, Schieve L, Rice C, Wiggins L, et al. Trends in the prevalence of autism spectrum disorder, cerebral palsy, hearing loss, intellectual disability, and vision impairment, metropolitan atlanta, 1991-2010. PloS one. 2015;10(4):e0124120.
8. Developmental Disabilities Monitoring Network Surveillance Year Principal I, Centers for Disease C, Prevention. Prevalence of autism spectrum disorder among children aged 8 years - autism and developmental disabilities monitoring network, 11 sites, United States, 2010. Morbidity and mortality weekly report Surveillance summaries. 2014;63(2):1-21.
9. Christensen DL, Baio J, Braun KV, Bilder D, Charles J, Constantino JN, et al. Prevalence and Characteristics of Autism Spectrum Disorder Among Children Aged 8 Years - Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2012. Morbidity and mortality weekly report Surveillance summaries. 2016;65(3):1-23.
10. Developmental disabilties Atlanta, GA: Centers for Disease Control and Prevention; 2016 [Available from: http://www.cdc.gov/ncbddd/developmentaldisabilities/index.html].
11. Rathod R, Kale A, Joshi S. Novel insights into the effect of vitamin B12 and omega-3 fatty acids on brain function. Journal of biomedical science. 2016;23(1):17.
12. Masi A, DeMayo MM, Glozier N, Guastella AJ. An Overview of Autism Spectrum Disorder, Heterogeneity and Treatment Options. Neurosci Bull. 2017.
13. Lyall K, Croen L, Daniels J, Fallin MD, Ladd-Acosta C, Lee BK, et al. The Changing Epidemiology of Autism Spectrum Disorders. Annu Rev Public Health. 2016.
14. Newschaffer CJ, Fallin D, Lee NL. Heritable and nonheritable risk factors for autism spectrum disorders. Epidemiol Rev. 2002;24(2):137-53.
15. Anjos T, Altmae S, Emmett P, Tiemeier H, Closa-Monasterolo R, Luque V, et al. Nutrition and neurodevelopment in children: focus on NUTRIMENTHE project. European journal of nutrition. 2013;52(8):1825-42.
16. Brown AS, Bottiglieri T, Schaefer CA, Quesenberry CP, Jr., Liu L, Bresnahan M, et al. Elevated prenatal homocysteine levels as a risk factor for schizophrenia. Archives of general psychiatry. 2007;64(1):31-9.

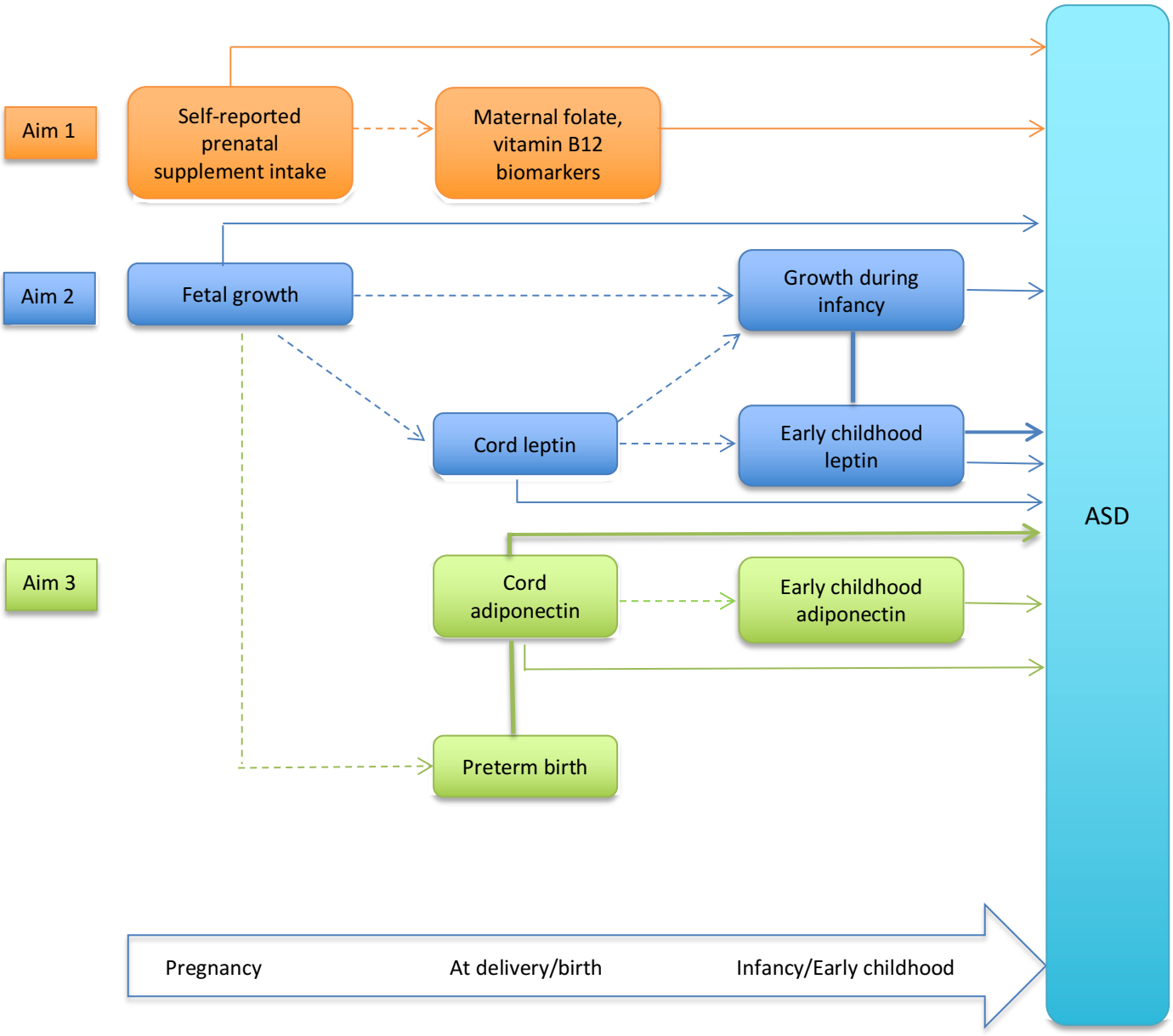
17. Rogers EJ. Has enhanced folate status during pregnancy altered natural selection and possibly Autism prevalence? A closer look at a possible link. Med Hypotheses. 2008;71(3):406-10.
18. James SJ, Melnyk S, Jernigan S, Pavliv O, Trusty T, Lehman S, et al. A functional polymorphism in the reduced folate carrier gene and DNA hypomethylation in mothers of children with autism. Am J Med Genet B Neuropsychiatr Genet. 2010;153B(6):1209-20.
19. Cusick SE, Georgieff MK. The Role of Nutrition in Brain Development: The Golden Opportunity of the "First 1000 Days". J Pediatr. 2016;175:16-21.
20. Schmidt RJ, Tancredi DJ, Ozonoff S, Hansen RL, Hartiala J, Allayee H, et al. Maternal periconceptional folic acid intake and risk of autism spectrum disorders and developmental delay in the CHARGE (CHildhood Autism Risks from Genetics and Environment) case-control study. The American journal of clinical nutrition. 2012;96(1):80-9.
21. Suren P, Roth C, Bresnahan M, Haugen M, Hornig M, Hirtz D, et al. Association between maternal use of folic acid supplements and risk of autism spectrum disorders in children. Jama. 2013;309(6):570-7.
22. Li M, Fallin MD, Riley A, Landa R, Walker SO, Silverstein M, et al. The Association of Maternal Obesity and Diabetes With Autism and Other Developmental Disabilities. Pediatrics. 2016;137(2):1-10.
23. Connolly N, Anixt J, Manning P, Ping ILD, Marsolo KA, Bowers K. Maternal metabolic risk factors for autism spectrum disorder-An analysis of electronic medical records and linked birth data. Autism Res. 2016;9(8):829-37.
24. Wang Y, Tang S, Xu S, Weng S, Liu Z. Maternal Body Mass Index and Risk of Autism Spectrum Disorders in Offspring: A Meta-analysis. Sci Rep. 2016;6:34248.
25. Mazaki-Tovi S, Romero R, Vaisbuch E, Erez O, Mittal P, Chaiworapongsa T, et al. Dysregulation of maternal serum adiponectin in preterm labor. J Matern Fetal Neonatal Med. 2009;22(10):887-904.
26. Kawwass JF, Summer R, Kallen CB. Direct effects of leptin and adiponectin on peripheral reproductive tissues: a critical review. Mol Hum Reprod. 2015;21(8):617-32.
27. Ashwood P, Kwong C, Hansen R, Hertz-Picciotto I, Croen L, Krakowiak P, et al. Brief report: plasma leptin levels are elevated in autism: association with early onset phenotype? J Autism Dev Disord. 2008;38(1):169-75.
28. Blardi P, de Lalla A, Ceccatelli L, Vanessa G, Auteri A, Hayek J. Variations of plasma leptin and adiponectin levels in autistic patients. Neuroscience letters. 2010;479(1):54-7.
29. Rodrigues DH, Rocha NP, Sousa LF, Barbosa IG, Kummer A, Teixeira AL. Changes in adipokine levels in autism spectrum disorders. Neuropsychobiology. 2014;69(1):6-10.
30. Al-Zaid FS, Alhader AA, Al-Ayadhi LY. Altered ghrelin levels in boys with autism: a novel finding associated with hormonal dysregulation. Sci Rep. 2014;4:6478.
31. Essa M.M., Braidy N., Al-Sharbati M.M., Al-Farsi YM, Ali A, Waly M.I., et al. Elevated plasma leptin levels in autisic children of Sultanate of Oman. International Journal of Biological & Medical Research. 2011;2(3):803-5.
32. Fujita-Shimizu A, Suzuki K, Nakamura K, Miyachi T, Matsuzaki H, Kajizuka M, et al. Decreased serum levels of adiponectin in subjects with autism. Progress in neuro-psychopharmacology & biological psychiatry. 2010;34(3):455-8.

33. Saito M, Nishimura K, Nozue H, Miyazono Y, Kamoda T. Changes in serum adiponectin levels from birth to term-equivalent age are associated with postnatal weight gain in preterm infants. Neonatology. 2011;100(1):93-8.
34. Oberthuer A, Donmez F, Oberhauser F, Hahn M, Hoppenz M, Hoehn T, et al. Hypoadiponectinemia in extremely low gestational age newborns with severe hyperglycemia--a matched-paired analysis. PloS one. 2012;7(6):e38481.
35. Kajantie E, Hytinantti T, Hovi P, Andersson S. Cord plasma adiponectin: a 20-fold rise between 24 weeks gestation and term. J Clin Endocrinol Metab. 2004;89(8):4031-6.
36. Lindsay RS, Walker JD, Havel PJ, Hamilton BA, Calder AA, Johnstone FD, et al. Adiponectin is present in cord blood but is unrelated to birth weight. Diabetes Care. 2003;26(8):2244-9.
37. Nakano Y, Itabashi K, Sakurai M, Aizawa M, Dobashi K, Mizuno K. Preterm infants have altered adiponectin levels at term-equivalent age even if they do not present with extrauterine growth restriction. Horm Res Paediatr. 2013;80(3):147-53.
38. Terrazzan AC, Procianoy RS, Silveira RC. Neonatal cord blood adiponectin and insulin levels in very low birth weight preterm and healthy full-term infants. J Matern Fetal Neonatal Med. 2014;27(6):616-20.
39. Yoshida T, Nagasaki H, Asato Y, Ohta T. The ratio of high-molecular weight adiponectin and total adiponectin differs in preterm and term infants. Pediatr Res. 2009;65(5):580-3.
40. Siahanidou T, Mandyla H, Papassotiriou GP, Papassotiriou I, Chrousos G. Circulating levels of adiponectin in preterm infants. Arch Dis Child Fetal Neonatal Ed. 2007;92(4):F286-90.
41. Karakosta P, Chatzi L, Plana E, Margioris A, Castanas E, Kogevinas M. Leptin levels in cord blood and anthropometric measures at birth: a systematic review and meta-analysis. Paediatr Perinat Epidemiol. 2011;25(2):150-63.
42. Schieve LA, Tian LH, Baio J, Rankin K, Rosenberg D, Wiggins L, et al. Population attributable fractions for three perinatal risk factors for autism spectrum disorders, 2002 and 2008 autism and developmental disabilities monitoring network. Ann Epidemiol. 2014;24(4):260-6.
43. Joseph RM, Korzeniewski SJ, Allred EN, O'Shea TM, Heeren T, Frazier JA, et al. Extremely low gestational age and very low birthweight for gestational age are risk factors for autism spectrum disorder in a large cohort study of 10-year-old children born at 23-27 weeks' gestation. American journal of obstetrics and gynecology. 2017;216(3):304 e1- e16.
44. Moore GS, Kneitel AW, Walker CK, Gilbert WM, Xing G. Autism risk in small- and large-for-gestational-age infants. American journal of obstetrics and gynecology. 2012;206(4):314 e1-9.
45. Padilla N, Eklof E, Martensson GE, Bolte S, Lagercrantz H, Aden U. Poor Brain Growth in Extremely Preterm Neonates Long Before the Onset of Autism Spectrum Disorder Symptoms. Cereb Cortex. 2017;27(2):1245-52.
46. Torrey EF, Dhavale D, Lawlor JP, Yolken RH. Autism and head circumference in the first year of life. Biol Psychiatry. 2004;56(11):892-4.
47. Dementieva YA, Vance DD, Donnelly SL, Elston LA, Wolpert CM, Ravan SA, et al. Accelerated head growth in early development of individuals with autism. Pediatr Neurol. 2005;32(2):102-8.

48. Dissanayake C, Bui QM, Huggins R, Loesch DZ. Growth in stature and head circumference in high-functioning autism and Asperger disorder during the first 3 years of life. Dev Psychopathol. 2006;18(2):381-93.
49. Darcy-Mahoney A, Minter B, Higgins M, Guo Y, Williams B, Head Zauche LM, et al. Probability of an Autism Diagnosis by Gestational Age. Newborn Infant Nurs Rev. 2016;16(4):322-6.
50. Schendel D, Bhasin TK. Birth weight and gestational age characteristics of children with autism, including a comparison with other developmental disabilities. Pediatrics. 2008;121(6):1155-64.
51. Kuzniewicz MW, Wi S, Qian Y, Walsh EM, Armstrong MA, Croen LA. Prevalence and neonatal factors associated with autism spectrum disorders in preterm infants. J Pediatr. 2014;164(1):20-5.
52. Angelidou A, Asadi S, Alysandratos KD, Karagkouni A, Kourembanas S, Theoharides TC. Perinatal stress, brain inflammation and risk of autism-review and proposal. BMC Pediatr. 2012;12:89.
53. Fezer GF, Matos MB, Nau AL, Zeigelboim BS, Marques JM, Liberalesso PBN. Perinatal Features of Children with Autism Spectrum Disorder. Rev Paul Pediatr. 2017;35(2):130-5.
54. Movsas TZ, Paneth N. The effect of gestational age on symptom severity in children with autism spectrum disorder. J Autism Dev Disord. 2012;42(11):2431-9.
55. Fuglestad AJ, Rao R, Georgieff MK. The role of nutrition in cognitive development [Available from: http://hirezz.com/satvenandmer.com/pdf/dha/Nutrition and Cognitive Development .pdf].
56. Langley-Evans SC. Developmental programming of health and disease. Proc Nutr Soc. 2006;65(1):97-105.
57. Georgieff MK. Nutrition and the developing brain: nutrient priorities and measurement. The American journal of clinical nutrition. 2007;85(2):614S-20S.
58. Nicholas LM, Morrison JL, Rattanatray L, Zhang S, Ozanne SE, McMillen IC. The early origins of obesity and insulin resistance: timing, programming and mechanisms. Int J Obes (Lond). 2016;40(2):229-38.
59. Neggers Y. The Relationship between Folic Acid and Risk of Autism Spectrum Disorders. Healthcare (Basel). 2014;2(4):429-44.
60. Ali A, Waly MI, Al-Farsi YM, Essa MM, Al-Sharbati MM, Deth RC. Hyperhomocysteinemia among Omani autistic children: a case-control study. Acta Biochim Pol. 2011;58(4):547-51.

Appendix

Figure 1-1 Nutritional and metabolic risk factors and ASD risk: A conceptual framework



Specific aim 1 is represented in orange; Specific aim 2 is represented in blue and Specific aim 3 is represented in green. Bold arrows represent mediation analysis. Only the parts with solid lines are the foci of this dissertation

CHAPTER 2

Literature review

2.1 Background and Significance

2.1.1 Definition and Diagnosis

ASD is a complex neurodevelopmental disorder affecting about 1.5% of the population (1). According to the *Diagnostic and Statistical Manual of Mental Disorders* (DSM), 5th edition, ASD is characterized by deficits in 1) social communication and social interaction, and 2) restricted repetitive patterns of behavior, interests, and activities (2-5). The recent DSM-5 definition encompasses the previous DSM-IV definition of autistic disorder (autism), Asperger's disorder, childhood disintegrative disorder, Rett's disorder, and pervasive developmental disorder not otherwise specified as a single diagnosis (5). However, for the purposes of this study, ASD will continue to be defined as described in DSM-IV since ASD cases in the BBC were diagnosed before DSM-5 was implemented (6-8).

One challenge to study ASD is the heterogeneity of impairments and co-morbidities, especially since autistic individuals often exhibit additional behavioral problems such as self-injury, aggression, tantrum, stereotypies, anxiety, sleep disturbances, feeding problems, non-compliance with instructions, and odd, repetitive behaviors such as hand flapping, echolalia, spinning objects, tip-toe walking and body rocking (4). National estimates suggest that 30% (historically 70%) of the children with ASD have intellectual disability (ID) and boys have a greater likelihood to have co-occurring ID when compared to girls (1, 5). Few studies to date were able to study refined subgroups of ASD due to lack of well-established approaches and sample size constraints.

Differential detection of ASD across populations due to parental awareness, and access to care has been an important concern. Several studies have shown that children who receive an early ASD diagnosis (between 18-24 months) and intervention have better outcomes with

communication, social interaction and cognitive development (9-15). However, the average age of formal diagnosis is 4 years, with economically disadvantaged and minority children identified even later (12, 16). To date, studies of ASD in urban, low income, minority children are quite limited, an important research gap to fill.

Diagnosing ASD is challenging given the lack of pathognomonic signs or biological biomarkers and is further complicated by the variety of common co-morbidities such as attention deficit hyperactivity disorder (ADHD), seizures and ID (16-18). As a result, the procedure for receiving ASD diagnosis involves a two-step process (19, 20). Level 1 universal screening is used to identify children with any type of developmental delays and assess a possibility of ASD (20). A few examples of level 1 screening instruments are Ages and Stages Questionnaire and BRIGANCE (19, 21). The American Academy of Pediatrics has recommended universal ASD screening for children between 18 and 24 months, in addition to a routine developmental surveillance (22). Level 2 screening is mainly for those that are already identified as at risk for developmental disabilities. It is also used for differentiating children with ASD from those with other type of neurodevelopmental disorders (19, 23). The discovery and utility of biomarkers to help early detection and diagnosis of ASD is increasingly becoming possible by rapid advancement in biomedical research and biotechnology and is emerging to be an exciting field to explore.

2.1.2 Prevalence and Public Health Implications

The dramatic increase in the number of children with ASD in the past few decades in the U.S. has justifiably raised concerns. The prevalence of ASD was about five per 10,000 individuals in 1980s, which increased to 4.2 per 1,000 in 1996 and eventually to about 14.6 per 1,000 in

2010 (one in 68) (1, 24-26). This average annual increase of 9.3% and cumulative increase of 269% (from 4.2/1,000 in 1996 to 15.5/1,000 in 2010) in prevalence has been attributed to a greater awareness, increase in risk factors, differences in methodologies used for estimating prevalence, changes in diagnostic practice or service availability, but true increase in ASD cases cannot be completely ruled out (2, 25-27). Much of the spike in ASD was observed in milder cases, with less drastic changes in prevalence noted in ASD cases with ID (26). ASD mostly is a life-long impairment and can have significant implications on parents, siblings, teachers and society as a whole (28). Financial burden of ASD can be tremendous on families. Recent estimates suggest that the annual direct medical, non-medical and productivity costs amounts to $268 billion in 2015 and is projected to be $461 billion for 2025 (29). The lifetime cost of supporting an individual with ASD and ID is $2.4 million and ASD without ID is $1.4 million (28).

2.1.3 Theory and Etiology

Several theories on ASD have been proposed ever since Leo Kanner (1943) and Hans Asperger (1944) described it independently and almost simultaneously (30). Most of the theories in the past explained ASD using the lens of behavioral psychology (31, 32). This slowly expanded to include underlying determinants of behavioral manifestations such as brain anatomy and physiology, genetics, prenatal factors and their interplay, although these factors have not been completely integrated with what is known about ASD behavior (30, 32). While the proposed theories are useful in understanding one aspect of ASD, not many attempts have been made to construct theories that postulate the complex pathological processes to explain the pathogenesis of ASD from its root causes to changes in the brain and resulting cognitive mechanisms, and the resulting behavior (33, 34).

Heterogeneity in clinical manifestation of ASD has long been an obstacle in explicating the underlying pathophysiology (35). The key to understanding the heterogeneity is elucidating how behavioral phenotypes are related to neurodevelopment, anatomical abnormalities and autism's fundamental causes. Ideally, a full account of the neurobiological basis of ASD and its manifestation in behavior across different levels of development would be desirable (36). However, even with the current state of limited knowledge, there is a critical need for a unified model that links neurodevelopmental and behavioral manifestations implicated in ASD, so as to properly understand the disorder as well as design treatment programs (36, 37). To fill this gap, an integrated model has been adapted from the ones proposed by Frith (2012) and Niculea and Paval (2016) (33, 38). It is important to note that this overarching model is merely a tool that "connect the dots" between different levels of deterministic factors and is not meant to be comprehensive in listing all the factors involved or provide definitive explanation (38).

Behavioral factors: ASD is primarily diagnosed based on behavioral criteria, rather than biochemical, neuroanatomical or physiological indices (39). As observed in figure 2-1, there is a high degree of "fanning-out" or divergence of clinical manifestations at the behavioral level from the cognitive level (32). Separating behavior from the underlying mechanism is an important goal of this model. One of the interesting issues with measuring behavior alone is that, no matter how reliably measured, it doesn't reveal its cause (38). Considering the variety of factors involved in ASD etiology, it is not surprising to observe a whole range of behavioral manifestations that may seem unrelated at the first glance (40). The timing of exposure, type of risk factors involved, alterations in brain connectivity can all have a role to play in the

manifestation of behavioral symptoms (33). Behaviors with same underlying cause may look different when manifested in different individuals (38) and further, ASD is associated with co-morbidities such as ADHD, motor incoordination and psychiatric symptoms that further complicates linking the behavior to neurobiological causes (30).

Cognitive factors: The next tier in the model is cognition, which serves as an interface between core dysfunction in the brain and behavior and this bridge may be critical in explaining heterogeneity in ASD (30, 40). Cognitively, ASD is perceived as a disorder with impairment in central coherence, executive functioning, theory of mind and empathizing – with none of these theories being mutually exclusive or mutually dependent (32). The following section provides a broad overview of well-accepted cognitive theories in the context of ASD-

- **Mindblindness Theory** or lack of theory of mind model posits that individuals with ASD have difficulty in understanding the minds of other people, specifically their emotions, feelings, beliefs and thoughts (41). Cognitive capacity to infer other's mental states is fundamental for social competence and profound difficulties in social interaction and communications observed in those with ASD is believed to stem from impairment of theory of mind (42). Research studies have repeatedly shown that children with ASD typically fail the theory of mind tasks, whereas their neurotypical counterparts or those with Down's syndrome pass the test. This has helped to further the understanding that ASD is not simple lack of sociability, rather a condition characterized by sociocognitive impairments (43). Theory of mind deficits may have their origins early in life and are

thought to explain language abnormalities in autism (e.g. muteness, language delays, echolalia and idiosyncratic use of language) (44, 45).

- **Weak Central Coherence Theory** is characterized by imbalance in information integration and lack of tendency to draw together diverse information to create higher-level meaning (46). The notion of lack of central coherence highlights a cognitive style in some with ASD who have a tendency to miss the "big picture" and rather focus on extreme details of information (47, 48). Further, individuals with ASD may also have difficulty integrating information in the realms of surrounding context (49).
- **Executive function** is an overarching term for functions such as working memory, impulse control, set-shifting, initiation and monitoring of action and all of these functions primarily involve activity in the prefrontal cortex of the brain (30). The deficit of executive function in ASD is characterized by the need for sameness, difficulty in switching attention, tendency to perseverate and lack of impulse control and these symptoms draw parallel to frontal lobe damage and frontal dementia (40, 50). While executive function impairment is common in ASD, it is not a universal feature thus limiting its potential to be used as a diagnostic marker (30).

Anatomical factors: This tier in the model illustrates many-to-one mapping from anatomical to cognitive level, where few core dysfunctions feed into a single node at the cognitive level. Earlier studies on brain anatomy and ASD expected to find a dramatic lesion in an otherwise normally developed brain. However, it was soon recognized that looking for anatomical anomalies specific and universal across ASD and identifying a localized lesion, such as in brain

areas associated with social/attention deficits, was inappropriate and unfruitful (32, 40). Subsequently, there was a paradigm shift with studies starting to focus on "systems-level neural system abnormalities." In line with this, cortical underconnectivity theory was proposed, which posited that the functional connectivity between frontal and posterior brain regions was lower in those with ASD (4, 48, 51). This has been proposed as a unifying theory that explains a range of deficits at the levels of psychological function, cortical function and cortical anatomy (52). Beyond cortical underconnectivity, other anatomical abnormalities are noted in cerebellum and cerebral cortex, however there is variability in direction and magnitude (5). Further, increased brain size – both in weight and volume, is often observed with ASD and is thought to be due to lack of pruning (i.e. elimination of faulty connections to optimize neural functioning) that occurs during normal reorganization of brain structure in childhood (30, 40).

Genetic, epigenetic and environmental factors: Epidemiological studies have shown that both genetic and environmental factors play a crucial role in ASD etiology (18). In the model described in figure 2-1, the farthest tier is the multitude of genetic, epigenetic factors that may be causally linked to ASD. The constellation of symptoms, its severity, and its inconsistent response to treatment across individuals with autism is suggestive that a multitude of genetic factors and multiple pathways are involved in ASD (53, 54). ASD is a complex trait with many different genetic, non-genetic risk factors at this level that converges to a limited number of core dysfunctions and therefore is unlikely to have one-to-one mapping (32, 35).

Genetics: The role of genetic factors in ASD has strongly been supported with heritability estimates ranging between 50% and 95% (5). In the past decade, several studies have identified

rare genetic variations, suggesting that polygenic and epistatic genetic factors may predispose a person to ASD (5). However, a lack of full concordance in monozygotic twins and varying concordance rates by diagnosis (55, 56) implies that genetic factors may not be sole cause of ASD (18). Further, genetic factors alone could not have driven the prevalence to epidemic proportions in just 10-20 years (57).

Epigenetics: The mechanism for the interaction of multiple early life factors with underlying genetics is unclear, but, it is postulated that epigenetics might play a role (18). Epigenetic mechanisms allow for heritable changes in the phenotype or gene expression without alteration in the primary DNA sequence and are hypothesized to be the link between gene and environment interaction (18, 58, 59). Both epigenetic and individual risk factors are necessary to allow the expression of an individual's genetic liability (60). Most critical periods associated with epigenetic regulation in ASD are believed to occur during 1) prenatal period, when active cellular proliferation and differentiation occurs; and 2) during early postnatal period, when methylation patterns are necessary to establish normal neuron network (2). DNA methylation is a commonly studied epigenetic modification that influences neurobiological processes and is important for regulating gene expression (59, 61). Studies have observed epigenetic modifications (both hypo- and hypermethylation) in brains of those with ASD (5). DNA methylation depends on the dietary sources of essential nutrients, especially folate and vitamin B12, during prenatal and early postnatal periods (18, 59, 62, 63) and recent evidence suggests that metabolic disturbances in one-carbon pathways (figure 2-2) are often observed in ASD (64, 65). As such, compared to other nutrients, folate and vitamin B12 status are worthy of particular attention.

Environmental factors: For the purposes of this model, environmental factors are described broadly to include non-genetic, biological, toxic, infectious and/or immune, and social factors (66). The absence of a single genetic cause and incomplete penetrance of known genetic factors, provides a hint that environmental factors are important for ASD risk (67). Many of these genetic risk factors are present in individuals without ASD, suggesting that while these mutations may increase ASD risk, it is likely that other risk factors are also necessary (67). Similarly, exposure to high risk environmental factors does not always result in ASD (68).

A pool of biological factors has been considered in the context of ASD. These include, but are not limited to, candidate risk factors such as advanced maternal age (69-77), advanced paternal age (78-80), maternal metabolic risk factors such as obesity (81), diabetes (82, 83), nutritional status (84-87), and prenatal maternal inflammatory disease (56, 88, 89). Environmental toxicants including exposure to air pollution (90-92) and pesticides (93, 94) have also been implicated in some studies.

It is notable to mention that social factors have been implicated in ASD. Studies in the U.S. have demonstrated preponderance towards those from a higher social class as evidenced by high socio-economic status, parental education and intellect level (71, 75, 95-98); on the other hand, some studies (mainly from Europe) have shown the contrary (99-101). Place of residence has also been linked to ASD risk, with urbanites having a greater likelihood of having ASD compared to the rural population (70, 102). Racial/ethnic disparities have been observed in many studies, with some reporting that blacks have greater rates (71, 74, 95), whereas others reporting whites/Asians having greater prevalence (25, 26, 74, 75, 97).

Linking the tiers of an integrated multilevel model: In summary, this integrated model (figure 2-1) demonstrates the involvement and interaction of predisposing factors across multiple levels and developmental periods leading to abnormal brain development and subsequent altered cognition and behavior. The model is a visual depiction of how small perturbations in genetic and epigenetic processes along with a complex interplay of multiple early life risk factors can have a downstream impact on developing brains, potentially giving rise to a spectrum of behavioral syndromes. The effect sizes for each of these factors may be small; however, an individual's genetic predisposition combined with environmental triggers may possibly create a 'perfect storm' to disrupt normal neurodevelopment (18).

The advantage of this model is that it provides an overview of pathological processes involved as opposed to focusing on one aspect of the deficit. Conducting studies using the multilevel integrated model in a life-course framework will shed light on developmental processes and will inform how genetic/epigenetic/environmental factors and morphological findings links to behavioral correlates (103). One challenge however, is that designing a study with multiple tiers may require elaborate models based on genetic, environmental, neurochemical, neurophysiological and behavioral manipulations, which may be beyond the scope of many studies – including this study.

Hence considering the availability of data, resources and time, the current study will focus on several important, yet understudied preconceptional/prenatal influences in ASD, namely nutritional (specifically folate and vitamin B12) and metabolic biomarkers (especially adipokines such as leptin and adiponectin). If one were to fit in the key components of this dissertation into the stated model (figure 2-1), it will feature in the bottom most tier along with

genetic influences. Anatomical and cognitive factors, although important, was beyond the scope of this dissertation; however, their implicit roles have been acknowledged when extrapolating the relationship between nutrition, metabolic factors and ASD. The next section will expand further on nutritional and metabolic predictors of ASD.

2.2 Nutrition in growth and neurodevelopment

Maternal and early postnatal nutrition affects the structure and function of offspring's brain development as well as later cognitive performance (65). Compared to the rest of the body, the brain develops at a rapid pace *in utero* and during the first 2 years of life making it more vulnerable to sub-optimal nutritional status (104, 105). While almost all nutrients have a role to play in ensuring proper brain development, some are more intricately involved than others. Specifically, nutrients in one-carbon metabolism, often dubbed "epi-nutrients," act as a primary methyl donor for DNA synthesis and methylation (figure 2-2) (65, 106) and are intricately involved during critical periods of brain development (107, 108). Exposure to deficient or excess levels of these nutrients during critical windows can permanently alter the brain structure thereby having a lasting impact on cognitive development (108, 109). A host of transporters and regulators play an important role in safeguarding the brain such that it does not receive too much or too little of each nutrient (107, 108).

2.2.1 Folate and vitamin B12 metabolism

Folate, vitamin B12, along with vitamins B6 and B2, are sources of co-enzymes that are involved in one carbon metabolism (110). Folate is a water-soluble vitamin that occurs naturally in foods such as leafy vegetables, legumes and red meat (111, 112). It is also produced synthetically as folic acid, which is used in supplements and food fortification programs (58).

The bioavailability of folic acid is ~70% greater than naturally available folate (113) and folic acid has to be reduced to tetrahydrofolate before it can be utilized by the body (114). As a methyl donor, folate is intricately involved in major cellular pathways including DNA synthesis and maintenance, DNA, RNA and protein methylation (58, 115). The metabolic demand for folate is high especially during pregnancy due to rapid cellular growth and development (116, 117). Placental folate transfer begins early in pregnancy and is actively transported, as evidenced by markedly higher levels of folate in cord blood when compared to maternal levels (117, 118).

Vitamin B12 is obtained by consuming animal source foods such as meat and dairy (119). Vitamin B12 (also called cobalamin) is a cobalt-containing compound that is transported by transcobalamin II, produced by liver and placenta (120). It is a co-factor for the enzyme methionine synthase in one-carbon metabolism (121). Vitamin B12 is critical for brain development and has a variety of functions such as transmethylation and maintenance of equilibrium between neurotrophic and neurotoxic factors in the central nervous system (122). Vitamin B12 also plays an important role in the formation of S-adenosylethionine (SAM) (figure 2-2) and evidence suggests that low dietary vitamin B12 is linked to developmental and neurological disorders (122). During pregnancy, vitamin B12 requirements are increased (123), while the plasma vitamin B12 level drops, possibly as a physiological response to pregnancy (124). Similar to folate, vitamin B12 is actively transported using specific-receptor carriers (120). In the U.S., the prevalence of vitamin B12 deficiency in pregnant women is relatively low (~6.6%) (125).

2.2.1.1 Role of folate in neurodevelopmental conditions

The incidence of both global and gene-specific changes in methylation patterns has instigated a lot of interest in understanding the primary pathways for one-carbon metabolites such as folate, vitamin B12 and homocysteine (2). Suboptimal folate status during the prenatal period has been associated with neurodevelopmental impairments such as neural tube defects (NTD), ID and other adverse health outcomes such as congenital heart defects, fetal growth retardation, low birth weight and preterm delivery - although the underlying mechanism for any of these conditions have not been elucidated (65, 84, 126, 127).

2.2.1.1.1 Folate and NTD

NTD are complex multifactorial disorders that impact neurulation of the brain and spinal cord, occurring between 21-28 days post-conception. While the etiology of NTD is still unclear, it is believed that both genetic and non-genetic (e.g. maternal nutrition status) factors may be implicated (128, 129). Genetic polymorphisms affecting critical components of folate metabolism have also shown to be associated with an increased the risk of NTD (130). Several trials in the 1980s and 1990s showed that exogenous supplementation of folic acid prevents NTD (128, 129). Based on this evidence the U.S. Food and Drug Administration (FDA) implemented mandatory flour fortification of folic acid in 1998. Since then, approximately 1,300 NTD births have been averted every year (131). Subsequently, there has been a sizeable increase in the intake of folic acid, which has drastically improved the folate status in the general population (129, 132). Vitamin B12 deficiency has also been shown to be associated with NTD, independent of folate (133, 134). Several studies have shown that mothers with

lowest vitamin B12 concentration have higher odds of having NTD-affected infants when compared to those with the highest levels (135, 136).

2.2.1.1.2 Folate and Down's Syndrome

There is some evidence that suggests that abnormal folate metabolism may be implicated in Down's Syndrome (137). Specifically, studies have shown that mothers of infants with Down's Syndrome have elevated plasma homocysteine when compared to the controls (138). Homocysteine is inversely correlated to folate status and is a sensitive marker of folate status. Higher frequency of polymorphisms in genes encoding the folate metabolizing enzymes (methylene tetrahydrofolate reductase (*MTHFR*) and methionine synthase reductase (MTRR)) was observed in mothers of Down's Syndrome babies (139, 140). However, not all studies have confirmed these findings (141-143).

2.2.1.1.3 Folate and ASD

In the past decade, there has been a great interest in assessing the impact of maternal folic acid consumption and ASD risk. A number of epidemiological and animal studies have been published. Below is the summary of the emerging research in this field -

Evidence from human studies: Recent epidemiological studies have shown that folic acid intake may be protective against ASD (84, 85, 87, 144). Specifically, mothers of children with ASD reported using preconception/prenatal vitamins or reported having higher levels of dietary folic acid intake and/or folic acid supplementation (84, 85, 87). Further, the greatest risk reduction was observed in subjects that were genetically susceptible to less efficient folate metabolism (84). On the other hand, two large studies conducted in the Danish Birth Cohort found no association between folic acid supplement intake before and during early pregnancy and

subsequent ASD risk in offspring (145, 146). Findings from the Generation R study demonstrated an association between folic acid supplementation and decreased ASD risk; however, this finding was not corroborated when biomarkers (maternal plasma folate levels) were assessed in the context of child's autistic traits (147). Similarly, Braun et al. showed that prenatal vitamin intake, when compared to those who did not use supplements, was associated with reduced odds of elevated Social Responsiveness Scale (SRS) scores, a scale used to identify ASD-typical behaviors. However, no association was found between maternal whole blood folate concentration and SRS scores (148). DeSoto et al. reported a contradictory finding and noted that mothers of children with ASD were more likely to report folic acid supplement intake during pregnancy (149).

A few authors have speculated that these incongruent associations could potentially be due to residual confounding (24, 146, 147). Multivitamin supplement use is closely tied to maternal education, which may be related to health conscious behaviors that could have lowered ASD risk (24, 147, 150, 151). Further, the timing of supplement intake and biomarker assessment is thought to play a role in the association with ASD risk (147, 148). Other potential reasons for these inconsistencies are elaborated in chapter 7, including lack of consideration of full range of biomarker levels from insufficiency to excess in the data analyses (152).

Evidence from ecological studies: Using Rochester Epidemiological Project data, Beard et al. showed that in Olmstead County, MN, the increased ASD incidence paralleled the prescription of high dose (1 mg) of folic acid supplementation (153). Others, such as Rogers and Leeming et al., independently speculated that the number of ASD cases have increased significantly paralleling folic acid fortification in the U.S. and explained a potential biological plausibility

involving folic acid metabolism and altered natural selection by increasing survival of infants possessing *MTHFR* C677T polymorphism (154-156). Along these lines, studies have shown that children diagnosed with ASD may have altered folate or methionine metabolism (157).

Evidence from animal models: Animal models have supported the hypothesis that elevated folic acid may be associated with adverse neurodevelopmental outcomes. Barua et al. showed that there is altered expression of several genes in the frontal cortex and cerebral hemisphere of pups, when the mothers were exposed to very high folic acid supplementation during pregnancy (158, 159). Similarly, high maternal folic acid exposure also resulted in altered DNA methylation and gene expression in offspring's cerebellum (160). Continuing such high doses through post-weaning periods resulted in behavior alterations, when compared to the offspring whose mothers were not supplemented such high doses (158). The authors hypothesized that behavior change may be possible because of altered gene expression and aberrant methylation that is potentially linked to folate over-supplementation (158).

Similarly, Girotto et al. showed that large doses of folic acid can alter brain synaptic transmission and increase offspring's susceptibility to seizures (161). Research in the chick embryo showed that folic acid potentially inhibits neurite extension, synaptogenesis and growth cone motility (162, 163). Bahous et al. demonstrated that high dietary folate is linked to pseudo-*MTHFR* deficiency, embryonic growth delay and offspring memory impairment (164).

2.2.1.2 Role of Vitamin B12 in ASD

It is well known that vitamin B12 is intimately involved in one-carbon metabolism along with folate; yet, there is a dearth of research on the role of vitamin B12 status on the developing brain (165). Several studies have shown that vitamin B12 deficiency can

detrimentally impact intracerebral functioning and is known to be associated with reduced levels of neurotrophins such as nerve growth factor and brain-derived neurotrophic factor (BDNF). The deficiency of the latter has been linked to increased oxidative stress (166) and severe retardation of myelination in the nervous system (167, 168).

A few studies have looked at biomarkers of one-carbon metabolites (e.g. homocysteine, methylmalonic acid, S-adenosylmethionine, S-adenosylhomocysteine) in children with ASD and, the methodologies and findings have been largely inconsistent (2, 169, 170). For example, some studies have shown a decline in serum folate and/or vitamin B12 (171, 172) among ASD children, while others have not confirmed a particular trend for these biomarkers (64, 169, 173-175). Nevertheless, lack of research on the association between vitamin B12 and ASD has been identified as an important research gap (24).

2.2.1.3 *MTHFR* genotype and ASD

MTHFR, an enzyme involved in one-carbon metabolism is at the crossroads of DNA methylation and synthesis (176). While some frequency of T allele and TT homozygosity exists in every population, the prevalence is highest among Hispanics and lowest in African Americans (177). One of the widely studied polymorphisms of *MTHFR* that affects neurodevelopment is *MTHFR* C677T (60). Human carriers of *MTHFR* C677T polymorphisms have reduced enzyme activity (by about 60%) and they are frequently seen to have increased plasma homocysteine (62, 176, 178) and decreased folate levels in circulation (179). Several studies have observed higher frequency of the C677T allele in children with ASD (174, 180). In a meta-analysis conducted by Pu et al., *MTHFR* C677T polymorphism was associated with increased risk among children, however, only in countries without folic acid fortification (60). Along these lines,

Schmidt et al., showed that an increased ASD risk was observed with *MTHFR* 677TT maternal genotype, only when mothers did not use preconception/prenatal supplements (84).

2.3 Role of Metabolic factors in ASD risk

Adipose tissue is a highly active endocrine organ that produces signaling molecules called adipokines, which are implicated in adiposity, inflammation, food intake and insulin sensitivity (181, 182). Emerging evidence suggests that adipose tissue may play an important role linking poor fetal growth and subsequent development of adverse conditions (183). Adipose tissue programming has its origins in prenatal and postnatal periods. There are 20 different types of adipokines that are broadly categorized into the following: 1) primarily produced in the adipose tissue (e.g. leptin, resistin); 2) exclusively produced in the adipose tissue (e.g. adiponectin); 3) mainly produced in other tissues or organs, but is also produced by adipose tissue (e.g. TNF-α) (182, 184). The source of adipokines may change over the course of the lifespan as observed in the case of adiponectin that is secreted in multiple tissues in fetal life (e.g. skeletal muscle fibers, small intestine and arterial walls) (185), whereas produced exclusively by adipose tissue in adulthood (184).

The role of adipokines in neurological dysfunction is increasingly recognized and is thought to facilitate the cross-communication between immune system and nervous system (66). Adipokines are pleiotropic and can influence the development and function of the nervous system. Specifically, they are involved in progenitor cell differentiation, cellular migration within the nervous system, synaptic formation, as well as higher order neurological function such as cognition and memory (66). They have the ability to control the nature, duration and intensity of the immune response (66).

Adipokines are intricately involved in metabolic conditions (such as obesity and diabetes) and are important risk factors for adverse outcomes that are often accompanied by other complications such as hyperglycemia, insulin resistance and untoward fetal consequences (186, 187). While there are several adipokines, for the purposes of this study, two of these were mainly considered: leptin, a pro-inflammatory cytokine; and adiponectin, an anti-inflammatory cytokine. Leptin and adiponectin are metabolic counterparts, which act in parallel yet in opposing directions (188). Both leptin and adiponectin are critical for fetal and postnatal development (189, 190). They have a multitude of other functions such as energy homeostasis and are associated with insulin resistance and BMI (191, 192).

The next section begins with a discussion of fetal and infant growth, followed by the physiology of adipokines in early life, as well as other factors that perturb the balances of these adipokines. Given that adipokines are intricately involved in growth and maturation, the discussion on prenatal and postnatal growth is placed first to provide some context, before delving deep into subsequent discussions on adipokines.

2.3.1 Fetal and infant growth

2.3.1.1 Fetal growth

Intrauterine growth restriction (IUGR) is defined as the failure of the fetus to achieve its intrinsic growth potential, possibly attributed to anatomical and/or functional disorders and diseases in the feto-placental-maternal unit (190, 193, 194). Small for gestational age (SGA), defined as birth weight <10th percentile of the population, is often used as a proxy for IUGR (194). IUGR can be categorized into: 1) symmetrical IUGR with weight, length and head circumference diminished, usually indicative of a process originating early in pregnancy; 2)

asymmetrical IUGR, characterized by brain sparing and a normal head circumference. The latter process may occur much later in pregnancy and is often related to impaired uteroplacental function or nutrient deficiency (190). IUGR represents a tremendous heterogeneity in terms of etiology, severity, body proportionality and the prognosis of IUGR infants may vary depending on the cause (195). In developed countries like the U.S., IUGR is mostly a manifestation of maternal, fetal and uterine factors that may have led to poor placental function (194). The metabolic conditions associated with IUGR may have an endocrine origin, which may be accompanied by alteration in subsequent hormonal bioavailability (190). Further, IUGR may initiate changes in fetal metabolism in order to provide immediate survival advantage, which may or may not be beneficial in the long-term (196). IUGR is known to compromise fetal adipose tissue development with IUGR infants having a marked reduction in body fat mass (190).

IUGR and neurodevelopment: Compromised neurodevelopment is one of the notable outcomes observed in IUGR. Compared to children that are appropriate for gestational age (AGA), those with SGA may have impaired neurodevelopment and are often associated with immature neurobehavioral scores, lower IQ and language problems, executive function, visual motor skills, poor academic outcomes and behaviours problems (193, 194, 197-199). SGA infants may also have reduced head circumference and diminished total and regional brain volumes compared to normal infants (194).

IUGR and ASD: Emerging research suggests that SGA could be a risk factor for ASD (200-203). While the underlying mechanism is yet to be elucidated, the association is thought to be due to an insult that could have limited fetal growth as well as impacted neurological development

(202). Specifically, studies have hypothesized that genetic polymorphism, prenatal risk factors such as fetal hypoxia, placental pathology, pre-eclampsia or infections during pregnancy or maternal risk factors such as smoking and substance abuse could have links to both SGA and neuropsychiatric morbidity (201, 202).

While the association between SGA and ASD may seem biologically plausible, some studies have not replicated these findings or have reported inconsistent associations (72, 204-206). For example, Langridge et al. demonstrated that the percentage of optimal birth weight, a measure of fetal growth (207), was not associated with ASD, especially among those without ID (204). Glasson et al. also showed no association between SGA and ASD (72), while Schnedel et al., noted that the relationship was observed only in girls and not in boys (206). Similarly, Larrson et al. observed that the association between fetal growth and ASD attenuated after adjusting for other covariates (205). The reason for this inconsistency may be due to methodological differences, lack of control for both prenatal and postnatal confounding factors such as infancy rapid weight gain, and sample size variations (201, 206). Further, studies could have considered IUGR as one homogeneous group, which could have precluded them from observing associations.

2.3.1.2 Growth in infancy

Fetal growth restriction has short- and long-term auxological and metabolic consequences (208). A well-known short-term consequence of IUGR is postnatal catch-up growth, which is a compensatory process to the thinness or smallness at birth (208, 209). About 70%-90% of SGA infants demonstrate catch-up growth during first few years of life, typically between 6 months to 2 years (210). Fetal growth restriction has been shown to correlate with

growth velocity at one month of age (211). However, the extent of catch-up growth that is related to IUGR and the exact time window when it becomes detrimental is unknown (208).

Weight gain during infancy and ASD: Rapid weight gain during infancy and early childhood has been associated with the risk of ASD (212-214). Studies have shown that accelerated weight gain may not be an isolated phenomenon, but is suggestive of a macrosomic endophenotype that may include rapid increase in head circumference and length (215-217). There is a correlation between neural and non-neural endophenotypes, with weight strongly predicting head circumference at all ages (214). Post-birth macrocephaly (defined as cranial circumference >97th percentile) is one of the characteristic anatomical features that have been linked to ASD in at least 14% to 36% of the subjects (212, 214, 216, 218-220). The precise time of onset of overgrowth is still unclear; but evidence suggests that overgrowth is noticeable between 6-12 months, which predates the onset of any overt behavioural symptoms (215, 221).

Early generalized overgrowth is a unique morphological feature of ASD and may not be observed in other developmental disabilities (215). Studies have shown that rapid overgrowth predicts later behavioural and cognitive development with steeper increase in body size associated with lower verbal and nonverbal score at 4 years of age (215) and increased head circumference linked to delayed onset of language (222), but this association has not been consistent (221). Several neurobiological factors including growth factors, neurotrophic factors, hormones and neurotransmitters have been hypothesized to play a role in excessive growth, although direct evidence is still being developed (214, 216). Since there are no known biomarkers for ASD, early life growth pattern alone, or in combination with neurobiological factors can potentially have a high predictive value in detecting ASD risk (214, 215).

2.3.2 Leptin

Leptin is a peptide hormone that is predominantly secreted by adipose tissue and has been studied in the context of regulating satiety, energy homeostasis, body composition, and overall fetal growth and development (188, 223, 224). Circulating leptin levels reflects body weight and adiposity, during fetal as well as late infancy time points (225). Leptin has been identified as one of the critical hormonal factors that may mediate developmental malprogramming (226-228). Alterations in leptin availability during the periods of developmental plasticity can underlie some of the adverse developmental changes (226). Leptin has recently been shown to regulate neuroendocrine functions and the immune system (188, 229, 230), making it a pleiotropic hormone (231).

Maternal and fetal leptin: Leptin has an important role in pregnancy, as evidenced by a two-fold increase in levels during the prenatal period, paralleling gradual increase in body mass index throughout gestation (224). Leptin levels are altered in pregnancy-related pathologies such as diabetes mellitus and placental insufficiency in preeclampsia (232, 233). Many studies have shown that maternal leptin levels are not correlated with cord leptin levels (234, 235).

Leptin is produced by maternal and fetal adipose tissues as well as placenta, with most of the placental leptin (~95%) delivered into maternal circulation and only ~5% secreted into fetal circulation (224). Fetal leptin is independent of maternal levels and is produced by white adipose tissue (236, 237), although some studies have noted a positive correlation between placental and cord leptin (224). Leptin production may start very early in pregnancy, with its presence observed as early as 6-10 weeks of gestation (224, 236). Fetal levels may be low during the first half of the gestation and increase rapidly during the last part of third trimester, paralleling the significant increase of adipose mass (224).

Umbilical cord leptin is strongly correlated with gestational age, birth weight and head circumference (227, 238-240). Preterm babies have lower cord leptin when compared to term infants (224, 237, 241). Serum leptin levels dramatically increase after ~34 weeks of gestation (241-243), prior to which babies exhibit negligible leptin levels (241). Studies have repeatedly shown that umbilical cord leptin is markedly different between SGA, AGA and large for gestational age (LGA) babies (236), with the former group having the lowest levels of leptin (224, 239, 244-246). On the other hand, LGA babies have higher cord leptin levels than AGA, which correlates well with the ponderal index (224). The lower leptin level in IUGR has been attributed to reduced fat mass and/or diminished placental production (190). Other factors that influence cord leptin levels include gender, family history of obesity and maternal gestational diabetes (224).

Cord and early childhood leptin: Cord blood leptin levels are inversely related to the rate of change in BMI (247, 248). In other words, lower cord leptin levels mediate faster weight and height gain at 6 months of age (224, 240, 249, 250). Emerging evidence suggests that leptin during neonatal period does not inhibit appetite, but rather promotes rapid growth and weight gain (224). This may be an adaptive leptin resistance response, possibly reflecting catch-up growth (224). The diminished leptin levels at birth may provide a cue for catch-up growth by reduced inhibition of satiety (251). Further, lower cord leptin levels are associated with higher leptin levels at 3 years of age and higher degree of obesity and linear growth (240).

Studies have assessed leptin levels in breastfed vs. formula fed infants, however the evidence has been equivocal (224). Some studies have reported lower leptin (252, 253) or no difference (254) in breastfed infants whereas others have reported higher levels (255). In the

latter study, the authors had speculated that leptin's presence in human milk might have contributed to higher leptin concentration in breast-fed infants (255). There is some indication that breast milk leptin may play a role in growth, appetite regulation, at least during the first few months of life (224).

The role of fetal leptin may be very different from that of mature leptin (236). Leptin at 3 years of age is positively correlated with BMI and measures of overall and central adiposity (240), possibly indicating the onset of leptin resistance (256). In a rat-model, Coupe et al. demonstrated that rapid postnatal growth (subsequent to IUGR) could trigger reduced leptin sensitivity (257). Thus, hyperleptinemia may indeed be a consequence of adverse fetal or early postnatal environment (257, 258). Mechanistically, leptin resistance has been attributed to a variety of factors including compromised transport of leptin across the blood brain barrier, impaired negative feedback mechanism, endoplasmic reticulum stress or intracellular leptin signal saturation (230, 256). In summary, the auxological and metabolic alterations from prenatal to postnatal period can be recapitulated as follows: SGA children have low cord leptin levels, which in turn may be associated with accelerated postnatal weight gain, which could predispose individuals to leptin resistance and potentially subsequent adverse health outcomes (228).

Leptin in neurodevelopment: Leptin has an important role in neurodevelopment and psychopathology of conditions including depression, anxiety, schizophrenia, ASD and Rett syndrome (233). Emerging research suggests that leptin is secreted in the brain where it seems to possess paracrine and/autocrine functions (233). Leptin can also cross the blood-brain barrier using a receptor-mediator transport (259). Leptin receptors are expressed in different

regions including, but not limited to, the midbrain, hippocampus, hindbrain, basolateral amygdyla, hypothalamus (231, 233) and has an important effect on proliferation, maintenance of glial and neuronal cells (227). Beyond its role as a metabolic regulator, leptin promotes establishment of hypothalamic circuitry, as well as cortical and hippocampal development (227, 230). Rodents with leptin insensitivity have been observed to possess deficits in long-term potentiation and long-term depression (260). Animal model suggests that brain weight as well as brain protein and DNA contents are diminished in leptin deficiency (224).

Leptin's impact on brain development may be mediated by its role in activating proinflammatory cytokines, which are known to be increased in ASD (229, 233). In a leptin resistant state, there is increased production of leptin, which are associated with an increase in proinflammatory cytokines (233). Inflammatory cytokines are implicated in impaired higher neurological functions such as memory and cognition, in addition to being involved in impaired brain development, synaptic functioning including processes of differentiation, migration, proliferation and impairments in behavior (261). Thus, abnormal inflammatory activity and imbalance of cytokines during development can adversely impact brain development, neural activity and could contribute to behavioral and neurological dysfunction in ASD (66, 261).

Recent studies have shown that children with ASD have significantly higher plasma leptin than controls, with more pronounced effects observed in those with early onset ASD (188, 229, 262-264). In a small subset of subjects, genetic correlation has been observed between ASD and leptin coding (265). A study conducted on autopsy tissues showed that there is a marked increase in leptin levels in anterior cingulate gyrus of the subjects with ASD (266).

Further, leptin is also known to suppress serotonin synthesis, which is reported in ASD, possibly suggesting a biological mechanism through which leptin may be involved (233).

2.3.3 Adiponectin

Adiponectin is a benign adipokine that possesses anti-diabetic, anti-atherogenic and anti-inflammatory properties (267). Adiponectin is an abundantly circulated and constitutes of 0.01% of total serum protein (268). Adiponectin exists in three major forms: trimer, hexamer and high molecular weight (HMW) multimer (269, 270). The biological functions vary by the type of adiponectin multimer (269) with the HMW being the most biologically active form (270). The anti-inflammatory properties of adiponectin are exerted by suppressing TNF-α secretion, by inhibiting its transcription and stimulating IL-10 and IL-1 receptor antagonist production. IL-10 is an anti-inflammatory cytokine that inhibits the production of pro-inflammatory cytokines (271).

Maternal and fetal adiponectin: Maternal adiponectin levels are altered throughout pregnancy, mirroring maternal insulin sensitivity (184). When compared to pregravid state, serum adiponectin levels are increased in early pregnancy, followed by a decline through the rest of the pregnancy (184). Maternal adiponectin levels are altered in conditions such as gestational diabetes, preeclampsia and preterm labor (181, 184, 272, 273). Maternal and fetal adiponectin have very different roles to play. For example, maternal adiponectin decreases placental insulin signaling and regulates fetal growth (274); whereas, fetal adiponectin increases fetal adiposity and growth (184).

Cord adiponectin levels are 2-3 times higher than maternal adiponectin (271). However, the correlation between maternal and fetal adiponectin is inconclusive (184, 185, 240, 275-

278). Emerging evidence suggests that adiponectin is mainly derived from the fetus and not from maternal or placenta tissues (185, 275). This is supported by the possibility that HMW multimer, the main form of adiponectin in the fetus, cannot pass through the placental barrier (278, 279).

Adiponectin expression in brown adipose tissue is observed as early as 14 weeks. Cord adiponectin levels increase with gestational age, with almost a 20-fold increase observed between 24 and 40 weeks (185, 280). Studies have shown that beyond adipocytes, fetal adiponectin is expressed in multiple tissues of mesodermic origin (such as skeletal muscle fibers, smooth muscle cells of small intestine and arterial walls and connective tissues) and ectodermal origin (epidermis and lens) (185). The pattern of adiponectin expression in the fetus is markedly different than adults. While higher adiponectin in adults is associated with lower fat percentage, an opposite relationship is observed prenatally, with higher adiponectin levels associated with higher percentage body fat in the fetus (281) and anthropometric measurements (278). The higher levels of adiponectin in newborns, when compared to adults may be due a variety of factors including lack of adipocyte hypertrophy, relatively low percentage of body fat or a different distribution of fat depots in newborn (275).

Adiponectin levels are related to both gestational age and birth weight (184, 240, 282), and studies have shown that preterm infants having substantially lower adiponectin when compared to their term counterparts (283-285). Recently, Terrazzan et al. showed that cord adiponectin levels are lower in very low birth weight preterm infants, when compared to full-term infants (286). Similarly, Visentin et al. showed that adiponectin levels were lower in IUGR fetuses, when compared to SGA and AGA (183). Many studies have hypothesized that

adiponectin may play an important role in fetal growth, although the precise mechanism is unclear (185, 281). Higher cord adiponectin is associated with slower weight gain during the first six months of life (240, 278).

***Adiponectin in infancy and early childhood*:** Adiponectin levels begin to increase during the first month of life, before it begins to decline (239). Specifically, between ages 1 and 2, fasting adiponectin levels decrease by almost 25%, which has been attributed to increase in adiposity (287). Decline in adiponectin is steep for children that were SGA and that exhibited rapid catch-up growth (287, 288). Hypoadiponetinemia in children is thought to be a consequence of increase in body fat with several studies showing that adiponectin is inversely associated with central adiposity (239, 240, 289). Studies on human milk adiponectin and infant growth has been conflicting with some research showing that higher adiponectin is associated with lower infant weight and weight-for-length *z*-scores during the first six months of life (290); whereas others demonstrating that lower adiponectin exposure were associated with lower weight by 2 years of age (291, 292).

Children who were born SGA have lower adiponectin levels, even when compared to obese children – who are known to possess lower adiponectin levels (288). Postnatal catch-up growth is associated with lower adiponectin levels. It has thus been speculated that intrauterine programming may permanently affect adiponectin secretion (288).

Adiponectin in neurodevelopment: Earlier studies speculated that adiponectin cannot pass through the blood brain barrier and thus is not found in the brain (293). However, recent studies have demonstrated that adiponectin is detectable in the brain after an IV injection of full-length adiponectin in an animal model (293, 294). There is a lack of clarity on whether

adiponectin is synthesized intrathecally or whether it flows into the intrathecal space from plasma crossing the blood brain barrier (293).

Compared to plasma, cerebrospinal fluid adiponectin is 100-times lower, but this is compensated by higher affinity to adiponectin receptors AdipoR1 and AdipoR2 that are found in the cortex, hypothalamus, pituitary glands and the cerebrospinal fluid (270, 295-297). Adiponectin has specific functions including regulation of neurogenesis, neurotrophic actions, promotion of adaptive neuroplasticity and protection of human neuroblastoma cells against cytotoxicity and neurotoxicity (295, 296, 298). Adiponectin deficiency is thought to curtail dendritic growth and spine density in hippocampal dentate gyrus in which neural progenitor cell proliferation and differentiation is inhibited (297).

Adiponectin is known to exhibit neuroprotective functions with adiponectin treatment in mice shown to protect the integrity of blood brain barrier and improve neurobehavioral performance and focal cerebral neurogenesis (297). Adiponectin may also possess higher brain functions. For example, diminished adiponectin levels are associated with clinically significant affective episodes and subjects with major depressive symptoms are known to have lower adiponectin levels (298). In addition, adiponectin knockout mice have exhibited depressive-like behavior (297). Further, hypoadiponectenemia is thought to increase sympathetic nervous system activity – which is observed in depression (299).

Adiponectin and ASD: Emerging evidence suggests that adiponectin levels may be altered in subjects diagnosed with ASD (192, 262). Fujita-Shimizu et al. noted that adiponectin levels are lower in children with ASD, when compared to normal controls. They also observed a negative correlation between adiponectin and measure of social development in children, assessed using

Autism Diagnostic Interview Revised (192). The authors speculated that altered serotonin neurotransmission, observed in children with ASD, could have impacted serum adiponectin levels (192). Rodrigues et al. showed that adiponectin was associated with severity of symptoms assessed by the Social Responsiveness Scale, although there were no differences in adiponectin levels between cases and controls (262). In a longitudinal study, Blardi et al. observed that adiponectin levels declined in subjects with Rett syndrome over time (191); however, the authors did not find a difference in adiponectin levels in ASD subjects, after excluding those with Rett Syndrome (188). It is interesting to note that adiponectin levels are diminished in those with ADHD (300).

2.5 Role of Timing in ASD

Perinatal period, infancy and early childhood demonstrate heightened plasticity that paves the way for subsequent development of anatomic, physiological and behavioral conditions (301, 302). During this period of opportunity and vulnerability, several aspects of the brain undergo rapid anatomic and functional expansion (302). Studies have consistently shown that insults that retard or accelerate development during specific windows of the "first 1000 days" may reprogram tissue structure and function leading to abnormal architecture of the brain (303). These in turn predispose an individual to future behavioral problems, learning difficulties, atypical or delayed cognitive development (303).

The brain is a heterogeneous organ comprised of anatomic regions that have their own developmental trajectories (304). The majority of the brain's structure and capacity is established during fetal life and early childhood. Maintaining a well-orchestrated developmental trajectory is not only important for establishing behaviors aided by these

regions, but it is also critical to ensure a time-coordinated development of brain areas that will soon start to work together as neural circuits (304).

There are “critical periods” and “sensitive periods” along the developmental trajectory of brain regions (304). A sensitive period is an epoch when the brain is more vulnerable to environmental factors, but is not necessarily deterministic. Critical period is an early life epoch when an impact in the brain (or brain regions) by an environmental factor may have an irreversible impact (304). Given that brain is not a homogeneous organ, the vulnerability of a developing brain is determined by factors such as timing of the insult and the extent of impact (305, 306). Among the external factors that influence early brain development, optimal nutrition and reduction of toxic stress and inflammation have been thought to have profound effects (304).

2.5.1 Timing of ASD onset

A centrally important question with regard to ASD is whether its onset occurs in prenatal period or the first few months of the postnatal life or years after birth (307, 308). Although the precise timing is unknown, emerging evidence is pointing to prenatal period as a vulnerable window for ASD onset (307-309). While earlier studies highlighted the vulnerability of early organogenesis (308, 310), recent studies are highlighting the importance of mid- and late-gestation periods (311-313). The developing brain is particularly vulnerable to insults between later half of gestation (24 and 42 weeks) and early postnatal period, because of the rapid development of several neurological processes (including synapse formation and myelination) (109). Despite the brain’s plasticity to recover and repair itself in early life, insults during this critical period may have a profound impact, which may result in brain dysfunction

(109). A study by Arora et al. on the timing of exposure during discrete prenatal and postnatal periods supported the critical role of later time periods in pregnancy (311). A study conducted by Beversdorf et al. noted that significant increases in stressors were reported between 21-32 weeks of gestation among mothers of ASD children when compared to the controls (314).

While the prenatal period is important, another viewpoint suggests that the vulnerable window could depend on 1) the risk factor, and 2) susceptibility for ASD at a given time, and this time period could stretch from conception to first years of life (315). Several postnatal factors have been studied in ASD, including respiratory infection, urinary infection and auditory deficit (316, 317). A recent study by Hazlett et al. suggested that very early postnatal hyper-expansion of cortical surface areas may be critical for ASD development (318). Thus hyper-expansion of cortical surface may be one of the early steps that trigger the cascade of events leading to brain over-growth, characteristic of ASD (318).

From the developmental stand point, late fetal to early postnatal period marks the rapid differentiation of brain regions including striatum, cerebellum, and limbic system including hippocampus, dopaminergic and glutamatergic neurotransmission systems, and prefrontal cortex, which may all be implicated in ASD (302, 306). The integrity of these regions is key for establishing connections for maturation of structures that subsequently support complex behaviors, including working memory and executive function (302). The limbic system has especially been of interest for ASD researchers because of the ways in which this region has been connected in social and emotional functioning of humans and primates (319). The critical brain wide processes including myelination, synaptogenesis, and development of the dopamine neurotransmitter system that are acutely impacted in ASD, are also spurred late in gestation

(302, 320-322). In summary, the existing evidence suggests that timing matters in ASD. However, more research is needed to identify the critical time periods for specific environmental risk or protective factors in early life that are deterministic to ASD, and to ascertain sensitive time windows that may be conducive for specific types of intervention.

2.6 References

1. Christensen DL, Baio J, Braun KV, Bilder D, Charles J, Constantino JN, et al. Prevalence and Characteristics of Autism Spectrum Disorder Among Children Aged 8 Years - Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2012. Morbidity and mortality weekly report Surveillance summaries. 2016;65(3):1-23.
2. Schaevitz LR, Berger-Sweeney JE. Gene-environment interactions and epigenetic pathways in autism: the importance of one-carbon metabolism. ILAR journal / National Research Council, Institute of Laboratory Animal Resources. 2012;53(3-4):322-40.
3. Veenstra-VanderWeele J, Blakely RD. Networking in autism: leveraging genetic, biomarker and model system findings in the search for new treatments. Neuropsychopharmacology. 2012;37(1):196-212.
4. Kana RK, Libero LE, Moore MS. Disrupted cortical connectivity theory as an explanatory model for autism spectrum disorders. Phys Life Rev. 2011;8(4):410-37.
5. Lyall K, Croen L, Daniels J, Fallin MD, Ladd-Acosta C, Lee BK, et al. The Changing Epidemiology of Autism Spectrum Disorders. Annu Rev Public Health. 2016.
6. Sandin S, Lichtenstein P, Kuja-Halkola R, Larsson H, Hultman CM, Reichenberg A. The familial risk of autism. Jama. 2014;311(17):1770-7.
7. Hallmayer J, Cleveland S, Torres A, Phillips J, Cohen B, Torigoe T, et al. Genetic heritability and shared environmental factors among twin pairs with autism. Archives of general psychiatry. 2011;68(11):1095-102.
8. Murray MJ. Attention-deficit/Hyperactivity Disorder in the context of Autism spectrum disorders. Current psychiatry reports. 2010;12(5):382-8.
9. Howard JS, Sparkman CR, Cohen HG, Green G, Stanislaw H. A comparison of intensive behavior analytic and eclectic treatments for young children with autism. Res Dev Disabil. 2005;26(4):359-83.
10. McEachin JJ, Smith T, Lovaas OI. Long-term outcome for children with autism who received early intensive behavioral treatment. Am J Ment Retard. 1993;97(4):359-72; discussion 73-91.
11. Sallows GO, Graupner TD. Intensive behavioral treatment for children with autism: four-year outcome and predictors. Am J Ment Retard. 2005;110(6):417-38.
12. Barton ML, Dumont-Mathieu T, Fein D. Screening young children for autism spectrum disorders in primary practice. J Autism Dev Disord. 2012;42(6):1165-74.
13. Dawson G. Early behavioral intervention, brain plasticity, and the prevention of autism spectrum disorder. Dev Psychopathol. 2008;20(3):775-803.
14. Dawson G, Rogers S, Munson J, Smith M, Winter J, Greenson J, et al. Randomized, controlled trial of an intervention for toddlers with autism: the Early Start Denver Model. Pediatrics. 2010;125(1):e17-23.
15. Lovaas OI. Behavioral treatment and normal educational and intellectual functioning in young autistic children. J Consult Clin Psychol. 1987;55(1):3-9.
16. Gura GF, Champagne MT, Blood-Siegfried JE. Autism spectrum disorder screening in primary care. J Dev Behav Pediatr. 2011;32(1):48-51.

17. Sunita, Bilszta JL. Early identification of autism: a comparison of the Checklist for Autism in Toddlers and the Modified Checklist for Autism in Toddlers. J Paediatr Child Health. 2013;49(6):438-44.
18. Ciernia AV, LaSalle J. The landscape of DNA methylation amid a perfect storm of autism aetiologies. Nat Rev Neurosci. 2016;17(7):411-23.
19. Norris M, Lecavalier L. Screening accuracy of Level 2 autism spectrum disorder rating scales. A review of selected instruments. Autism. 2010;14(4):263-84.
20. Hampton J, Strand PS. A Review of Level 2 Parent-Report Instruments Used to Screen Children Aged 1.5-5 for Autism: A Meta-Analytic Update. J Autism Dev Disord. 2015;45(8):2519-30.
21. Barnard-Brak L, Richman DM, Chesnut SR, Little TD. Social Communication Questionnaire scoring procedures for autism spectrum disorder and the prevalence of potential social communication disorder in ASD. Sch Psychol Q. 2016;31(4):522-33.
22. Council on Children With D, Section on Developmental Behavioral P, Bright Futures Steering C, Medical Home Initiatives for Children With Special Needs Project Advisory C. Identifying infants and young children with developmental disorders in the medical home: an algorithm for developmental surveillance and screening. Pediatrics. 2006;118(1):405-20.
23. Duda M, Daniels J, Wall DP. Clinical Evaluation of a Novel and Mobile Autism Risk Assessment. J Autism Dev Disord. 2016;46(6):1953-61.
24. DeVilbiss EA, Gardner RM, Newschaffer CJ, Lee BK. Maternal folate status as a risk factor for autism spectrum disorders: a review of existing evidence. The British journal of nutrition. 2015:1-10.
25. Developmental Disabilities Monitoring Network Surveillance Year Principal I, Centers for Disease C, Prevention. Prevalence of autism spectrum disorder among children aged 8 years - autism and developmental disabilities monitoring network, 11 sites, United States, 2010. Morbidity and mortality weekly report Surveillance summaries. 2014;63(2):1-21.
26. Van Naarden Braun K, Christensen D, Doernberg N, Schieve L, Rice C, Wiggins L, et al. Trends in the prevalence of autism spectrum disorder, cerebral palsy, hearing loss, intellectual disability, and vision impairment, metropolitan atlanta, 1991-2010. PloS one. 2015;10(4):e0124120.
27. Tordjman S, Somogyi E, Coulon N, Kermarrec S, Cohen D, Bronsard G, et al. Gene x Environment interactions in autism spectrum disorders: role of epigenetic mechanisms. Front Psychiatry. 2014;5:53.
28. Buescher AV, Cidav Z, Knapp M, Mandell DS. Costs of autism spectrum disorders in the United Kingdom and the United States. JAMA Pediatr. 2014;168(8):721-8.
29. Leigh JP, Du J. Brief Report: Forecasting the Economic Burden of Autism in 2015 and 2025 in the United States. J Autism Dev Disord. 2015;45(12):4135-9.
30. Hill EL, Frith U. Understanding autism: insights from mind and brain. Philos Trans R Soc Lond B Biol Sci. 2003;358(1430):281-9.
31. Belmonte MK, Allen G, Beckel-Mitchener A, Boulanger LM, Carper RA, Webb SJ. Autism and abnormal development of brain connectivity. J Neurosci. 2004;24(42):9228-31.
32. Belmonte MK, Cook EH, Jr., Anderson GM, Rubenstein JL, Greenough WT, Beckel-Mitchener A, et al. Autism as a disorder of neural information processing: directions for research and targets for therapy. Mol Psychiatry. 2004;9(7):646-63.

33. Niculae AS, Paval D. From molecules to behavior: An integrative theory of autism spectrum disorder. Med Hypotheses. 2016;97:74-84.
34. Boucher J. The Autistic Spectrum: Characteristics, Causes and Practical Issues: SAGE; 2009.
35. Chaste P, Leboyer M. Autism risk factors: genes, environment, and gene-environment interactions. Dialogues Clin Neurosci. 2012;14(3):281-92.
36. Frith U. Cognitive explanations of autism. Acta Paediatr Suppl. 1996;416:63-8.
37. Wozniak RH, Leezenbaum NB, Northrup JB, West KL, Iverson JM. The development of autism spectrum disorders: variability and causal complexity. Wiley Interdiscip Rev Cogn Sci. 2017;8(1-2).
38. Frith U. Why we need cognitive explanations of autism. Q J Exp Psychol (Hove). 2012;65(11):2073-92.
39. Moy SS, Nadler JJ. Advances in behavioral genetics: mouse models of autism. Mol Psychiatry. 2008;13(1):4-26.
40. Frith U, Happe F. Autism spectrum disorder. Curr Biol. 2005;15(19):R786-90.
41. Fletcher-Watson S, McConnell F, Manola E, McConachie H. Interventions based on the Theory of Mind cognitive model for autism spectrum disorder (ASD). Cochrane Database Syst Rev. 2014(3):CD008785.
42. Senju A. Spontaneous theory of mind and its absence in autism spectrum disorders. Neuroscientist. 2012;18(2):108-13.
43. Happe F, Conway JR. Recent progress in understanding skills and impairments in social cognition. Curr Opin Pediatr. 2016;28(6):736-42.
44. Frith U. Mind blindness and the brain in autism. Neuron. 2001;32(6):969-79.
45. Hamilton K, Hoogenhout M, Malcolm-Smith S. Neurocognitive considerations when assessing Theory of Mind in Autism Spectrum Disorder. J Child Adolesc Ment Health. 2016;28(3):233-41.
46. Levy F. Theories of autism. Aust N Z J Psychiatry. 2007;41(11):859-68.
47. Cognitive Theories Explaining ASD: Interactive Autism Network; [Available from: https://iancommunity.org/cs/understanding_research/cognitive_theories_explaining_asds.
48. Just MA, Cherkassky VL, Keller TA, Kana RK, Minshew NJ. Functional and anatomical cortical underconnectivity in autism: evidence from an FMRI study of an executive function task and corpus callosum morphometry. Cereb Cortex. 2007;17(4):951-61.
49. Ellis Weismer S, Haebig E, Edwards J, Saffran J, Venker CE. Lexical Processing in Toddlers with ASD: Does Weak Central Coherence Play a Role? J Autism Dev Disord. 2016;46(12):3755-69.
50. Rajendran G, Mitchell P. Cognitive theories of autism. Developmental Review. 2007;27(2):224-60.
51. Just MA, Cherkassky VL, Keller TA, Minshew NJ. Cortical activation and synchronization during sentence comprehension in high-functioning autism: evidence of underconnectivity. Brain. 2004;127(Pt 8):1811-21.
52. Just MA, Keller TA, Malave VL, Kana RK, Varma S. Autism as a neural systems disorder: a theory of frontal-posterior underconnectivity. Neuroscience and biobehavioral reviews. 2012;36(4):1292-313.

53. Gliga T, Jones EJ, Bedford R, Charman T, Johnson MH. From early markers to neuro-developmental mechanisms of autism. Dev Rev. 2014;34(3):189-207.
54. Hendren RL, Bertoglio K, Ashwood P, Sharp F. Mechanistic biomarkers for autism treatment. Med Hypotheses. 2009;73(6):950-4.
55. Field SS. Interaction of genes and nutritional factors in the etiology of autism and attention deficit/hyperactivity disorders: a case control study. Med Hypotheses. 2014;82(6):654-61.
56. Rangasamy S, D'Mello SR, Narayanan V. Epigenetics, autism spectrum, and neurodevelopmental disorders. Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics. 2013;10(4):742-56.
57. Deth R, Muratore C, Benzecry J, Power-Charnitsky VA, Waly M. How environmental and genetic factors combine to cause autism: A redox/methylation hypothesis. Neurotoxicology. 2008;29(1):190-201.
58. Crider KS, Yang TP, Berry RJ, Bailey LB. Folate and DNA methylation: a review of molecular mechanisms and the evidence for folate's role. Adv Nutr. 2012;3(1):21-38.
59. Montano C, Taub MA, Jaffe A, Briem E, Feinberg JI, Trygvadottir R, et al. Association of DNA Methylation Differences With Schizophrenia in an Epigenome-Wide Association Study. JAMA Psychiatry. 2016;73(5):506-14.
60. Pu D, Shen Y, Wu J. Association between MTHFR gene polymorphisms and the risk of autism spectrum disorders: a meta-analysis. Autism Res. 2013;6(5):384-92.
61. McCullough LE, Miller EE, Mendez MA, Murtha AP, Murphy SK, Hoyo C. Maternal B vitamins: effects on offspring weight and DNA methylation at genomically imprinted domains. Clin Epigenetics. 2016;8:8.
62. DeVilbiss EA, Gardner RM, Newschaffer CJ, Lee BK. Maternal folate status as a risk factor for autism spectrum disorders: a review of existing evidence. The British journal of nutrition. 2015;114(5):663-72.
63. Kok DE, Dhonukshe-Rutten RA, Lute C, Heil SG, Uitterlinden AG, van der Velde N, et al. The effects of long-term daily folic acid and vitamin B12 supplementation on genome-wide DNA methylation in elderly subjects. Clin Epigenetics. 2015;7:121.
64. Pasca SP, Dronca E, Kaucsar T, Craciun EC, Endreffy E, Ferencz BK, et al. One carbon metabolism disturbances and the C677T MTHFR gene polymorphism in children with autism spectrum disorders. J Cell Mol Med. 2009;13(10):4229-38.
65. Schaevitz L, Berger-Sweeney J, Ricceri L. One-carbon metabolism in neurodevelopmental disorders: using broad-based nutraceutics to treat cognitive deficits in complex spectrum disorders. Neuroscience and biobehavioral reviews. 2014;46 Pt 2:270-84.
66. Goines PE, Ashwood P. Cytokine dysregulation in autism spectrum disorders (ASD): possible role of the environment. Neurotoxicol Teratol. 2013;36:67-81.
67. Onore C, Careaga M, Ashwood P. The role of immune dysfunction in the pathophysiology of autism. Brain Behav Immun. 2012;26(3):383-92.
68. Caspi A, Moffitt TE. Gene-environment interactions in psychiatry: joining forces with neuroscience. Nat Rev Neurosci. 2006;7(7):583-90.
69. Sandin S, Hultman CM, Kolevzon A, Gross R, MacCabe JH, Reichenberg A. Advancing maternal age is associated with increasing risk for autism: a review and meta-analysis. Journal of the American Academy of Child and Adolescent Psychiatry. 2012;51(5):477-86 e1.

70. Leonard H, Glasson E, Nassar N, Whitehouse A, Bebbington A, Bourke J, et al. Autism and intellectual disability are differentially related to sociodemographic background at birth. PloS one. 2011;6(3):e17875.
71. Croen LA, Grether JK, Selvin S. Descriptive epidemiology of autism in a California population: who is at risk? J Autism Dev Disord. 2002;32(3):217-24.
72. Glasson EJ, Bower C, Petterson B, de Klerk N, Chaney G, Hallmayer JF. Perinatal factors and the development of autism: a population study. Archives of general psychiatry. 2004;61(6):618-27.
73. Croen LA, Najjar DV, Fireman B, Grether JK. Maternal and paternal age and risk of autism spectrum disorders. Archives of pediatrics & adolescent medicine. 2007;161(4):334-40.
74. DiGuiseppi CG, Daniels JL, Fallin DM, Rosenberg SA, Schieve LA, Thomas KC, et al. Demographic profile of families and children in the Study to Explore Early Development (SEED): Case-control study of autism spectrum disorder. Disability and health journal. 2016.
75. Windham GC, Anderson MC, Croen LA, Smith KS, Collins J, Grether JK. Birth prevalence of autism spectrum disorders in the San Francisco Bay area by demographic and ascertainment source characteristics. J Autism Dev Disord. 2011;41(10):1362-72.
76. Shelton JF, Tancredi DJ, Hertz-Picciotto I. Independent and dependent contributions of advanced maternal and paternal ages to autism risk. Autism Res. 2010;3(1):30-9.
77. King MD, Fountain C, Dakhlallah D, Bearman PS. Estimated autism risk and older reproductive age. American journal of public health. 2009;99(9):1673-9.
78. Lee BK, McGrath JJ. Advancing parental age and autism: multifactorial pathways. Trends Mol Med. 2015;21(2):118-25.
79. Idring S, Magnusson C, Lundberg M, Ek M, Rai D, Svensson AC, et al. Parental age and the risk of autism spectrum disorders: findings from a Swedish population-based cohort. Int J Epidemiol. 2014;43(1):107-15.
80. Quinlan CA, McVeigh KH, Driver CR, Govind P, Karpati A. Parental Age and Autism Spectrum Disorders Among New York City Children 0-36 Months of Age. Matern Child Health J. 2015;19(8):1783-90.
81. Suren P, Gunnes N, Roth C, Bresnahan M, Hornig M, Hirtz D, et al. Parental obesity and risk of autism spectrum disorder. Pediatrics. 2014;133(5):e1128-38.
82. Li M, Fallin MD, Riley A, Landa R, Walker SO, Silverstein M, et al. The Association of Maternal Obesity and Diabetes With Autism and Other Developmental Disabilities. Pediatrics. 2016;137(2):1-10.
83. Xiang AH, Wang X, Martinez MP, Walthall JC, Curry ES, Page K, et al. Association of maternal diabetes with autism in offspring. Jama. 2015;313(14):1425-34.
84. Schmidt RJ, Hansen RL, Hartiala J, Allayee H, Schmidt LC, Tancredi DJ, et al. Prenatal vitamins, one-carbon metabolism gene variants, and risk for autism. Epidemiology. 2011;22(4):476-85.
85. Suren P, Roth C, Bresnahan M, Haugen M, Hornig M, Hirtz D, et al. Association between maternal use of folic acid supplements and risk of autism spectrum disorders in children. Jama. 2013;309(6):570-7.
86. Lyall K, Munger KL, O'Reilly EJ, Santangelo SL, Ascherio A. Maternal dietary fat intake in association with autism spectrum disorders. American journal of epidemiology. 2013;178(2):209-20.

87. Schmidt RJ, Tancredi DJ, Ozonoff S, Hansen RL, Hartiala J, Allayee H, et al. Maternal periconceptional folic acid intake and risk of autism spectrum disorders and developmental delay in the CHARGE (CHildhood Autism Risks from Genetics and Environment) case-control study. The American journal of clinical nutrition. 2012;96(1):80-9.
88. Le Belle JE, Sperry J, Ngo A, Ghochani Y, Laks DR, Lopez-Aranda M, et al. Maternal inflammation contributes to brain overgrowth and autism-associated behaviors through altered redox signaling in stem and progenitor cells. Stem cell reports. 2014;3(5):725-34.
89. Patterson PH. Maternal infection and immune involvement in autism. Trends Mol Med. 2011;17(7):389-94.
90. Roberts AL, Lyall K, Hart JE, Laden F, Just AC, Bobb JF, et al. Perinatal air pollutant exposures and autism spectrum disorder in the children of Nurses' Health Study II participants. Environmental health perspectives. 2013;121(8):978-84.
91. Roberts S. Have the short-term mortality effects of particulate matter air pollution changed in Australia over the period 1993-2007? Environmental pollution. 2013;182:9-14.
92. Volk HE, Lurmann F, Penfold B, Hertz-Picciotto I, McConnell R. Traffic-related air pollution, particulate matter, and autism. JAMA Psychiatry. 2013;70(1):71-7.
93. Roberts EM, English PB, Grether JK, Windham GC, Somberg L, Wolff C. Maternal residence near agricultural pesticide applications and autism spectrum disorders among children in the California Central Valley. Environmental health perspectives. 2007;115(10):1482-9.
94. Shelton JF, Geraghty EM, Tancredi DJ, Delwiche LD, Schmidt RJ, Ritz B, et al. Neurodevelopmental disorders and prenatal residential proximity to agricultural pesticides: the CHARGE study. Environmental health perspectives. 2014;122(10):1103-9.
95. Bhasin TK, Schendel D. Sociodemographic risk factors for autism in a US metropolitan area. J Autism Dev Disord. 2007;37(4):667-77.
96. Maenner MJ, Arneson CL, Durkin MS. Socioeconomic disparity in the prevalence of autism spectrum disorder in Wisconsin. WMJ : official publication of the State Medical Society of Wisconsin. 2009;108(5):253-5.
97. Thomas P, Zahorodny W, Peng B, Kim S, Jani N, Halperin W, et al. The association of autism diagnosis with socioeconomic status. Autism. 2012;16(2):201-13.
98. Durkin MS, Maenner MJ, Meaney FJ, Levy SE, DiGuiseppi C, Nicholas JS, et al. Socioeconomic inequality in the prevalence of autism spectrum disorder: evidence from a U.S. cross-sectional study. PloS one. 2010;5(7):e11551.
99. Delobel-Ayoub M, Ehlinger V, Klapouszczak D, Maffre T, Raynaud JP, Delpierre C, et al. Socioeconomic Disparities and Prevalence of Autism Spectrum Disorders and Intellectual Disability. PloS one. 2015;10(11):e0141964.
100. Rai D, Lewis G, Lundberg M, Araya R, Svensson A, Dalman C, et al. Parental socioeconomic status and risk of offspring autism spectrum disorders in a Swedish population-based study. Journal of the American Academy of Child and Adolescent Psychiatry. 2012;51(5):467-76 e6.
101. Emerson E. Deprivation, ethnicity and the prevalence of intellectual and developmental disabilities. Journal of epidemiology and community health. 2012;66(3):218-24.
102. Lauritsen MB, Astrup A, Pedersen CB, Obel C, Schendel DE, Schieve L, et al. Urbanicity and autism spectrum disorders. J Autism Dev Disord. 2014;44(2):394-404.

103. Sussman D, Leung RC, Vogan VM, Lee W, Trelle S, Lin S, et al. The autism puzzle: Diffuse but not pervasive neuroanatomical abnormalities in children with ASD. Neuroimage Clin. 2015;8:170-9.
104. Nyaradi A, Li J, Hickling S, Foster J, Oddy WH. The role of nutrition in children's neurocognitive development, from pregnancy through childhood. Frontiers in human neuroscience. 2013;7:97.
105. Black MM. Effects of vitamin B12 and folate deficiency on brain development in children. Food and nutrition bulletin. 2008;29(2 Suppl):S126-31.
106. Meiser J, Vazquez A. Give it or take it: the flux of one-carbon in cancer cells. FEBS J. 2016;283(20):3695-704.
107. Fuglestad AJ, Rao R, Georgieff MK. The role of nutrition in cognitive development [Available from: http://hirezz.com/satvenandmer.com/pdf/dha/Nutrition and Cognitive Development .pdf.
108. Georgieff MK, Brunette KE, Tran PV. Early life nutrition and neural plasticity. Dev Psychopathol. 2015;27(2):411-23.
109. Georgieff MK. Nutrition and the developing brain: nutrient priorities and measurement. The American journal of clinical nutrition. 2007;85(2):614S-20S.
110. Selhub J. Folate, vitamin B12 and vitamin B6 and one carbon metabolism. J Nutr Health Aging. 2002;6(1):39-42.
111. Supplements OoD. Folate: ODS, NIH; 2016 [Available from: https://ods.od.nih.gov/factsheets/Folate-HealthProfessional/.
112. Ami N, Bernstein M, Boucher F, Rieder M, Parker L, Canadian Paediatric Society DT, et al. Folate and neural tube defects: The role of supplements and food fortification. Paediatr Child Health. 2016;21(3):145-54.
113. De-Regil LM, Fernandez-Gaxiola AC, Dowswell T, Pena-Rosas JP. Effects and safety of periconceptional folate supplementation for preventing birth defects. Cochrane Database Syst Rev. 2010(10):CD007950.
114. Smith AD, Kim YI, Refsum H. Is folic acid good for everyone? The American journal of clinical nutrition. 2008;87(3):517-33.
115. Appling DR. Compartmentation of folate-mediated one-carbon metabolism in eukaryotes. FASEB J. 1991;5(12):2645-51.
116. Plumptre L, Masih SP, Ly A, Aufreiter S, Sohn KJ, Croxford R, et al. High concentrations of folate and unmetabolized folic acid in a cohort of pregnant Canadian women and umbilical cord blood. The American journal of clinical nutrition. 2015;102(4):848-57.
117. Solanky N, Requena Jimenez A, D'Souza SW, Sibley CP, Glazier JD. Expression of folate transporters in human placenta and implications for homocysteine metabolism. Placenta. 2010;31(2):134-43.
118. Kalhan SC. One carbon metabolism in pregnancy: Impact on maternal, fetal and neonatal health. Mol Cell Endocrinol. 2016;435:48-60.
119. Finkelstein JL, Layden AJ, Stover PJ. Vitamin B-12 and Perinatal Health. Adv Nutr. 2015;6(5):552-63.
120. Dominguez-Salas P, Cox SE, Prentice AM, Hennig BJ, Moore SE. Maternal nutritional status, C(1) metabolism and offspring DNA methylation: a review of current evidence in human subjects. Proc Nutr Soc. 2012;71(1):154-65.

121. Kalhan SC. One-carbon metabolism, fetal growth and long-term consequences. Nestle Nutr Inst Workshop Ser. 2013;74:127-38.
122. Bailey RL, Durazo-Arvizu RA, Carmel R, Green R, Pfeiffer CM, Sempos CT, et al. Modeling a methylmalonic acid-derived change point for serum vitamin B-12 for adults in NHANES. The American journal of clinical nutrition. 2013;98(2):460-7.
123. Milman N, Byg KE, Bergholt T, Eriksen L, Hvas AM. Cobalamin status during normal pregnancy and postpartum: a longitudinal study comprising 406 Danish women. Eur J Haematol. 2006;76(6):521-5.
124. Varela-Moreiras G, Murphy MM, Scott JM. Cobalamin, folic acid, and homocysteine. Nutrition reviews. 2009;67 Suppl 1:S69-72.
125. Fothergill A, Finkelstein J. Vitamin B12 Status in Women of Reproductive Age, NHANES 2013–2014. The FASEB Journal. 2017;31.
126. Bailey LB, Stover PJ, McNulty H, Fenech MF, Gregory JF, 3rd, Mills JL, et al. Biomarkers of Nutrition for Development-Folate Review. J Nutr. 2015;145(7):1636S-80S.
127. Tamura T, Goldenberg RL, Chapman VR, Johnston KE, Ramey SL, Nelson KG. Folate status of mothers during pregnancy and mental and psychomotor development of their children at five years of age. Pediatrics. 2005;116(3):703-8.
128. Barua S, Kuizon S, Junaid MA. Folic acid supplementation in pregnancy and implications in health and disease. Journal of biomedical science. 2014;21:77.
129. Pitkin RM. Folate and neural tube defects. The American journal of clinical nutrition. 2007;85(1):285S-8S.
130. Crider KS, Bailey LB, Berry RJ. Folic acid food fortification-its history, effect, concerns, and future directions. Nutrients. 2011;3(3):370-84.
131. Williams J, Mai CT, Mulinare J, Isenburg J, Flood TJ, Ethen M, et al. Updated estimates of neural tube defects prevented by mandatory folic Acid fortification - United States, 1995-2011. MMWR Morb Mortal Wkly Rep. 2015;64(1):1-5.
132. Heseker HB, Mason JB, Selhub J, Rosenberg IH, Jacques PF. Not all cases of neural-tube defect can be prevented by increasing the intake of folic acid. The British journal of nutrition. 2009;102(2):173-80.
133. Simpson JL, Bailey LB, Pietrzik K, Shane B, Holzgreve W. Micronutrients and women of reproductive potential: required dietary intake and consequences of dietary deficiency or excess. Part I--Folate, Vitamin B12, Vitamin B6. J Matern Fetal Neonatal Med. 2010;23(12):1323-43.
134. Ray JG, Blom HJ. Vitamin B12 insufficiency and the risk of fetal neural tube defects. QJM. 2003;96(4):289-95.
135. Ray JG, Wyatt PR, Thompson MD, Vermeulen MJ, Meier C, Wong PY, et al. Vitamin B12 and the risk of neural tube defects in a folic-acid-fortified population. Epidemiology. 2007;18(3):362-6.
136. Molloy AM, Kirke PN, Troendle JF, Burke H, Sutton M, Brody LC, et al. Maternal vitamin B12 status and risk of neural tube defects in a population with high neural tube defect prevalence and no folic Acid fortification. Pediatrics. 2009;123(3):917-23.
137. Cuckle HS. Primary prevention of Down's syndrome. Int J Med Sci. 2005;2(3):93-9.

138. James SJ, Pogribna M, Pogribny IP, Melnyk S, Hine RJ, Gibson JB, et al. Abnormal folate metabolism and mutation in the methylenetetrahydrofolate reductase gene may be maternal risk factors for Down syndrome. The American journal of clinical nutrition. 1999;70(4):495-501.
139. O'Leary VB, Parle-McDermott A, Molloy AM, Kirke PN, Johnson Z, Conley M, et al. MTRR and MTHFR polymorphism: link to Down syndrome? Am J Med Genet. 2002;107(2):151-5.
140. Hobbs CA, Sherman SL, Yi P, Hopkins SE, Torfs CP, Hine RJ, et al. Polymorphisms in genes involved in folate metabolism as maternal risk factors for Down syndrome. Am J Hum Genet. 2000;67(3):623-30.
141. Coppede F. The genetics of folate metabolism and maternal risk of birth of a child with Down syndrome and associated congenital heart defects. Front Genet. 2015;6:223.
142. Amorim MR, Castilla EE, Orioli IM. Is there a familial link between Down's syndrome and neural tube defects? Population and familial survey. BMJ. 2004;328(7431):84.
143. Chango A, Fillon-Emery N, Mircher C, Blehaut H, Lambert D, Herbeth B, et al. No association between common polymorphisms in genes of folate and homocysteine metabolism and the risk of Down's syndrome among French mothers. The British journal of nutrition. 2005;94(2):166-9.
144. Levine SZ, Kodesh A, Viktorin A, Smith L, Uher R, Reichenberg A, et al. Association of Maternal Use of Folic Acid and Multivitamin Supplements in the Periods Before and During Pregnancy With the Risk of Autism Spectrum Disorder in Offspring. JAMA Psychiatry. 2018.
145. Virk J, Liew Z, Olsen J, Nohr EA, Catov JM, Ritz B. Preconceptional and prenatal supplementary folic acid and multivitamin intake and autism spectrum disorders. Autism. 2016;20(6):710-8.
146. Strom M, Granstrom C, Lyall K, Ascherio A, Olsen SF. Research Letter: Folic acid supplementation and intake of folate in pregnancy in relation to offspring risk of autism spectrum disorder. Psychol Med. 2017:1-7.
147. Steenweg-de Graaff J, Ghassabian A, Jaddoe VW, Tiemeier H, Roza SJ. Folate concentrations during pregnancy and autistic traits in the offspring. The Generation R Study. European journal of public health. 2015;25(3):431-3.
148. Braun JM, Froehlich T, Kalkbrenner A, Pfeiffer CM, Fazili Z, Yolton K, et al. Brief report: are autistic-behaviors in children related to prenatal vitamin use and maternal whole blood folate concentrations? J Autism Dev Disord. 2014;44(10):2602-7.
149. DeSoto MC, Hitlan RT. Synthetic folic acid supplementation during pregnancy may increase the risk of developing autism. Journal of Pediatric Biochemistry. 2012:251-61.
150. Rock CL. Multivitamin-multimineral supplements: who uses them? The American journal of clinical nutrition. 2007;85(1):277S-9S.
151. Nilsen RM, Vollset SE, Gjessing HK, Magnus P, Meltzer HM, Haugen M, et al. Patterns and predictors of folic acid supplement use among pregnant women: the Norwegian Mother and Child Cohort Study. The American journal of clinical nutrition. 2006;84(5):1134-41.
152. Daniels JL. Considerations for Studying Folate Beyond the Typical Range of Exposure. Paediatric and perinatal epidemiology. 2017.
153. Beard CM, Panser LA, Katusic SK. Is excess folic acid supplementation a risk factor for autism? Med Hypotheses. 2011;77(1):15-7.
154. Leeming RJ, Lucock M. Autism: Is there a folate connection? J Inherit Metab Dis. 2009;32(3):400-2.

155. Rogers EJ. Has enhanced folate status during pregnancy altered natural selection and possibly Autism prevalence? A closer look at a possible link. Med Hypotheses. 2008;71(3):406-10.
156. Beaudet AL, Goin-Kochel RP. Some, but not complete, reassurance on the safety of folic acid fortification. The American journal of clinical nutrition. 2010;92(6):1287-8.
157. Neggers Y. The Relationship between Folic Acid and Risk of Autism Spectrum Disorders. Healthcare (Basel). 2014;2(4):429-44.
158. Barua S, Chadman KK, Kuizon S, Buenaventura D, Stapley NW, Ruocco F, et al. Increasing maternal or post-weaning folic acid alters gene expression and moderately changes behavior in the offspring. PloS one. 2014;9(7):e101674.
159. Barua S, Kuizon S, Chadman KK, Flory MJ, Brown WT, Junaid MA. Single-base resolution of mouse offspring brain methylome reveals epigenome modifications caused by gestational folic acid. Epigenetics Chromatin. 2014;7(1):3.
160. Barua S, Kuizon S, Brown WT, Junaid MA. DNA Methylation Profiling at Single-Base Resolution Reveals Gestational Folic Acid Supplementation Influences the Epigenome of Mouse Offspring Cerebellum. Front Neurosci. 2016;10:168.
161. Girotto F, Scott L, Avchalumov Y, Harris J, Iannattone S, Drummond-Main C, et al. High dose folic acid supplementation of rats alters synaptic transmission and seizure susceptibility in offspring. Sci Rep. 2013;3:1465.
162. Wiens D. Could folic acid influence growth cone motility during the development of neural connectivity? Neurogenesis (Austin). 2016;3(1):e1230167.
163. Wiens D, DeWitt A, Kosar M, Underriner C, Finsand M, Freese M. Influence of Folic Acid on Neural Connectivity during Dorsal Root Ganglion Neurogenesis. Cells Tissues Organs. 2016;201(5):342-53.
164. Bahous RH, Jadavji NM, Deng L, Cosin-Tomas M, Lu J, Malysheva O, et al. High dietary folate in pregnant mice leads to pseudo-MTHFR deficiency and altered methyl metabolism, with embryonic growth delay and short-term memory impairment in offspring. Hum Mol Genet. 2017;26(5):888-900.
165. Zhang Y, Hodgson NW, Trivedi MS, Abdolmaleky HM, Fournier M, Cuenod M, et al. Decreased Brain Levels of Vitamin B12 in Aging, Autism and Schizophrenia. PloS one. 2016;11(1):e0146797.
166. Rathod R, Kale A, Joshi S. Novel insights into the effect of vitamin B12 and omega-3 fatty acids on brain function. Journal of biomedical science. 2016;23(1):17.
167. Lovblad K, Ramelli G, Remonda L, Nirkko AC, Ozdoba C, Schroth G. Retardation of myelination due to dietary vitamin B12 deficiency: cranial MRI findings. Pediatric radiology. 1997;27(2):155-8.
168. Dror DK, Allen LH. Effect of vitamin B12 deficiency on neurodevelopment in infants: current knowledge and possible mechanisms. Nutrition reviews. 2008;66(5):250-5.
169. Adams JB, Audhya T, McDonough-Means S, Rubin RA, Quig D, Geis E, et al. Nutritional and metabolic status of children with autism vs. neurotypical children, and the association with autism severity. Nutr Metab (Lond). 2011;8(1):34.
170. Pu D, Shen Y, Wu J. Association between MTHFR gene polymorphisms and the risk of Autism Spectrum Disorders: A Meta-Analysis. Autism Research. 2013;6(384 - 392).

171. Tu WJ, Yin CH, Guo YQ, Li SO, Chen H, Zhang Y, et al. Serum homocysteine concentrations in Chinese children with autism. Clin Chem Lab Med. 2013;51(2):e19-22.
172. Ali A, Waly MI, Al-Farsi YM, Essa MM, Al-Sharbati MM, Deth RC. Hyperhomocysteinemia among Omani autistic children: a case-control study. Acta Biochim Pol. 2011;58(4):547-51.
173. Ramaekers VT, Blau N, Sequeira JM, Nassogne MC, Quadros EV. Folate receptor autoimmunity and cerebral folate deficiency in low-functioning autism with neurological deficits. Neuropediatrics. 2007;38(6):276-81.
174. Frustaci A, Neri M, Cesario A, Adams JB, Domenici E, Dalla Bernardina B, et al. Oxidative stress-related biomarkers in autism: systematic review and meta-analyses. Free Radic Biol Med. 2012;52(10):2128-41.
175. Melnyk S, Fuchs GJ, Schulz E, Lopez M, Kahler SG, Fussell JJ, et al. Metabolic imbalance associated with methylation dysregulation and oxidative damage in children with autism. J Autism Dev Disord. 2012;42(3):367-77.
176. Rai V. Association of methylenetetrahydrofolate reductase (MTHFR) gene C677T polymorphism with autism: evidence of genetic susceptibility. Metab Brain Dis. 2016;31(4):727-35.
177. Wang X, Fu J, Li Q, Zeng D. Geographical and Ethnic Distributions of the MTHFR C677T, A1298C and MTRR A66G Gene Polymorphisms in Chinese Populations: A Meta-Analysis. PloS one. 2016;11(4):e0152414.
178. Kaluzna-Czaplinska J, Zurawicz E, Michalska M, Rynkowski J. A focus on homocysteine in autism. Acta Biochim Pol. 2013;60(2):137-42.
179. O'Donovan CB, Walsh MC, Forster H, Woolhead C, Celis-Morales C, Fallaize R, et al. The impact of MTHFR 677C --> T risk knowledge on changes in folate intake: findings from the Food4Me study. Genes Nutr. 2016;11:25.
180. Mohammad NS, Jain JM, Chintakindi KP, Singh RP, Naik U, Akella RR. Aberrations in folate metabolic pathway and altered susceptibility to autism. Psychiatr Genet. 2009;19(4):171-6.
181. Mazaki-Tovi S, Romero R, Vaisbuch E, Erez O, Mittal P, Chaiworapongsa T, et al. Dysregulation of maternal serum adiponectin in preterm labor. J Matern Fetal Neonatal Med. 2009;22(10):887-904.
182. Maiorana A, Del Bianco C, Cianfarani S. Adipose Tissue: A Metabolic Regulator. Potential Implications for the Metabolic Outcome of Subjects Born Small for Gestational Age (SGA). Rev Diabet Stud. 2007;4(3):134-46.
183. Visentin S, Lapolla A, Londero AP, Cosma C, Dalfra M, Camerin M, et al. Adiponectin levels are reduced while markers of systemic inflammation and aortic remodelling are increased in intrauterine growth restricted mother-child couple. Biomed Res Int. 2014;2014:401595.
184. Aye IL, Powell TL, Jansson T. Review: Adiponectin--the missing link between maternal adiposity, placental transport and fetal growth? Placenta. 2013;34 Suppl:S40-5.
185. Corbetta S, Bulfamante G, Cortelazzi D, Barresi V, Cetin I, Mantovani G, et al. Adiponectin expression in human fetal tissues during mid- and late gestation. J Clin Endocrinol Metab. 2005;90(4):2397-402.
186. Korkmaz L, Bastug O, Kurtoglu S. Maternal Obesity and its Short- and Long-Term Maternal and Infantile Effects. J Clin Res Pediatr Endocrinol. 2016;8(2):114-24.

187. Gaillard R. Maternal obesity during pregnancy and cardiovascular development and disease in the offspring. Eur J Epidemiol. 2015;30(11):1141-52.
188. Blardi P, de Lalla A, Ceccatelli L, Vanessa G, Auteri A, Hayek J. Variations of plasma leptin and adiponectin levels in autistic patients. Neurosci Lett. 2010;479(1):54-7.
189. Kiess W, Petzold S, Topfer M, Garten A, Bluher S, Kapellen T, et al. Adipocytes and adipose tissue. Best Pract Res Clin Endocrinol Metab. 2008;22(1):135-53.
190. Briana DD, Malamitsi-Puchner A. Intrauterine growth restriction and adult disease: the role of adipocytokines. Eur J Endocrinol. 2009;160(3):337-47.
191. Blardi P, de Lalla A, D'Ambrogio T, Vonella G, Ceccatelli L, Auteri A, et al. Long-term plasma levels of leptin and adiponectin in Rett syndrome. Clin Endocrinol (Oxf). 2009;70(5):706-9.
192. Fujita-Shimizu A, Suzuki K, Nakamura K, Miyachi T, Matsuzaki H, Kajizuka M, et al. Decreased serum levels of adiponectin in subjects with autism. Prog Neuropsychopharmacol Biol Psychiatry. 2010;34(3):455-8.
193. Kolevzon A, Gross R, Reichenberg A. Prenatal and perinatal risk factors for autism: a review and integration of findings. Archives of pediatrics & adolescent medicine. 2007;161(4):326-33.
194. Hunter DS, Hazel SJ, Kind KL, Owens JA, Pitcher JB, Gatford KL. Programming the brain: Common outcomes and gaps in knowledge from animal studies of IUGR. Physiol Behav. 2016;164(Pt A):233-48.
195. Kramer MS, Olivier M, McLean FH, Willis DM, Usher RH. Impact of intrauterine growth retardation and body proportionality on fetal and neonatal outcome. Pediatrics. 1990;86(5):707-13.
196. Beltrand J, Verkauskiene R, Nicolescu R, Sibony O, Gaucherand P, Chevenne D, et al. Adaptive changes in neonatal hormonal and metabolic profiles induced by fetal growth restriction. J Clin Endocrinol Metab. 2008;93(10):4027-32.
197. Yang S, Fombonne E, Kramer MS. Duration of gestation, size at birth and later childhood behaviour. Paediatric and perinatal epidemiology. 2011;25(4):377-87.
198. Murray E, Fernandes M, Fazel M, Kennedy SH, Villar J, Stein A. Differential effect of intrauterine growth restriction on childhood neurodevelopment: a systematic review. BJOG. 2015;122(8):1062-72.
199. von Beckerath AK, Kollmann M, Rotky-Fast C, Karpf E, Lang U, Klaritsch P. Perinatal complications and long-term neurodevelopmental outcome of infants with intrauterine growth restriction. American journal of obstetrics and gynecology. 2013;208(2):130 e1-6.
200. Gardener H, Spiegelman D, Buka SL. Perinatal and neonatal risk factors for autism: a comprehensive meta-analysis. Pediatrics. 2011;128(2):344-55.
201. Lampi KM, Lehtonen L, Tran PL, Suominen A, Lehti V, Banerjee PN, et al. Risk of autism spectrum disorders in low birth weight and small for gestational age infants. J Pediatr. 2012;161(5):830-6.
202. Moore GS, Kneitel AW, Walker CK, Gilbert WM, Xing G. Autism risk in small- and large-for-gestational-age infants. American journal of obstetrics and gynecology. 2012;206(4):314 e1-9.
203. Limperopoulos C. Autism spectrum disorders in survivors of extreme prematurity. Clin Perinatol. 2009;36(4):791-805, vi.

204. Langridge AT, Glasson EJ, Nassar N, Jacoby P, Pennell C, Hagan R, et al. Maternal conditions and perinatal characteristics associated with autism spectrum disorder and intellectual disability. PloS one. 2013;8(1):e50963.
205. Larsson HJ, Eaton WW, Madsen KM, Vestergaard M, Olesen AV, Agerbo E, et al. Risk factors for autism: perinatal factors, parental psychiatric history, and socioeconomic status. American journal of epidemiology. 2005;161(10):916-25; discussion 26-8.
206. Schendel D, Bhasin TK. Birth weight and gestational age characteristics of children with autism, including a comparison with other developmental disabilities. Pediatrics. 2008;121(6):1155-64.
207. Blair EM, Liu Y, de Klerk NH, Lawrence DM. Optimal fetal growth for the Caucasian singleton and assessment of appropriateness of fetal growth: an analysis of a total population perinatal database. BMC Pediatr. 2005;5(1):13.
208. Beltrand J, Nicolescu R, Kaguelidou F, Verkauskiene R, Sibony O, Chevenne D, et al. Catch-up growth following fetal growth restriction promotes rapid restoration of fat mass but without metabolic consequences at one year of age. PloS one. 2009;4(4):e5343.
209. Verkauskiene R, Beltrand J, Claris O, Chevenne D, Deghmoun S, Dorgeret S, et al. Impact of fetal growth restriction on body composition and hormonal status at birth in infants of small and appropriate weight for gestational age. Eur J Endocrinol. 2007;157(5):605-12.
210. Cho WK, Suh BK. Catch-up growth and catch-up fat in children born small for gestational age. Korean J Pediatr. 2016;59(1):1-7.
211. Larsen T, Greisen G, Petersen S. Intrauterine growth correlation to postnatal growth--influence of risk factors and complications in pregnancy. Early Hum Dev. 1997;47(2):157-65.
212. Dissanayake C, Bui QM, Huggins R, Loesch DZ. Growth in stature and head circumference in high-functioning autism and Asperger disorder during the first 3 years of life. Dev Psychopathol. 2006;18(2):381-93.
213. Torrey EF, Dhavale D, Lawlor JP, Yolken RH. Autism and head circumference in the first year of life. Biol Psychiatry. 2004;56(11):892-4.
214. Mraz KD, Green J, Dumont-Mathieu T, Makin S, Fein D. Correlates of head circumference growth in infants later diagnosed with autism spectrum disorders. J Child Neurol. 2007;22(6):700-13.
215. Campbell DJ, Chang J, Chawarska K. Early generalized overgrowth in autism spectrum disorder: prevalence rates, gender effects, and clinical outcomes. Journal of the American Academy of Child and Adolescent Psychiatry. 2014;53(10):1063-73 e5.
216. Sacco R, Militerni R, Frolli A, Bravaccio C, Gritti A, Elia M, et al. Clinical, morphological, and biochemical correlates of head circumference in autism. Biol Psychiatry. 2007;62(9):1038-47.
217. van Daalen E, Swinkels SH, Dietz C, van Engeland H, Buitelaar JK. Body length and head growth in the first year of life in autism. Pediatr Neurol. 2007;37(5):324-30.
218. Courchesne E, Carper R, Akshoomoff N. Evidence of brain overgrowth in the first year of life in autism. Jama. 2003;290(3):337-44.
219. Sacco R, Gabriele S, Persico AM. Head circumference and brain size in autism spectrum disorder: A systematic review and meta-analysis. Psychiatry Res. 2015;234(2):239-51.

220. Dementieva YA, Vance DD, Donnelly SL, Elston LA, Wolpert CM, Ravan SA, et al. Accelerated head growth in early development of individuals with autism. Pediatr Neurol. 2005;32(2):102-8.
221. Chawarska K, Campbell D, Chen L, Shic F, Klin A, Chang J. Early generalized overgrowth in boys with autism. Archives of general psychiatry. 2011;68(10):1021-31.
222. Lainhart JE, Bigler ED, Bocian M, Coon H, Dinh E, Dawson G, et al. Head circumference and height in autism: a study by the Collaborative Program of Excellence in Autism. Am J Med Genet A. 2006;140(21):2257-74.
223. Karakosta P, Chatzi L, Plana E, Margioris A, Castanas E, Kogevinas M. Leptin levels in cord blood and anthropometric measures at birth: a systematic review and meta-analysis. Paediatric and perinatal epidemiology. 2011;25(2):150-63.
224. Alexe DM, Syridou G, Petridou ET. Determinants of early life leptin levels and later life degenerative outcomes. Clin Med Res. 2006;4(4):326-35.
225. Ibanez L, Sebastiani G, Diaz M, Gomez-Roig MD, Lopez-Bermejo A, de Zegher F. Low body adiposity and high leptinemia in breast-fed infants born small-for-gestational-age. J Pediatr. 2010;156(1):145-7.
226. Reynolds CM, Segovia SA, Vickers MH. Experimental Models of Maternal Obesity and Neuroendocrine Programming of Metabolic Disorders in Offspring. Front Endocrinol (Lausanne). 2017;8:245.
227. Vickers MH, Sloboda DM. Leptin as mediator of the effects of developmental programming. Best Pract Res Clin Endocrinol Metab. 2012;26(5):677-87.
228. Vickers MH. Developmental programming and adult obesity: the role of leptin. Curr Opin Endocrinol Diabetes Obes. 2007;14(1):17-22.
229. Ashwood P, Kwong C, Hansen R, Hertz-Picciotto I, Croen L, Krakowiak P, et al. Brief report: plasma leptin levels are elevated in autism: association with early onset phenotype? J Autism Dev Disord. 2008;38(1):169-75.
230. Bouret SG. Crossing the border: developmental regulation of leptin transport to the brain. Endocrinology. 2008;149(3):875-6.
231. Bouret SG. Neurodevelopmental actions of leptin. Brain Res. 2010;1350:2-9.
232. Hauguel-de Mouzon S, Lepercq J, Catalano P. The known and unknown of leptin in pregnancy. American journal of obstetrics and gynecology. 2006;194(6):1537-45.
233. Valleau JC, Sullivan EL. The impact of leptin on perinatal development and psychopathology. Journal of chemical neuroanatomy. 2014;61-62:221-32.
234. Hassink SG, de Lancey E, Sheslow DV, Smith-Kirwin SM, O'Connor DM, Considine RV, et al. Placental leptin: an important new growth factor in intrauterine and neonatal development? Pediatrics. 1997;100(1):E1.
235. Laml T, Hartmann BW, Ruecklinger E, Preyer O, Soeregi G, Wagenbichler P. Maternal serum leptin concentrations do not correlate with cord blood leptin concentrations in normal pregnancy. J Soc Gynecol Investig. 2001;8(1):43-7.
236. Lepercq J, Challier JC, Guerre-Millo M, Cauzac M, Vidal H, Hauguel-de Mouzon S. Prenatal leptin production: evidence that fetal adipose tissue produces leptin. J Clin Endocrinol Metab. 2001;86(6):2409-13.

237. Ertl T, Funke S, Sarkany I, Szabo I, Rascher W, Blum WF, et al. Postnatal changes of leptin levels in full-term and preterm neonates: their relation to intrauterine growth, gender and testosterone. Biol Neonate. 1999;75(3):167-76.
238. Donnelly JM, Lindsay KL, Walsh JM, Horan M, Molloy EJ, McAuliffe FM. Fetal metabolic influences of neonatal anthropometry and adiposity. BMC Pediatr. 2015;15:175.
239. Bozzola E, Meazza C, Arvigo M, Travaglino P, Pagani S, Stronati M, et al. Role of adiponectin and leptin on body development in infants during the first year of life. Ital J Pediatr. 2010;36:26.
240. Mantzoros CS, Rifas-Shiman SL, Williams CJ, Fargnoli JL, Kelesidis T, Gillman MW. Cord blood leptin and adiponectin as predictors of adiposity in children at 3 years of age: a prospective cohort study. Pediatrics. 2009;123(2):682-9.
241. Stoll-Becker S, Kreuder J, Reiss I, Etspuler J, Blum WF, Gortner L. Influence of gestational age and intrauterine growth on leptin concentrations in venous cord blood of human newborns. Klin Padiatr. 2003;215(1):3-8.
242. Ng PC, Lam CW, Lee CH, Wong GW, Fok TF, Chan IH, et al. Leptin and metabolic hormones in preterm newborns. Arch Dis Child Fetal Neonatal Ed. 2000;83(3):F198-202.
243. Jaquet D, Leger J, Levy-Marchal C, Oury JF, Czernichow P. Ontogeny of leptin in human fetuses and newborns: effect of intrauterine growth retardation on serum leptin concentrations. J Clin Endocrinol Metab. 1998;83(4):1243-6.
244. Pighetti M, Tommaselli GA, D'Elia A, Di Carlo C, Mariano A, Di Carlo A, et al. Maternal serum and umbilical cord blood leptin concentrations with fetal growth restriction. Obstet Gynecol. 2003;102(3):535-43.
245. Valuniene M, Verkauskiene R, Boguszewski M, Dahlgren J, Lasiene D, Lasas L, et al. Leptin levels at birth and in early postnatal life in small- and appropriate-for-gestational-age infants. Medicina (Kaunas). 2007;43(10):784-91.
246. Yildiz L, Avci B, Ingec M. Umbilical cord and maternal blood leptin concentrations in intrauterine growth retardation. Clin Chem Lab Med. 2002;40(11):1114-7.
247. Parker M, Rifas-Shiman SL, Belfort MB, Taveras EM, Oken E, Mantzoros C, et al. Gestational glucose tolerance and cord blood leptin levels predict slower weight gain in early infancy. J Pediatr. 2011;158(2):227-33.
248. Ong KK, Ahmed ML, Sherriff A, Woods KA, Watts A, Golding J, et al. Cord blood leptin is associated with size at birth and predicts infancy weight gain in humans. ALSPAC Study Team. Avon Longitudinal Study of Pregnancy and Childhood. J Clin Endocrinol Metab. 1999;84(3):1145-8.
249. Chaoimh CN, Murray DM, Kenny LC, Irvine AD, Hourihane JO, Kiely M. Cord blood leptin and gains in body weight and fat mass during infancy. Eur J Endocrinol. 2016;175(5):403-10.
250. Kaar JL, Brinton JT, Crume T, Hamman RF, Glueck DH, Dabelea D. Leptin levels at birth and infant growth: the EPOCH study. J Dev Orig Health Dis. 2014;5(3):214-8.
251. Ong KK, Ahmed ML, Emmett PM, Preece MA, Dunger DB. Association between postnatal catch-up growth and obesity in childhood: prospective cohort study. BMJ. 2000;320(7240):967-71.
252. Petridou E, Mantzoros CS, Belechri M, Skalkidou A, Dessypris N, Papathoma E, et al. Neonatal leptin levels are strongly associated with female gender, birth length, IGF-I levels and formula feeding. Clinical endocrinology. 2005;62(3):366-71.

253. Lonnerdal B, Havel PJ. Serum leptin concentrations in infants: effects of diet, sex, and adiposity. The American journal of clinical nutrition. 2000;72(2):484-9.
254. Camurdan Duyan A, Sahin F, Camurdan MO, Bideei A, Cinaz P. Role of leptin in growth and adiposity in early infancy: impact of nutritional pattern. Indian Pediatr. 2007;44(9):687-90.
255. Savino F, Costamagna M, Prino A, Oggero R, Silvestro L. Leptin levels in breast-fed and formula-fed infants. Acta Paediatr. 2002;91(9):897-902.
256. Boeke CE, Mantzoros CS, Hughes MD, S LR-S, Villamor E, Zera CA, et al. Differential associations of leptin with adiposity across early childhood. Obesity (Silver Spring). 2013;21(7):1430-7.
257. Coupe B, Grit I, Hulin P, Randuineau G, Parnet P. Postnatal growth after intrauterine growth restriction alters central leptin signal and energy homeostasis. PloS one. 2012;7(1):e30616.
258. Coupe B, Grit I, Darmaun D, Parnet P. The timing of "catch-up growth" affects metabolism and appetite regulation in male rats born with intrauterine growth restriction. Am J Physiol Regul Integr Comp Physiol. 2009;297(3):R813-24.
259. Pan W, Hsuchou H, Tu H, Kastin AJ. Developmental changes of leptin receptors in cerebral microvessels: unexpected relation to leptin transport. Endocrinology. 2008;149(3):877-85.
260. Irving AJ, Harvey J. Leptin regulation of hippocampal synaptic function in health and disease. Philos Trans R Soc Lond B Biol Sci. 2014;369(1633):20130155.
261. Tonhajzerova I, Ondrejka I, Mestanik M, Mikolka P, Hrtanek I, Mestanikova A, et al. Inflammatory Activity in Autism Spectrum Disorder. Adv Exp Med Biol. 2015;861:93-8.
262. Rodrigues DH, Rocha NP, Sousa LF, Barbosa IG, Kummer A, Teixeira AL. Changes in adipokine levels in autism spectrum disorders. Neuropsychobiology. 2014;69(1):6-10.
263. Al-Zaid FS, Alhader AA, Al-Ayadhi LY. Altered ghrelin levels in boys with autism: a novel finding associated with hormonal dysregulation. Sci Rep. 2014;4:6478.
264. Essa M.M., Braidy N., Al-Sharbati M.M., Al-Farsi YM, Ali A, Waly M.I., et al. Elevated plasma leptin levels in autisic children of Sultanate of Oman. International Journal of Biological & Medical Research. 2011;2(3):803-5.
265. Bochukova EG, Huang N, Keogh J, Henning E, Purmann C, Blaszczyk K, et al. Large, rare chromosomal deletions associated with severe early-onset obesity. Nature. 2010;463(7281):666-70.
266. Vargas DL, Nascimbene C, Krishnan C, Zimmerman AW, Pardo CA. Neuroglial activation and neuroinflammation in the brain of patients with autism. Ann Neurol. 2005;57(1):67-81.
267. Challa AS, Evagelidou EN, Cholevas VI, Kiortsis DN, Giapros VI, Drougia AA, et al. Growth factors and adipocytokines in prepubertal children born small for gestational age: relation to insulin resistance. Diabetes Care. 2009;32(4):714-9.
268. Koerner A, Kratzsch J, Kiess W. Adipocytokines: leptin--the classical, resistin--the controversical, adiponectin--the promising, and more to come. Best Pract Res Clin Endocrinol Metab. 2005;19(4):525-46.
269. Liu M, Liu F. Regulation of adiponectin multimerization, signaling and function. Best Pract Res Clin Endocrinol Metab. 2014;28(1):25-31.
270. Steinberg GR, Kemp BE. Adiponectin: starving for attention. Cell Metab. 2007;6(1):3-4.

271. Sartori C, Lazzeroni P, Merli S, Patianna VD, Viaroli F, Cirillo F, et al. From Placenta to Polycystic Ovarian Syndrome: The Role of Adipokines. Mediators Inflamm. 2016;2016:4981916.
272. Mazaki-Tovi S, Romero R, Vaisbuch E, Erez O, Mittal P, Chaiworapongsa T, et al. Maternal serum adiponectin multimers in gestational diabetes. J Perinat Med. 2009;37(6):637-50.
273. Mazaki-Tovi S, Romero R, Vaisbuch E, Kusanovic JP, Erez O, Gotsch F, et al. Maternal serum adiponectin multimers in preeclampsia. J Perinat Med. 2009;37(4):349-63.
274. Qiao L, Wattez JS, Lee S, Guo Z, Schaack J, Hay WW, Jr., et al. Knockout maternal adiponectin increases fetal growth in mice: potential role for trophoblast IGFBP-1. Diabetologia. 2016;59(11):2417-25.
275. Sivan E, Mazaki-Tovi S, Pariente C, Efraty Y, Schiff E, Hemi R, et al. Adiponectin in human cord blood: relation to fetal birth weight and gender. J Clin Endocrinol Metab. 2003;88(12):5656-60.
276. Kotani Y, Yokota I, Kitamura S, Matsuda J, Naito E, Kuroda Y. Plasma adiponectin levels in newborns are higher than those in adults and positively correlated with birth weight. Clinical endocrinology. 2004;61(4):418-23.
277. Pardo IM, Geloneze B, Tambascia MA, Barros-Filho AA. Hyperadiponectinemia in newborns: relationship with leptin levels and birth weight. Obes Res. 2004;12(3):521-4.
278. Zhang ZQ, Lu QG, Huang J, Jiao CY, Huang SM, Mao LM. Maternal and cord blood adiponectin levels in relation to post-natal body size in infants in the first year of life: a prospective study. BMC Pregnancy Childbirth. 2016;16(1):189.
279. Zheng J, Xiao X, Zhang Q, Mao L, Li M, Yu M, et al. Correlation of high-molecular-weight adiponectin and leptin concentrations with anthropometric parameters and insulin sensitivity in newborns. Int J Endocrinol. 2014;2014:435376.
280. Kajantie E, Hytinantti T, Hovi P, Andersson S. Cord plasma adiponectin: a 20-fold rise between 24 weeks gestation and term. J Clin Endocrinol Metab. 2004;89(8):4031-6.
281. Mazaki-Tovi S, Kanety H, Pariente C, Hemi R, Schiff E, Sivan E. Cord blood adiponectin in large-for-gestational age newborns. American journal of obstetrics and gynecology. 2005;193(3 Pt 2):1238-42.
282. Cekmez F, Pirgon O, Tanju A, Ipcioglu OM. Cord plasma concentrations of visfatin, adiponectin and insulin in healthy term neonates: positive correlation with birthweight. Int J Biomed Sci. 2009;5(3):257-60.
283. Nakano Y, Itabashi K, Sakurai M, Aizawa M, Dobashi K, Mizuno K. Preterm infants have altered adiponectin levels at term-equivalent age even if they do not present with extrauterine growth restriction. Horm Res Paediatr. 2013;80(3):147-53.
284. Oberthuer A, Donmez F, Oberhauser F, Hahn M, Hoppenz M, Hoehn T, et al. Hypoadiponectinemia in extremely low gestational age newborns with severe hyperglycemia--a matched-paired analysis. PloS one. 2012;7(6):e38481.
285. Siahanidou T, Mandyla H, Papassotiriou GP, Papassotiriou I, Chrousos G. Circulating levels of adiponectin in preterm infants. Arch Dis Child Fetal Neonatal Ed. 2007;92(4):F286-90.
286. Terrazzan AC, Procianoy RS, Silveira RC. Neonatal cord blood adiponectin and insulin levels in very low birth weight preterm and healthy full-term infants. J Matern Fetal Neonatal Med. 2014;27(6):616-20.

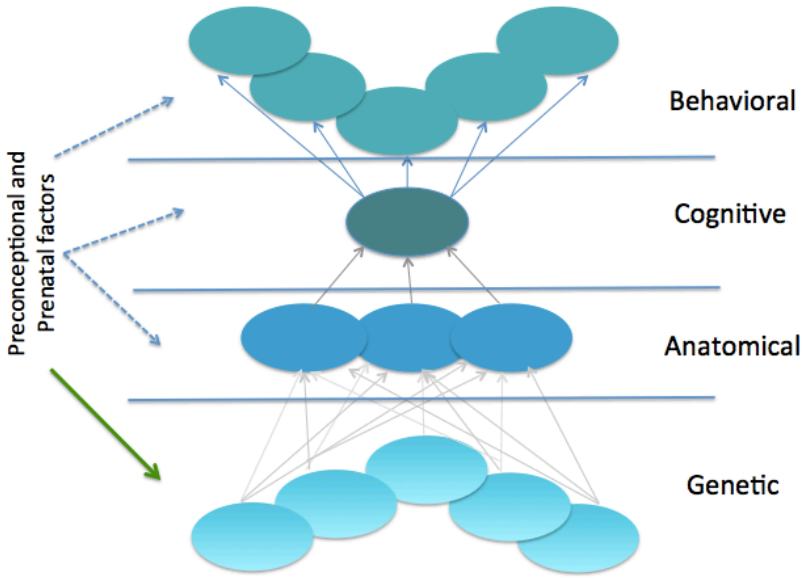
287. Iniguez G, Soto N, Avila A, Salazar T, Ong K, Dunger D, et al. Adiponectin levels in the first two years of life in a prospective cohort: relations with weight gain, leptin levels and insulin sensitivity. J Clin Endocrinol Metab. 2004;89(11):5500-3.
288. Cianfarani S, Martinez C, Maiorana A, Scire G, Spadoni GL, Boemi S. Adiponectin levels are reduced in children born small for gestational age and are inversely related to postnatal catch-up growth. J Clin Endocrinol Metab. 2004;89(3):1346-51.
289. Stefan N, Bunt JC, Salbe AD, Funahashi T, Matsuzawa Y, Tataranni PA. Plasma adiponectin concentrations in children: relationships with obesity and insulinemia. J Clin Endocrinol Metab. 2002;87(10):4652-6.
290. Woo JG, Guerrero ML, Altaye M, Ruiz-Palacios GM, Martin LJ, Dubert-Ferrandon A, et al. Human milk adiponectin is associated with infant growth in two independent cohorts. Breastfeed Med. 2009;4(2):101-9.
291. Woo JG, Guerrero ML, Guo F, Martin LJ, Davidson BS, Ortega H, et al. Human milk adiponectin affects infant weight trajectory during the second year of life. J Pediatr Gastroenterol Nutr. 2012;54(4):532-9.
292. Weyermann M, Brenner H, Rothenbacher D. Adipokines in human milk and risk of overweight in early childhood: a prospective cohort study. Epidemiology. 2007;18(6):722-9.
293. Thundyil J, Pavlovski D, Sobey CG, Arumugam TV. Adiponectin receptor signalling in the brain. Br J Pharmacol. 2012;165(2):313-27.
294. Schulz C, Paulus K, Lehnert H. Adipocyte-brain: crosstalk. Results Probl Cell Differ. 2010;52:189-201.
295. Waragai M, Ho G, Takamatsu Y, Sekiyama K, Sugama S, Takenouchi T, et al. Importance of adiponectin activity in the pathogenesis of Alzheimer's disease. Ann Clin Transl Neurol. 2017;4(8):591-600.
296. Ng RC, Cheng OY, Jian M, Kwan JS, Ho PW, Cheng KK, et al. Chronic adiponectin deficiency leads to Alzheimer's disease-like cognitive impairments and pathologies through AMPK inactivation and cerebral insulin resistance in aged mice. Mol Neurodegener. 2016;11(1):71.
297. Ng RC, Chan KH. Potential Neuroprotective Effects of Adiponectin in Alzheimer's Disease. Int J Mol Sci. 2017;18(3).
298. Machado-Vieira R, Gold PW, Luckenbaugh DA, Ballard ED, Richards EM, Henter ID, et al. The role of adipokines in the rapid antidepressant effects of ketamine. Mol Psychiatry. 2017;22(1):127-33.
299. Lehto SM, Huotari A, Niskanen L, Tolmunen T, Koivumaa-Honkanen H, Honkalampi K, et al. Serum adiponectin and resistin levels in major depressive disorder. Acta Psychiatr Scand. 2010;121(3):209-15.
300. Ozcan O, Arslan M, Gungor S, Yuksel T, Selimoglu MA. Plasma Leptin, Adiponectin, Neuropeptide Y Levels in Drug Naive Children With ADHD. J Atten Disord. 2015.
301. Moody L, Chen H, Pan YX. Early-Life Nutritional Programming of Cognition-The Fundamental Role of Epigenetic Mechanisms in Mediating the Relation between Early-Life Environment and Learning and Memory Process. Adv Nutr. 2017;8(2):337-50.
302. Krebs NF, Lozoff B, Georgieff MK. Neurodevelopment: The Impact of Nutrition and Inflammation During Infancy in Low-Resource Settings. Pediatrics. 2017;139(Suppl 1):S50-S8.

303. Van den Bergh BR. Developmental programming of early brain and behaviour development and mental health: a conceptual framework. Dev Med Child Neurol. 2011;53 Suppl 4:19-23.
304. Cusick SE, Georgieff MK. The Role of Nutrition in Brain Development: The Golden Opportunity of the "First 1000 Days". J Pediatr. 2016;175:16-21.
305. Prado EL, Dewey KG. Nutrition and brain development in early life. Nutrition reviews. 2014;72(4):267-84.
306. Georgieff MK. Iron assessment to protect the developing brain. The American journal of clinical nutrition. 2017;106(Suppl 6):1588S-93S.
307. Landrigan PJ. Environment and Autism. Arlington, VA: American Psychiatric Publishing; 2010.
308. Arndt TL, Stodgell CJ, Rodier PM. The teratology of autism. Int J Dev Neurosci. 2005;23(2-3):189-99.
309. Shi L, Fatemi SH, Sidwell RW, Patterson PH. Maternal influenza infection causes marked behavioral and pharmacological changes in the offspring. J Neurosci. 2003;23(1):297-302.
310. Ploeger A, Raijmakers ME, van der Maas HL, Galis F. The association between autism and errors in early embryogenesis: what is the causal mechanism? Biol Psychiatry. 2010;67(7):602-7.
311. Arora M, Reichenberg A, Willfors C, Austin C, Gennings C, Berggren S, et al. Fetal and postnatal metal dysregulation in autism. Nat Commun. 2017;8:15493.
312. Fatemi SH, Folsom TD, Reutiman TJ, Huang H, Oishi K, Mori S. Prenatal viral infection of mice at E16 causes changes in gene expression in hippocampi of the offspring. Eur Neuropsychopharmacol. 2009;19(9):648-53.
313. Raz R, Roberts AL, Lyall K, Hart JE, Just AC, Laden F, et al. Autism spectrum disorder and particulate matter air pollution before, during, and after pregnancy: a nested case-control analysis within the Nurses' Health Study II Cohort. Environmental health perspectives. 2015;123(3):264-70.
314. Beversdorf DQ, Manning SE, Hillier A, Anderson SL, Nordgren RE, Walters SE, et al. Timing of prenatal stressors and autism. J Autism Dev Disord. 2005;35(4):471-8.
315. Lyall K, Schmidt RJ, Hertz-Picciotto I. Maternal lifestyle and environmental risk factors for autism spectrum disorders. Int J Epidemiol. 2014;43(2):443-64.
316. Hadjkacem I, Ayadi H, Turki M, Yaich S, Khemekhem K, Walha A, et al. Prenatal, perinatal and postnatal factors associated with autism spectrum disorder. J Pediatr (Rio J). 2016;92(6):595-601.
317. Wang C, Geng H, Liu W, Zhang G. Prenatal, perinatal, and postnatal factors associated with autism: A meta-analysis. Medicine (Baltimore). 2017;96(18):e6696.
318. Hazlett HC, Gu H, Munsell BC, Kim SH, Styner M, Wolff JJ, et al. Early brain development in infants at high risk for autism spectrum disorder. Nature. 2017;542(7641):348-51.
319. Joseph RM. Neuropsychological frameworks for understanding autism. Int Rev Psychiatry. 1999;11(4):309-24.
320. Bourgeron T. A synaptic trek to autism. Curr Opin Neurobiol. 2009;19(2):231-4.
321. Zikopoulos B, Barbas H. Changes in prefrontal axons may disrupt the network in autism. J Neurosci. 2010;30(44):14595-609.

322. Kriete T, Noelle DC. Dopamine and the development of executive dysfunction in autism spectrum disorders. PloS one. 2015;10(3):e0121605.

Appendix

Figure 2-1 An integrated multilevel model depicting underlying abnormalities from genetic to behavioral factors in ASD



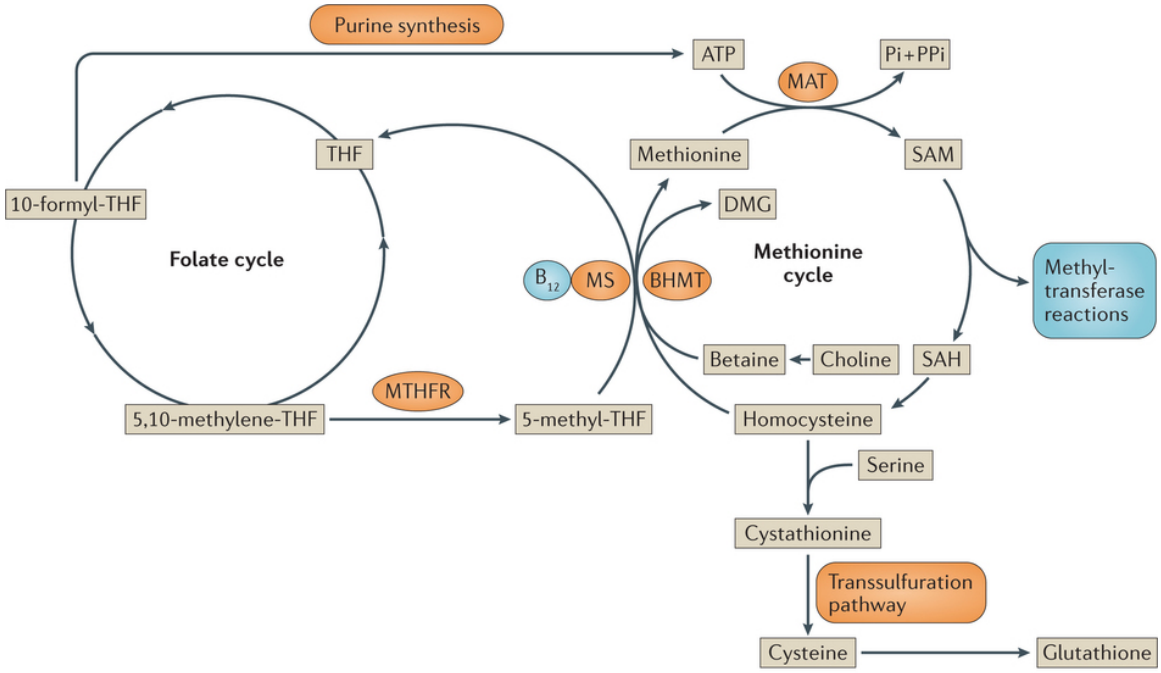
(Solid green line between preconceptional, prenatal factors and genetic factors will be the focus of this study)

Adapted from Frith, Niculea and Paval. The original caption from Frith: "The X-shape of the three-level framework illustrating a cognitive phenotype where there is multiple mapping between brain and behaviour via a singular node at the cognitive level. Any developmental disorder is likely to have more than one specific deficit, but for simplicity only one is shown in the diagram."

Source: Frith, U. (2012). Why we need cognitive explanations of autism. *Q J Exp Psychol (Hove), 65*(11), 2073-2092. doi:10.1080/17470218.2012.697178

Niculae, A. S., & Paval, D. (2016). From molecules to behavior: An integrative theory of autism spectrum disorder. *Med Hypotheses, 97*, 74-84. doi:10.1016/j.mehy.2016.10.016

Figure 2-2 One-carbon metabolism



Original caption from Yang and Vousden: "The methionine cycle produces *S*-adenosylmethionine (SAM), which is a ubiquitous methyl group donor that is used by a large family of SAM-dependent methyltransferases for the methylation of DNA, RNA, proteins and lipids. Methionine is converted into SAM by *S*-adenosylmethionine synthase (also known as methionine adenosyltransferase (MAT)) in an ATP-dependent process. The folate cycle (which is supported by serine-derived one-carbon units) helps to maintain cellular ATP levels through *de novo* purine synthesis. The by-product of the methyltransferase reactions, *S*-adenosylhomocysteine (SAH), is converted into homocysteine, a precursor for synthesis of cysteine and glutathione through the transsulfuration pathway. 5,10-methylene-tetrahydrofolate (5,10-methylene-THF) derived from the folate cycle can be irreversibly converted by methylenetetrahydrofolate reductase (*MTHFR*) into 5-methyl-THF, which then donates its methyl group to homocysteine in the methionine synthase (MS) reaction to produce

methionine and THF, a process that uses vitamin B12 as a cofactor. Choline can also contribute to homocysteine remethylation via the generation of betaine, which is converted into dimethylglycine (DMG) through a reaction catalysed by betaine-homocysteine *S*-methyltransferase (BHMT). DMG can further contribute one-carbon units to the mitochondrial folate cycle." Source: Yang, M. and K.H. Vousden, *Serine and one-carbon metabolism in cancer.* Nat Rev Cancer, 2016. **16**(10): p. 650-62.

CHAPTER 3

Research Design and Methods

3.1 Data Source: the Boston Birth Cohort

This dissertation used data from the Boston Birth Cohort (BBC), based at the Boston Medical Center (BMC). The BBC is an ongoing prospective cohort study, initiated by Dr. Xiaobin Wang in 1998 to study the environmental and genetic determinants of preterm delivery and later expanded to study pediatric outcomes. The BBC is uniquely poised to study ASD, since BBC is an enriched cohort due to oversampled preterm deliveries and preterm birth is a known risk factor for ASD. BBC is one of the largest birth cohorts that consist of predominantly urban low-income minority population. A majority of the BBC participants received health care through means-tested insurance programs such as Medicaid and MassHealth (1). Data collection in the BBC follows two Boston University Medical Center IRB approved protocols that govern baseline and postnatal follow-up data collection, respectively which are discussed below.

3.1.1 Baseline data collection

Between 1998 and 2015, 7,939 mother-child pairs were enrolled in the BBC. For every preterm (defined as <37 weeks) and/or low birth weight baby (defined as <2,500 g), approximately two term/normal birth weight babies (and their mothers) were enrolled in the study (2, 3). The preterm and/or LBW baby were matched with the full term/normal weight baby on delivery date, race/ethnicity and maternal age ($\pm$5 years). At the time of recruitment, a small percentage of pregnancies (<1%) were excluded from the study for the following reasons: conceived using in-vitro fertilization, multiple-gestation pregnancies, fetuses with chromosomal abnormalities or major birth defects, preterm delivery due to maternal trauma and women with congenital or acquired uterine lesions or incompetent cervix (4). Eligible women were invited to participate in the study 24-72 hours after delivery. Over 90% of those that were

approached agreed to participate and were initiated into the study (3, 5). Participants and non-participants did not differ on characteristics such as infant birth weight, maternal ethnicity, or other sociodemographic characteristics (3).

After obtaining signed informed consent from the mothers, using a standardized questionnaire, face-to-face interviews were conducted by trained research staff to collect epidemiological data. Detailed information was gathered on mother's general health, maternal reproductive history, smoking status, drug use, alcohol consumption, preconception and prenatal supplement intake, prenatal dietary intake, physical activity during index pregnancy and social and demographic characteristics. Maternal and infant medical records were reviewed using standardized abstraction forms to collect data on pre-pregnancy weight, height, gestational weight gain, and pregnancy related complications such as gestational diabetes, preeclampsia and adverse birth outcomes. Mothers and infants were assigned the same study identification number in order to ensure accurate linking of data (1).

3.1.2 Follow-up data collection

Children that were part of the BBC at baseline were invited to participate in a follow-up study designed to assess postnatal growth, health and developmental outcomes (6). About 2,932 children were eligible to participate in this postnatal follow-up study and were prospectively followed between 2003 and 2015 (5, 7, 8). The follow-up visits were scheduled in alignment with pediatric well-child visits during infancy and childhood (6-12 months, 2, 4 and 6 years) and venous blood sample was collected during one of those visits (6, 7). Postnatal demographic and environmental information were collected using a standardized questionnaire (4, 9). Mother-infant dyads that participated in the follow-up study were similar to the non-

participants, in terms of baseline characteristics, including maternal age, maternal education, household income, parity, child's sex and mode of delivery (5, 7). Children in the follow-up study were pediatric patients of the BMC with an average 39.3 (range: 1-463) visits to the BMC (1). The unique identification number used in the baseline study of the BBC was continued in the follow-up study to ensure that the data can be linked.

3.1.3 Ethical considerations

The baseline study protocol was approved by the Institutional Review Boards (IRB) at the BMC and the Massachusetts Department of Public Health. Written informed consent was obtained from all study participants. Each participant was apprised that their participation in the study was voluntary and that their information will remain anonymous. The funding for the baseline BBC study was provided by the March of Dimes Perinatal Epidemiological Research Initiative (PERI) grants (PI: Wang, 20-FY02-5), the National Institute of Environmental Health Sciences, NIH (PI: Wang, R21 ES11666), and the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, NIH (PI: Wang, R01 HD41702).

The IRB at the Boston University Medical Center and Children's Memorial Hospital in Chicago approved the postnatal follow-up data collection protocol. Informed consent was obtained prior to participation in the follow-up study (7). The funding for the follow-up study was provided by the Food Allergy Initiative and the National Institute of Allergy and Infectious Disease, NIH (PI Wang: R21 AI079872, U01 AI090727, 2R01 HD41702) and the Department of Defense (PI Wang: W81XWH-10-1-0123). Subsequently, the baseline and postnatal follow-up data collection was approved by the IRB at the Johns Hopkins Bloomberg School of Public Health (JHBSPH), when Dr. Wang moved to the Johns Hopkins University.

I have completed all the ethics training required by the JHBSPH and have completed the CITI certificate and obtained IRB approval as a research staff of the BBC. This study adhered to data procurement, management and analysis plan stipulated by the JHSPH IRB. To protect confidentiality of the study participants, I used the datasets without personal identifier and the data were stored on secure networks. Additionally, data will be reported only as aggregates.

3.2 Analytic Sample for this dissertation

This dissertation used data from the postnatal follow-up study, which began in 2003 and had a baseline sample of 2,932. Since the transition to ICD-10-CM began on October 1 2015, an end date cut-off of September 30 2015 was chosen for the follow-up study. Figure 3-1 summarizes the sample sizes for each specific aim. The sample size varied depending on the inclusion and exclusion criteria for each specific aim. There were two main reasons for excluding subjects: 1) missing data for one or more explanatory variables, and 2) competing diagnosis in the non-ASD subjects.

Nutritional, metabolic biomarkers and early life growth data were the key explanatory variables in this study. In the baseline and follow-up studies, biomarkers were assessed in a random sample of mothers and children. Thus, depending on the specific aim, lack of appropriate biomarker data was one of the important reasons for excluding subjects. Since the BBC oversampled preterm babies at enrolment, there were a considerable proportion of children with neurodevelopmental diagnosis. To allow cleaner comparison between cases and control, those with competing diagnosis such as Attention Deficit Hyperactivity Disorder (ADHD), Intellectual Disability (ID) and other developmental disabilities were excluded from the neurotypical group.

3.2.1 Analytic sample for Specific Aim 1

The base sample for the analyses in Aim 1 was 2,932. A total of 896 children were excluded because of lack of maternal plasma folate and vitamin B12 measures. An additional 189 were eliminated because of the missing third trimester prenatal supplement intake data. In the remaining 1,848 subjects, 167 had ADHD, 406 had other developmental disabilities and 8 had ID without co-occurring ASD. One subject had Ventricular Septal Defect (VSD), but was miscoded as having ASD. Additionally, 9 subjects were excluded because of lack of relevant EMR data such as age of ASD diagnosis, date of first and last EMR visit – which was used in the Cox Proportional Hazards model, to account for the variability in length to follow-up. The final analytic sample size for specific aim 1 was 1,257.

3.2.2 Analytic sample for Specific Aim 2

In the process of identifying subjects eligible for this specific aim, 1,554 children were excluded from the final analysis because of lack of one or more of the following explanatory variables: a) cord leptin, b) early childhood leptin, c) IUGR data, d) first year weight gain data. In addition, 534 children were eliminated since they were not neurotypical. In other words, they had other competing diagnosis such as ADHD (n=198) and other developmental disabilities (n=336) without a concurrent ASD diagnosis. Thus, the analytic sample for specific aim 2 was 844.

3.2.3 Analytic sample for Specific Aim 3

The lack of cord adiponectin and/or early childhood adiponectin resulted in exclusion of 1,546 subjects from the base sample (n=2,932). Subsequently, 539 children were excluded because they had other competing diagnosis such as ADHD (n=203) and other developmental disabilities (n=336), leaving an analytic sample of 847 for this sub-aim.

3.3. Measures

3.3.1 Outcome: Autism Spectrum Disorder

ASD diagnosis in this dissertation was based on EMR data, which documented pediatric inpatient, outpatient, and emergency room visits using International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM). EMR data was collected for every postnatal clinical visit between October 2003 and September 30 2015. Children ever diagnosed with autism (ICD-9 code 299.00), Asperger syndrome (299.80) and/or pervasive developmental disorder not otherwise specified (299.90) were categorized as having ASD (1, 8). Neurotypical children were those without ASD, ADHD (314.0–314.9), other developmental disorders (315.0–315.9), or ID (317–319). A sub-set of ASD children who were diagnosed by a specialist such as developmental behavioral pediatrician, pediatric neurologist and child psychologist and received ASD code at least on two separate occasions were identified as stringent cases and were used in sensitivity analysis.

3.3.2 Major Explanatory Variables

Considering the multifactorial etiology of ASD, this dissertation assessed the association between multiple explanatory variables, broadly categorized into nutritional and metabolic factors, and ASD. Nutritional factors were addressed from a maternal context and metabolic explanatory variables were addressed from the child's context. The latter was assessed at two time points – at birth and early childhood (prior to ASD diagnosis).

Specific Aim 1: Nutritional factors

The goal of specific aim 1 was to assess the association between maternal B-vitamin status during third trimester and risk of ASD. The key explanatory variables for this aim were: 1)

maternal plasma folate and vitamin B12, and 2) preconception and prenatal supplement intake, especially third trimester supplement intake.

Blood was drawn from mothers 24-72 hours after delivery. Plasma folate was subsequently measured using chemiluminescent immunoassay with diagnostic kits (Shenzhen New Industries Biomedical Engineering Co., Ltd. China), plasma vitamin B12 was measured using Beckman Coulter ACCESS Immunoassay System (Beckman-Coulter Canada, Mississauga, Canada) and the interassay coefficient of variation was less than 4% (9). Plasma homocysteine levels were measured using automatic clinical analyzers (Beckman-Coulter) (9). Although the blood was drawn post-birth, plasma folate is a marker of recent intake and is reflective of third trimester levels (10).

Maternal plasma folate levels were categorized based on the cut-offs suggested by the World Health Organization (WHO) (11). In addition, plasma folate and vitamin B12 levels were categorized in such a way that the lowest and highest deciles (bottom and top 10^{th} percentiles) were compared against the middle 80^{th} percentile. The joint effects of folate and vitamin B12 percentiles were assessed using the following categorizations: 1) Both folate and vitamin B12 (10^{th} – $<90^{th}$ percentile, referent category), 2) Folate ($<10^{th}$ percentile) and vitamin B12 (10^{th} – $<90^{th}$ percentile), 3) Folate ($\geq 90^{th}$ percentile*)* and vitamin B12 (10^{th} – $<90^{th}$ percentile), 4) vitamin B12 ($<10^{th}$ percentile) and folate (10^{th} – $<90^{th}$ percentile), 5) vitamin B12 ($\geq 90^{th}$ percentile) and folate (10^{th} – $<90^{th}$ percentile). Using an alternate approach the following categorizations were created: 1) Both folate and vitamin B12 (10^{th} – $<90^{th}$ percentile, referent category), 2) Either folate or vitamin B12 ($\geq 90^{th}$ percentile), and 3) Both folate and vitamin B12 ($\geq 90^{th}$ percentile).

Preconception and prenatal supplement intake data was gathered from mothers using a standardized questionnaire administered soon after birth. Preconception multivitamin intake was dichotomized (no vs. yes). The frequency of prenatal supplement intake during first, second and third trimesters were categorized as follows: ≤2 times/week, 3–5 times/week and >5 times/week.

Specific Aim 2: Leptin and early life growth pattern

This specific aim assessed the relationship between ASD and the following key explanatory variables: 1) cord and early childhood leptin levels, 2) fetal growth and weight gain during 1st year of life. Birth weight for gestational age, a proxy for fetal growth, was defined as follows: small for gestational age (<10th percentile), appropriate for gestational age (10th – 90th percentile) and large for gestational age (>90th percentile). Child's length (<2 years), height and weight measured during the well-child visits were used to assess the rate of weight of gain during infancy. The WHO reference values were used to calculate weight-for-age *z*-scores, defined as the changed in weight-for-age *z* scores from birth until the target time point. Based on this, the rate of weight gain was categorized as slow (weight gain *z* -score <-0.67), on track (−0.67 to 0.67), rapid (>0.67 to 1.28), and extremely rapid (>1.28).

Umbilical cord blood was collected at delivery and postnatal venous blood was collected during a follow-up visit. The blood samples were processed immediately and plasma was stored in a freezer at −80°C. Using a sandwich immunoassay based on flow metric xMAP technology, plasma leptin levels were measured on Luminex 200 machines (Luminex Corp., Austin, TX) and had an interassay coefficient of variation of 4.5% (12). Plasma leptin >3 SD above the mean were deemed unlikely and was reassigned a value of 3SD (9). To ensure

temporality, childhood leptin measurements prior to ASD diagnosis were only included in the analysis.

Specific Aim 3: Adiponectin

In this specific aim, the association between adiponectin and ASD was assessed at two time points. Adiponectin was assessed in umbilical cord sample drawn at delivery and venous blood sample collected during a subsequent early childhood follow-up visit. Adiponectin was measured using an immunoassay (ELISA) and had an inter-assay variation of <5.8% (9). The assays were run according to the manufacturer's recommendation and were measured in duplicates. Children that had adiponectin assessed after ASD diagnosis were excluded from the follow-up analysis. Adiponectin levels greater than 3SD above the mean were re-assigned to 3SD levels.

3.3.3 Other explanatory variables

Covariates for this dissertation were chosen *a priori* based on the earlier work in the BBC (1, 5, 9, 13) as well as studies looking at the relationships between nutritional, metabolic factors and ASD (14-16). These covariates were gathered from maternal interviews, using standardized questionnaire or medical record abstraction. While most of the same covariates were accounted for in the three specific aims, there were a few that were specific for each aim. Below, is the list of covariates, broadly categorized into maternal and child factors.

Maternal factors

Maternal age at delivery was defined as mother's age at delivery of the index pregnancy, calculated using mother's date of birth and infant's date of birth.

Parity captured the number of previous pregnancies, not including the index pregnancy and was categorized into zero, one, or two or more. This data was obtained from the baseline maternal questionnaire.

Educational attainment was categorized into high school or less and some college or more. This grouping was reconciled from the original five options that the mothers chose, including: no school/elementary school, some secondary school (9^{th} grade and above), high school graduate or GED, some college, college degree and above.

Smoking status was ascertained using one of the two responses – a) smoking 3 months before pregnancy or during pregnancy, or b) no smoking during preconception or pregnancy.

Race-ethnicity was categorized into black, white, Hispanic and Other. This categorization is based on 8 original responses (Black/African American, White, Hispanic, Asian, Haitian, Cape Verdian, Pacific Islander, and Multiple responses (mixed ethnicity)) that were recoded into the 4 categories.

Pre-pregnancy BMI was generated using self-reported height and weight and was categorized into the following: underweight (<18.5 kg/m^2), normal weight (≥ 18.5 to <25 kg/m^2), overweight (25 to <29.9 kg/m^2) and obesity (≥ 30 kg/m^2). Since underweight comprised a very small proportion of BBC women, they were combined with normal weight category. Depending on the specific aim, pre-pregnancy BMI was considered as a continuous variable (as in the case of specific aim 1).

Maternal diabetes status was categorized into the following: no pre-gestational or gestational diabetes, gestational diabetes and pre-gestational diabetes. Mothers ever diagnosed with diabetes mellitus complicating pregnancy (648.00 and 648.03) comprised gestational diabetes

cases and those ever diagnosed with diabetes (250.00 – 250.93) were identified as pre-gestational diabetes cases.

Maternal Methylene tetrahydrofolate reductase (*MTHFR*) C677T genotype was categorized into CC (wild type) vs. CT (heterozygous) vs. TT (homozygous). Considering the intimate role of *MTHFR* genotype in one-carbon metabolism (involving folate and vitamin B12), it was only included in specific aim 1 analyses.

Homocysteine, an amino acid that is closely involved in folate and vitamin B12 metabolism, was included in specific aim 1 analyses, along with plasma folate and vitamin B12. It was considered as a binary variable with the top 10th percentile, compared against the bottom 90th percentile. Plasma homocysteine levels were measured using automatic clinical analyzers (Beckman-Coulter).

Child factors

Sex was coded as 0 for female and 1 for male.

Gestational age at birth was used to categorize preterm birth into full term (≥37 weeks), late preterm (≥34 to <37 weeks) and early preterm (<34 weeks). Gestational age was characterized based on the first day of the last menstrual period data and early ultrasound data (3, 5).

Year of birth was coded as a dichotomous variable - children born between 1998-2006 vs. 2007-2013.

Follow-up time was captured as a continuous variable. Since BBC is a birth cohort, follow-up time was assessed as the age at which a child had the last EMR record (between Oct 2003 and Sep 2015).

Age of blood draw was calculated as the number of months between child's birth and age of first blood draw. This variable was accounted for in the analyses that included early childhood biomarkers as the key explanatory variable (specific aims 2 and 3).

Infant feeding was categorized into 1) formula feeding only, 2) both breastfeeding and formula feeding, and 3) breastfeeding only. This data was obtained from mothers using a standardized questionnaire during the first few years of study.

Cord and early childhood insulin was used as a covariate in specific aim 3. Similar to leptin and adiponectin, insulin was measured in umbilical cord and during early childhood using a sandwich immunoassay, with an interassay coefficient of 4.0%. Cord and early childhood insulin were used as a continuous variable.

Other relevant covariates: Information about paternal social characteristics might have had a role to play in the association with ASD, but were not included in this dissertation for the following reasons. First, data on paternal characteristics was added to baseline interview around mid-2000 and therefore, this information were unavailable for children enrolled in the cohort between 1998 and mid-2000. Second, the baseline and follow-up interviews were conducted with mothers, who may or may not have accurate data on paternal characteristics. Third, maternal and paternal sociodemographic characteristics are generally related and this was true in this dataset as well. Data showed that dad's age correlated well with mother's age (correlation coefficient: 0.74) and preliminary analysis adjusting for paternal age in specific aim 1 (along with other covariates) did not alter the association between maternal plasma folate, vitamin B12 and ASD. However, including paternal age resulted in losing ~1/4th of the sample size. Thus, only maternal characteristics were used as the primary explanatory variable.

3.4 Statistical Analysis

3.4.1 Data preparation

This dissertation used BBC data from the following sources: 1) enrollment log, 2) baseline maternal questionnaire, 3) baseline medical record abstraction sheet, 4) maternal biomarker analysis data, 5) first follow-up child health questionnaire, 6) cord and early childhood biomarker analysis data, and 7) EMR data for the children that were followed. Several major data preparation steps were performed prior to data analysis. All preparation was done using Stata 13.0 statistical software (College Station, TX).

3.4.1.1 Data Entry, Cleaning, and Management

The first step in the data preparation process was data entry and cleaning, which were done separately for each of the data source. The cleaned data from different sources were systematically merged and linked using the unique ID (described in sections 3.1.1 and 3.1.2). The baseline enrolment log was maintained as an excel file, which was converted into a Stata file. Records with implausible dates such as 01/01/1900 or infant's date of birth prior to study inception, such as 01/01/1998, were coded as missing.

The baseline maternal questionnaire was originally collected in paper-based forms. Digitization of paper-based forms began in late-2013 and was conducted using digital optical recognition software (Teleform). Trained data entry operators at the JHBSPH scanned the questionnaires and converted them into PDF using Teleform software. In order to ensure data quality, the original questionnaires were compared with the entry in PDF files and any uncertainties and errors were manually corrected. The medical record abstraction sheets were originally paper-based and data from selected questions (e.g. pregnancy and obstetric

complications) were manually entered. Maternal follow-up questionnaire data was digitized using Teleform (as described above).

The EMR data corresponding to diagnosis records, inpatient prescription and outpatient prescription records of the study children were obtained from the BMC data warehouse. The diagnosis records data contained information on discharge claims and/or diagnostic information for a child's clinic or hospital visit, as well as child's identifiers. Since the follow-up period began only in Oct 2003, visits prior to this time period were not captured in the EMR. Similarly, visits after September 2015 were also not considered for this dissertation.

3.4.1.2 Analysis and Handling of Missing Data

The outcome data was complete for all the participants. As described in section 3.3.2, subjects with missing data for the key explanatory variables were excluded from the analyses. These variables included biomarker data, maternal supplement intake, fetal growth pattern and weight gain during infancy. The extent of missingness was minimal and ranged from 0% to 4% for important covariates such as pre-pregnancy BMI, diabetes status, offspring's sex and gestational age.

3.4.2. Analyses for Specific Aim 1

The primary purpose of this sub-aim was to understand the relationship between maternal nutritional status measured during pregnancy and risk of ASD. It has 2 main objectives:

- To assess the association between maternal B-vitamin biomarkers measured at delivery and risk of ASD in children

- To assess the association between preconception and prenatal supplement intake and risk of ASD in children

Descriptive analyses were conducted to compare the characteristics of neurotypical children and those with ASD using chi-squared tests for categorical variables and ANOVA for continuous variables. I fitted a Cox proportional hazard regression model to estimate hazard ratios and account for variability in length of follow-up. Birth of the child was defined as the time of origin and the child's first postnatal visit recorded in the EMR was defined as the time of entry. The child exited the ASD risk pool if he/she had the event (ASD diagnosis) or was censored after his/her recorded last postnatal visit (Sep 30, 2015). Covariates specified in the previous section were included in the model. The final model adjusted for maternal covariates such as age, parity, education status, race/ethnicity, smoking status, pre-pregnancy BMI, diabetes status, *MTHFR*, homocysteine and offspring characteristics such as sex, gestational age and year of birth.

To assess robustness of results for sub-aim 1a, I used propensity score matching to assess similarity of distribution of observable characteristics among the exposed and unexposed. Matching was implemented to reduce the possibility of model extrapolations and misspecifications. Propensity scores were constructed using a majority of the variables used in the main Cox proportional hazard model, since the internal validity of propensity score matching depends on the covariates (17). The propensity score matching was conducted in R using *MatchIt* package (18) and used estimated average effects on "treatment on treated," defined as the averaged causal effect that might be observed if everyone in the group were exposed verses none in the exposed group were exposed (19). The nearest neighbor matching

(3:1) without replacement was used, since there were a large number of controls when compared to cases.

Next, I conducted a sensitivity analysis to assess the influence of potential EMR misclassification of ASD or neurotypical development. First, a sub-set of ASD subjects were identified using a stringent categorization, defined as receiving ASD codes on at least two separate occasions and having a diagnosis by a specialist. Similarly, a sub-set of the neurotypical children were identified by excluding those with any potential developmental disability indicators. The main analyses were repeated on this sub-set population defined based on stringent ASD and stringent neurotypical definitions.

3.4.3 Analyses for Specific Aim 2

Specific Aim 2 assessed the relationship between early life growth, leptin, and ASD risk. This aim had three objectives:

- To assess the association between cord, early childhood plasma leptin and ASD risk
- To assess the association between fetal growth, weight gain during first year of life and ASD risk
- To evaluate the mediating effects of early childhood leptin in the association between weight gain during first year of life and ASD risk

As discussed in specific aim 1, descriptive analyses using chi-squared tests (for categorical variables) and ANOVA (for continuous variables) were conducted before embarking on the main analysis. Correlation between cord and early childhood leptin was assessed. Since the distribution of cord and early childhood leptin was skewed, they were log transformed. Logistic regression models were constructed to assess the following relationships: 1) fetal growth and

ASD, 2) weight gain during first year of life and ASD, 3) cord leptin and ASD, and 4) early childhood plasma leptin and ASD. In each of these models, covariates were sequentially added to the base specification to assess the robustness of the coefficient (20).

1. The **cord leptin** model adjusted for maternal age at delivery, parity, smoking, maternal BMI, maternal diabetes status, education, race, child's sex and follow-up time (base model) and in addition, adjusted for gestational age.
2. The **early childhood leptin** model adjusted for child's sex, race, age of leptin measurement, follow-up time and breastfeeding status (base model), followed by sequential adjustment of gestational age, cord leptin levels and maternal age, maternal BMI and maternal diabetes status. Specifically, cord leptin was adjusted to tease apart the independent association of early childhood leptin and ASD.
3. The **fetal growth** model adjusted for covariates including maternal age at delivery, parity, smoking, education, maternal BMI, maternal diabetes status, race, child's sex and follow-up time (base model). In the subsequent model, preterm birth was adjusted in addition to all covariates specified above.
4. The **first-year weight gain** model adjusted for child's sex, race, follow-up time and breastfeeding status (base model). Next, the following covariates were adjusted in addition to ones specified in this base model: fetal growth pattern, gestational age and maternal age, maternal diabetes status and maternal BMI. Fetal growth pattern was adjusted to delineate the independent association between first year weight gain and ASD.

As described in the previous section, sensitivity analyses were conducted to assess the

robustness of the findings. All four models were repeated using a sub-set of subjects identified by stringent ASD definition. Next, the analyses were repeated using stringent control definition. Because of the small sample size, stringent cases and controls were not concurrently run in the same model.

Mediation analysis

The analyses for specific aim 2 focused on understanding the extent to which early childhood leptin mediates the association between weight gain during first year of life and ASD risk. A mediator is defined as one that is on the causal path between exposure and outcome and is caused by the exposure (21). Although there are several methods available for mediation analysis, KHB was chosen because it allowed categorical exposure and binary outcome. KHB model decomposes the logit coefficients into total, direct and indirect effects (22). The total effect is defined as the effect of treatment variable *X* (weight gain during infancy) on outcome variable *Y* (ASD), without mediating variable *M* (early childhood leptin). The direct effect is defined as the effect of treatment variable *X* on outcome *Y*, when controlling for mediating variable *M*. The indirect effect is defined as the effect of treatment variable *X* on outcome variable *Y* through mediating variable *M* (23). The mediation percentage is derived as the ratio of the indirect effect to the total effect (24).

3.4.4 Analyses for Specific Aim 3

In specific aim 3, I looked at the association between adiponectin and ASD, and assessed the joint effects of adiponectin and preterm birth on ASD. This aim has two main objectives:

- To evaluate the association between cord and early childhood plasma adiponectin and ASD risk

- To assess the joint effects of cord adiponectin and preterm birth on ASD risk

As discussed in other specific aims, descriptive statistical analysis was conducted as the first step. Correlation between cord and early childhood plasma adiponectin was assessed. Since the distribution of explanatory variables was skewed, they were log transformed prior to analysis. Two separate regression models (described below) were constructed and covariates were sequentially added.

1. **Cord adiponectin and ASD risk**: The base model adjusted for covariates including maternal age, education, parity, smoking status, race/ethnicity, pre-pregnancy BMI, diabetes status, child's sex and follow-up time. Then, the following covariates were individually added to the base model: 1) gestational age, 2) cord insulin, 3) cord leptin, and 4) weight gain during first year of life.
2. **Early childhood adiponectin and ASD risk**: The base model adjusted for maternal age, pre-pregnancy BMI, maternal diabetes status, race/ethnicity, child's sex, age of measurement of adiponectin, breast feeding status and follow-up time. The following covariates were adjusted sequentially: 1) gestational age, 2) early childhood insulin, 3) early childhood leptin, 4) weight gain during first year of life. In addition, cord adiponectin was adjusted to the base model, to make sure that the association between early childhood adiponectin and ASD, independent of cord adiponectin is captured. Sensitivity analysis was conducted using a sub-set sample, identified using stringent criteria for ASD and neurotypical children (as discussed in sections 3.4.2 and 3.4.3).
3. Next, the joint effects of cord adiponectin and preterm birth were assessed. The referent group was term children (≥37 weeks of gestation) with high adiponectin (>50th

percentile) levels. This groups was compared against the following groups – 1) preterm (<37 weeks) and high adiponectin, 2) term and low adiponectin (≤50th percentile), and 3) preterm and low adiponectin. Similar to the regression model, I first ran an unadjusted analysis and then sequentially added relevant covariates such as maternal age, education, parity, smoking status, race/ethnicity, pre-pregnancy BMI, diabetes status, child's sex and follow-up time.

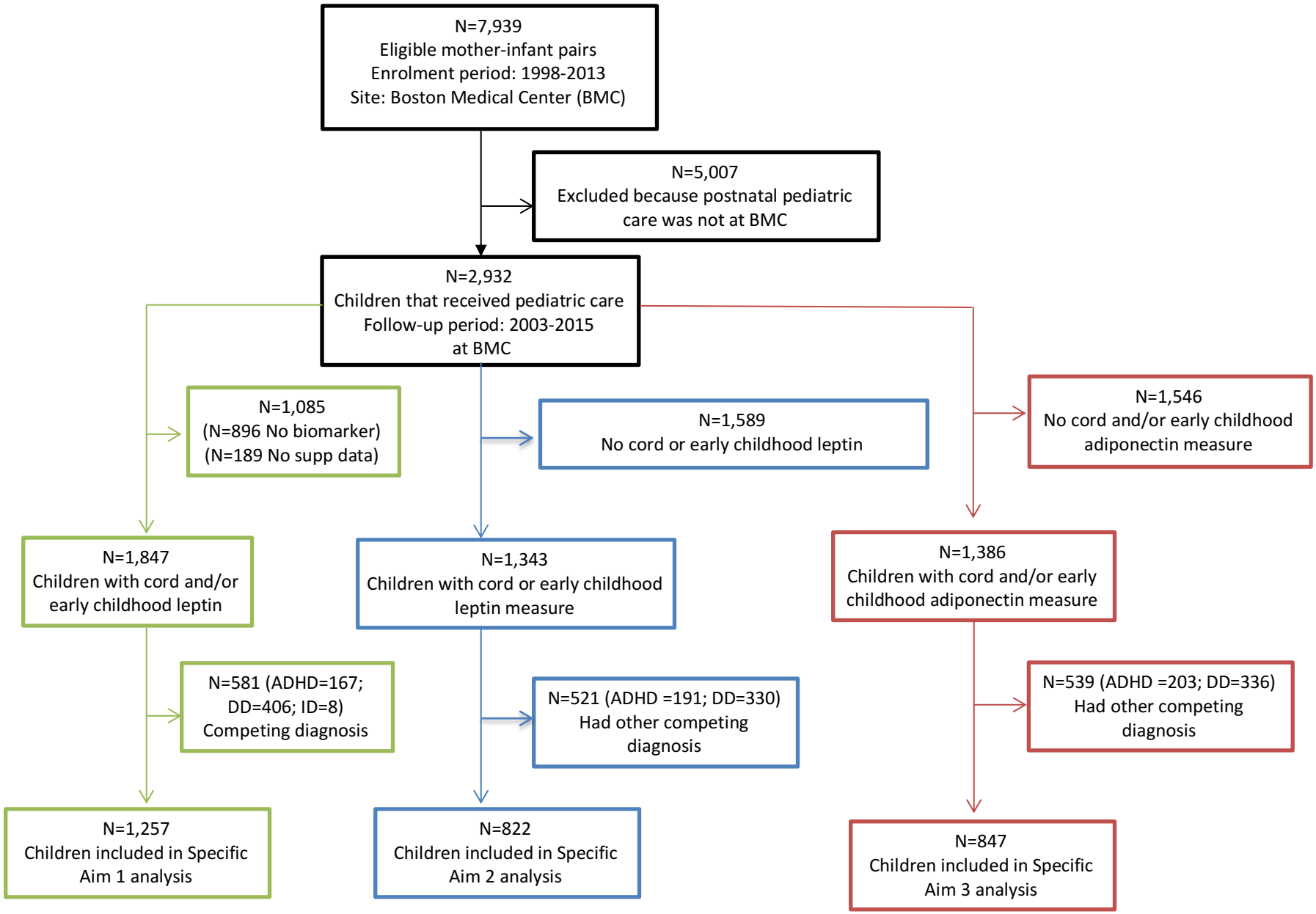
4. Finally, an exploratory mediation analysis was conducted. Since the temporality in the relationship between preterm birth and altered adiponectin levels are unclear, the mediation analysis was run both ways, as described below: 1) Cord adiponectin mediating the association between preterm birth and ASD; 2) Preterm birth mediating the association between cord adiponectin and ASD. Preterm was considered as a categorical variable (early preterm (<34 weeks), late preterm (≥34 to <37 weeks) and full term (≥37 weeks)), so that the impact of early vs. late preterm was captured. Cord adiponectin, the mediator, was treated as a continuous variable and the outcome ASD was a binary variable. The mediation analysis was run using Baron and Kenny mediation analysis. First, I adjusted for covariates that were accounted in the base regression model for cord adiponectin and ASD. The above analysis was then repeated to assess whether early childhood adiponectin is a potential mediator between preterm birth and ASD risk.

3.5 References

1. Brucato M, Ladd-Acosta C, Li M, Caruso D, Hong X, Kaczaniuk J, et al. Prenatal exposure to fever is associated with autism spectrum disorder in the boston birth cohort. Autism Res. 2017;10(11):1878-90.
2. Global BMIMC, Di Angelantonio E, Bhupathiraju Sh N, Wormser D, Gao P, Kaptoge S, et al. Body-mass index and all-cause mortality: individual-participant-data meta-analysis of 239 prospective studies in four continents. Lancet. 2016;388(10046):776-86.
3. Wang X, Zuckerman B, Pearson C, Kaufman G, Chen C, Wang G, et al. Maternal cigarette smoking, metabolic gene polymorphism, and infant birth weight. Jama. 2002;287(2):195-202.
4. Kumar R, Yu Y, Story RE, Pongracic JA, Gupta R, Pearson C, et al. Prematurity, chorioamnionitis, and the development of recurrent wheezing: a prospective birth cohort study. J Allergy Clin Immunol. 2008;121(4):878-84 e6.
5. Wang G, Divall S, Radovick S, Paige D, Ning Y, Chen Z, et al. Preterm birth and random plasma insulin levels at birth and in early childhood. Jama. 2014;311(6):587-96.
6. Hong X, Wang G, Liu X, Kumar R, Tsai HJ, Arguelles L, et al. Gene polymorphisms, breast-feeding, and development of food sensitization in early childhood. J Allergy Clin Immunol. 2011;128(2):374-81 e2.
7. Kumar R, Tsai HJ, Hong X, Liu X, Wang G, Pearson C, et al. Race, ancestry, and development of food-allergen sensitization in early childhood. Pediatrics. 2011;128(4):e821-9.
8. Li M, Fallin MD, Riley A, Landa R, Walker SO, Silverstein M, et al. The Association of Maternal Obesity and Diabetes With Autism and Other Developmental Disabilities. Pediatrics. 2016;137(2):1-10.
9. Wang G, Hu FB, Mistry KB, Zhang C, Ren F, Huo Y, et al. Association Between Maternal Prepregnancy Body Mass Index and Plasma Folate Concentrations With Child Metabolic Health. JAMA Pediatr. 2016;170(8):e160845.
10. Galloway M, Rushworth L. Red cell or serum folate? Results from the National Pathology Alliance benchmarking review. J Clin Pathol. 2003;56(12):924-6.
11. WHO. Serum and red blood cell folate concentrations for assessing folate status in populations. Vitamin and Mineral Nutrition Information System [Internet]. 2012. Available from: http://apps.who.int/iris/bitstream/10665/75584/1/WHO_NMH_NHD_EPG_12.1_eng.pdf.
12. Wang G, Johnson S, Gong Y, Polk S, Divall S, Radovick S, et al. Weight Gain in Infancy and Overweight or Obesity in Childhood across the Gestational Spectrum: a Prospective Birth Cohort Study. Sci Rep. 2016;6:29867.
13. Li M, Fallin MD, Riley A, Landa R, Walker SO, Silverstein M, et al. The Association of Maternal Obesity and Diabetes With Autism and Other Developmental Disabilities. Pediatrics. 2016;137(2):e20152206.
14. Suren P, Roth C, Bresnahan M, Haugen M, Hornig M, Hirtz D, et al. Association between maternal use of folic acid supplements and risk of autism spectrum disorders in children. Jama. 2013;309(6):570-7.
15. Schmidt RJ, Hansen RL, Hartiala J, Allayee H, Schmidt LC, Tancredi DJ, et al. Prenatal vitamins, one-carbon metabolism gene variants, and risk for autism. Epidemiology. 2011;22(4):476-85.

16. Schmidt RJ, Tancredi DJ, Ozonoff S, Hansen RL, Hartiala J, Allayee H, et al. Maternal periconceptional folic acid intake and risk of autism spectrum disorders and developmental delay in the CHARGE (CHildhood Autism Risks from Genetics and Environment) case-control study. The American journal of clinical nutrition. 2012;96(1):80-9.
17. Slade EP, Stuart EA, Salkever DS, Karakus M, Green KM, Ialongo N. Impacts of age of onset of substance use disorders on risk of adult incarceration among disadvantaged urban youth: a propensity score matching approach. Drug Alcohol Depend. 2008;95(1-2):1-13.
18. Ho DE, Imai K, King G, Stuart EA. Matching as nonparametric preprocessing for reducing model dependance in parametric causal inference. Political Analysis. 2007;15:199-236.
19. Harder VS, Stuart EA, Anthony JC. Adolescent cannabis problems and young adult depression: male-female stratified propensity score analyses. American journal of epidemiology. 2008;168(6):592-601.
20. Gelbach JB. When do covariates matter? And which ones, and how much? Journal of Labor Economics. 2014.
21. Richmond RC, Hemani G, Tilling K, Davey Smith G, Relton CL. Challenges and novel approaches for investigating molecular mediation. Hum Mol Genet. 2016;25(R2):R149-R56.
22. Karlson KB, Holm A, Breen R. Total, Direct, and Indirect effects in Logit models. Aarhus University; 2010.
23. Zhang Y, Liu Y, Wang J, Jia C. Mediation of smoking consumption on the association of perception of smoking risks with successful spontaneous smoking cessation. Int J Behav Med. 2014;21(4):677-81.
24. Linden A, Karlson KB. Using mediation analysis to identify causal mechanisms in disease management interventions. Health Serv Outcomes Res Method. 2013;13:86-108.

Figure 3-1 Flow chart showing the sample size for each specific aim



CHAPTER 4

Manuscript 1

Maternal Multivitamin Intake, Plasma Folate and Vitamin B12 Levels and Autism Spectrum Disorder Risk in Offspring

(This paper has been published and the reference is provided here: Raghavan R, Riley AW, Volk H, Caruso D, Hironaka L, Sices L, Hong X, Wang G, Ji Y, Brucato M, Wahl A, Stivers T, Pearson C, Zuckerman B, Stuart EA, Landa R, Fallin MD, Wang X. Maternal Multivitamin Intake, Plasma Folate and Vitamin B12 Levels and Autism Spectrum Disorder Risk in Offspring. *Paediatric and Perinatal Epidemiology.* 2018;32(1):100-111.)

4.1 Abstract

Background: To examine the prospective association between multivitamin supplementation during pregnancy and biomarker measures of maternal plasma folate and vitamin B12 levels at birth and child's Autism Spectrum Disorder (ASD) risk.

Methods: This report included 1257 mother-child pairs, who were recruited at birth and prospectively followed through childhood at the Boston Medical Center. ASD was defined from diagnostic codes in electronic medical records. Maternal multivitamin supplementation was assessed via questionnaire interview; maternal plasma folate and B12 were measured from samples taken 2-3 days after birth.

Results: Moderate (3-5 times/week) self-reported supplementation during pregnancy was associated with decreased risk of ASD, consistent with previous findings. Using this as the reference group, low (≤2 times/week) and high (>5 times/week) supplementation was associated with increased risk of ASD. Very high levels of maternal plasma folate at birth (≥60.3 nmol/L) had 2.5 times increased risk of ASD (95% confidence interval (CI) 1.3, 4.6) compared to folate levels in the middle 80th percentile, after adjusting for covariates including *MTHFR* genotype. Similarly, very high B12 (≥536.8 pmol/L) showed 2.5 times increased risk (95% CI 1.4, 4.5).

Conclusion: There was a "U" shaped relationship between maternal multivitamin supplementation frequency and ASD risk. Extremely high maternal plasma folate and B12 levels at birth were associated with ASD risk. This hypothesis-generating study does not question the importance of consuming adequate folic acid and vitamin B12 during pregnancy; rather, raises

new questions about the impact of extremely elevated levels of plasma folate and B12 exposure *in utero* on early brain development.

Key Words: Autism, folate, vitamin B12, prenatal supplement intake

4.2 Introduction

Autism Spectrum Disorder (ASD) is a heterogeneous group of neurodevelopmental conditions characterized by impaired social reciprocity, abnormal communication and repetitive or unusual behavior (1, 2). The prevalence of ASD was about five per 10,000 individuals in 1980s, but recent estimates in the U.S. suggest that it is now one in 68 individuals (3, 4). The etiology of ASD is complex, and includes the interplay of genetic and environmental factors (5, 6). Folate is an essential B vitamin involved in nucleic acid synthesis, DNA methylation, and repair (7). Folic acid is a synthetic form of folate that is commonly used to fortify foods, and consumed as nutritional supplements (8). In light of substantial evidence that folic acid supplementation reduces the risk of neural tube defects (NTD) in offspring, the US Public Health Service recommended in 1992 that women of reproductive age consume 400 µg/d before and during pregnancy. Subsequently, mandatory fortification of cereal grain products at a suggested level of 140 µg folic acid/100 g was implemented in the US in 1998 (9).

In the post-fortification era, serum folate levels in the US have increased 2.5 times across all life stages, including among pregnant women (10). A recent NHANES study showed that unmetabolized folic acid (either from folic acid supplementation or fortified grain products) has been detected in most of the U.S. population (11). Furthermore, data from the Boston Birth Cohort showed a wide range of individual variation in plasma folate levels ranging from insufficient to excessive levels (12). The association between folic acid intake during pregnancy and ASD risk in offspring has been equivocal. Several studies suggest that mothers who use a periconceptional multivitamin or folic acid supplementation are less likely to have offspring with ASD, (2, 13) yet, others have hypothesized an opposite relationship (14-16).

Preliminary studies that included both women's report of prenatal vitamin use and maternal biomarker data found a protective effect of prenatal vitamins intake on ASD based on report, but this relationship could not be confirmed using biomarker data (17, 18). Given this background, in this hypothesis-generating study, we evaluated the relationship between self-reported pregnancy multivitamin intake, and plasma folate levels in mothers at birth and ASD in offspring. Since both vitamin B12 and folate are intricately involved in one-carbon metabolism and there are very few studies on B12 status on human brain (19), we also explored the association between maternal B12 biomarker levels and ASD risk in offspring.

4.3 Methods

4.3.1 Participants and data collection procedure

The study included mother-infant pairs who were recruited at the Boston Medical Center (BMC) at the time of birth from 1998 to 2013 and followed up prospectively from 2003 to 2015 (supplemental figure 4-1), as described elsewhere (12, 20). Children who were not intending to receive pediatric care at the BMC were excluded. Mothers who had a post-birth blood sample for analyzing plasma folate and B12 and who had data on maternal multivitamin supplement intake during at least the third trimester were included in the analysis.

Mothers of newborns were approached 24-72 hours postpartum to participate in the study. After obtaining informed consent, a standard questionnaire was used to collect relevant maternal data including supplement intake. Maternal and infant medical records were reviewed using standardized abstraction forms to collect data on pre-pregnancy weight and pregnancy related complications. Maternal blood samples collected 24-72 hours post-delivery were later analyzed for maternal plasma folate, B12 and homocysteine levels. Children were

followed from birth through both study visits and clinical pediatric visits at the BMC. Electronic Medical Records (EMR) containing clinicians' primary and secondary diagnoses using ICD-9 codes were obtained for every postnatal clinical visit starting in 2003. The study was approved by the Institutional Review Boards of the Johns Hopkins Bloomberg School of Public Health and Boston University Medical Center.

4.3.2 Identification of children with ASD

Based on EMR, children who were ever diagnosed with autism (ICD-9 code 299.00), Asperger syndrome (299.80) and/or pervasive developmental disorder not otherwise specified (299.90) were categorized as having ASD, as described elsewhere (21). Children who concomitantly had ASD and ADHD, ASD and other developmental disabilities, or ASD and intellectual disabilities were classified as having ASD. Children without ASD, ADHD (314.0-314.9), other developmental (315.0 - 315.9), or intellectual disabilities (317 – 319) constituted the 'neurotypical' group. Children diagnosed with ADHD or other developmental or intellectual disabilities without concurrent ASD were excluded from the analysis. One subject who had Ventricular Septal Defect (VSD), but was miscoded as having ASD, was identified and excluded. For sensitivity analyses, children with ASD were restricted to those with an ASD code for at least 2 visits and where at least one visit was with a specialist (developmental behavioral pediatrician, pediatric neurologist or child psychologist). Sensitivity analyses were also implemented restricting neurotypical children to only those that did not have other competing diagnoses, including congenital anomalies and psychiatric or behavioral disorders (see supplemental table 4-1).

4.3.3 Exposures

Plasma folate was measured using chemiluminescent immunoassay with diagnostic kits (Shenzhen New Industries Biomedical Engineering Co., Ltd. China) and plasma B12 was measured using the Beckman Coulter ACCESS Immunoassay System (Beckman-Coulter Canada, Mississauga, Canada) using a MAGLUMI 2000 Analyzer. The interassay coefficient of variation was less than 4% (12). Mothers with the lowest and highest deciles (top and bottom 10^{th} percentiles) of the plasma folate (<14.7 and ≥60.3 nmol/L) and B12 (<247.0 and ≥536.8 pmol/L) distributions were compared against the middle 80^{th} percentile.

Preconception multivitamin intake was dichotomized (no vs. yes) and prenatal multivitamin supplement intake was coded as a categorical variable (supplement ≤2 times/week, 3-5 times/week and >5 times/week). Mothers ever diagnosed with diabetes (250.00-250.93) were identified as pregestational diabetes cases and those ever diagnosed with diabetes mellitus complicating pregnancy (648.00 and 648.03) comprised gestational diabetes cases.

4.3.4 Covariates

Plasma homocysteine levels were measured using automatic clinical analyzers (Beckman-Coulter). Homocysteine was considered as a binary variable with the top 10^{th} percentile of the distribution compared against the bottom 90^{th} percentile (<11.7 µmol/L). Other covariates were chosen *a priori* based on previous studies looking at maternal nutritional status and the risk of ASD (13, 22). Neonates who were delivered at or after 37 completed weeks of gestation were considered full term; those delivered <34 weeks, and ≥34 but <37 weeks of gestation were considered early and late preterm, respectively. Race-ethnicity was categorized into black, white, Hispanic and Other. Other covariates assessed were: child sex

(female vs. male), maternal age at delivery, smoking during pregnancy ("ever smoked" 3 months before pregnancy/during pregnancy vs. "no smoking" during preconception/ pregnancy), parity (not including the index pregnancy), maternal education (high school or less vs. some college or more), year of the baby's birth (1998-2006 vs. 2007-2013) and Methylene tetrahydrofolate reductase (*MTHFR*) C677T genotypes (CC vs. CT vs. TT).

4.3.5 Statistical Analyses

The primary outcome variable was ASD and exposures were maternal vitamin supplementation during preconception, 1st, 2nd and 3rd trimesters or maternal plasma folate and B12 levels in the days after birth. Preliminary data analysis was performed to compare neurotypical children and those with ASD using chi-squared tests for categorical variables and ANOVA for continuous variables. We fitted a Cox proportional hazard regression model to estimate hazard ratios and account for the variability in length of follow-up. Birth of the child was defined as the time of origin and the child's first postnatal visit recorded in the EMR was defined as the time of entry. The child exited the ASD risk pool if he/she had the event (ASD diagnosis) or was censored after his/her recorded last postnatal visit. In addition, the role of maternal *MTHFR* C677T genotype was examined via stratification and cross-product proportional hazard regression analyses. We tested the interaction of maternal folate (as a binary variable) and 1) B12 levels (as a binary variable) and 2) *MTHFR* C677T (as a categorical variable) on the risk of ASD.

4.4 Results

A total of 1257 mother-infant pairs were included in the analysis, of which 86 were ASD cases and 1171 were children with neurotypical development (supplemental figure 4-1).

Children with ASD had co-morbidities including ADHD (n=25), intellectual disabilities (n=39) and other developmental disabilities (n=76), that were not mutually exclusive. Table 4-1 describes the characteristics of mothers and children in each group. The frequency of multivitamin supplement intake during third trimester differed between mothers whose children had ASD when compared to those who had children with neurotypical development. They were also more likely to have higher pre-pregnancy BMI, pre-gestational/gestational diabetes and very high maternal B12 (≥90th percentile). The distribution of folate and B12 levels in our study population is consistent with the NHANES data for women of reproductive age (supplemental figure 4-2) and detailed comparisons are provided in supplemental tables 4-2 and 4-3. To understand the impact of differential follow-up, analyses were conducted on this cohort comparing baseline characteristics of the children included in the analyses and those excluded from the analyses.

Maternal multivitamin supplement intake preconception was not statistically significantly associated with the risk of ASD in children (Table 4-2). Consistent with previous research, (13) 1st trimester supplement intake (≥ 3 times/week) was protective against ASD risk when compared to those who reported taking a supplement <3 times/week; or conversely, low levels of multivitamin supplement intake were associated with increased ASD risk. When supplement intake was stratified by intake frequency, a “U” shaped relationship was observed, with maternal supplement intake ≤2 times/week and >5 times/week during both demonstrating statistically significantly increased risk for ASD (Table 4-2 and Figure 4-1). This “U” shaped relationship was consistent across all trimesters and became stronger after adjusting for potential confounders.

Elevated maternal plasma B12 (>600 pmol/L) compared to non-elevated (≥200 – ≤600 pmol/L) levels was associated with increase in risk of ASD in offspring in both unadjusted and adjusted models (Table 4-3) (23). Deficient B12 (<200 pmol/L) compared to normal levels was not associated with ASD risk (Table 4-3).

The risk of ASD in children along the continuum of plasma folate and B12 is presented in Figure 4-2 and supplemental tables 4-4 and 4-5. Using the WHO suggested threshold, the risk of ASD was not significantly different between children when their mothers had possibly deficient (<13.5 nmol/L) or excess (>45.3 nmol/L) plasma folate levels after birth, compared to mothers who had normal levels (≥13.5 to ≤45.3) (Table 4-3). However, the risk of ASD among mother-child pairs with higher levels of plasma folate suggests association at increasing folate levels.

We categorized and compared the lowest and highest deciles with the middle 80th percentiles for maternal folate (<14.7 and ≥60.3 nmol/L) and B12 levels (<247.0 and ≥536.8 pmol/L). Mothers with plasma folate levels in the highest 10th percentile, when compared with the middle 80th percentile, had a significant increase in the risk of children's ASD in both unadjusted and adjusted models (Table 4-3). Mothers who had plasma folate levels in the lowest decile did not have increased ASD risk in children in the adjusted model (Table 4-3). Plasma B12 levels in the top decile, when compared to the middle 80%, was associated with about two and half times increased risk (Table 4-3). However, the risk of ASD in children whose mothers had lowest levels of plasma B12 was not significantly different than the referent group in both models. Similarly, elevated maternal plasma homocysteine when compared to non-elevated levels did not alter ASD risk even after accounting for confounders (Table 4-3).

We compared self-reported maternal multivitamin supplement intake to measured biomarker levels after birth. Nearly all mothers took supplements during pregnancy; the frequencies of use for 1st, 2nd and 3rd trimesters were 86.2%, 90.2% and 89.1% respectively. Within each supplement intake category, there was a range in maternal plasma folate and B12 levels (supplemental table 4-6, supplemental figures 4-3 and 4-4). There were no differences in the mean plasma folate levels between non-supplement users and different levels of supplement users, except those who had supplements 3-5 times/week. The percentage of mothers with elevated plasma folate and B12 did not vary between different levels of supplement intake. When supplement intake was stratified by parity, a greater percentage of nulliparous women were likely to consume supplements >5 times/week in the 1st and 3rd trimesters (supplemental table 4-7).

We assessed the joint effects of maternal plasma folate and B12 levels on the risk of ASD in children by considering mothers who had both biomarkers in the middle 80th percentile at delivery as the referent category (Table 4-3). The risk of ASD in children was not different between mothers with only one or the other biomarker in an extreme decile compared to mothers who had both biomarkers in the middle 80th percentile, after adjusting for confounders. For mothers who had *at least one* biomarker level (plasma folate and/or B12) in the lowest decile, the risk of ASD in children was not different, nor was it for *both* in the lowest decile (Table 4-3). Mothers who had *at least* one or *both* biomarkers in the top decile did have an increased risk for ASD in their offspring in adjusted models (Table 4-3). There was an interaction between maternal folate and B12 ($P < 0.01$).

MTHFR genotype was available for 96.5% of the mothers (n=1213) of which 794 (65.9%), 347 (28.6%) and 72 (5.9%) had CC, CT and TT genotype respectively. In this sample, maternal *MTHFR* genotype did not differ between children with neurotypical development and those with ASD (Supplemental Table 4-8). No differences in genotype were observed by maternal folate and B12 levels (Supplemental Table 4-9). The geometric means of plasma folate were also not significantly different across different genotypes (Supplemental Table 4-10) and there was no interaction between maternal folate and *MTHFR* genotype status on ASD risk.

To assess the influence of EMR misclassification of ASD or neurotypical development on these findings, a sensitivity analysis (Supplemental Table 4-11) was conducted applying stringent criteria for case diagnosis (ASD code for at least 2 visits, including a specialist) and for children with neurotypical development (excluding potential developmental disability indications) (Supplemental Table 4-1). The results demonstrate slightly stronger associations in this smaller sample; mothers who had plasma folate in the top decile had more than two-fold greater ASD risk (HR 2.4, 95% CI 1.1, 5.0) and with plasma B12 in the top decile had almost four times increased risk (HR 3.9, 95% CI 2.0, 7.7), after adjusting for confounders. Consistent with results in the full sample, the risk of ASD was highest when mothers had elevated levels of both plasma folate and B12 (HR 16.4, 95% CI 6.5, 41.7). Results from alternative analyses using propensity score matching (Supplemental Table 4-13) and logistic regression (Supplemental Table 4-14) were consistent in direction and statistical significance.

4.5 Comment

4.5.1 Main findings

The results show that moderate intake (3-5 times/week) of multivitamin supplements during pregnancy is associated with decreased risk of ASD in offspring, consistent with the previous literature (2, 13). Upon further examination of the risk at the low and high ends of intake, the study results further suggest that while infrequent intake (≤2 times/week) of multivitamin supplements is associated with increased risk of ASD, as has been reported previously, high frequency of multivitamin supplement intake (>5 times/week) is also associated with increased risk of ASD in children in this cohort, compared to moderate intake. This is the first study to report on the prospective association between measured maternal plasma folate and B12 biomarkers at birth and risk of ASD in offspring in a large, prospective US birth cohort. Consistent with the "U-shaped" finding for supplement intake, the biomarker analyses showed that very high levels of maternal plasma folate and B12 (≥90th percentile) at birth were also associated with increased risk of ASD in offspring.

With regard to post-delivery biomarkers, which are reasonably consistent with third trimester pregnancy levels, (24, 25) maternal plasma folate and B12 in the highest decile (≥90th percentile) were each associated with increased risk of ASD in children. A moderate level of self-reported supplement intake (3-5 times/week) is protective against ASD, but that an increased risk is observed with higher and lower intake of multivitamin supplements, and with very high concentrations of plasma folate (≥90th percentile) and B12 (≥90th percentile) biomarkers at birth. This observation suggests that while deficiency is detrimental, excess nutrient status might also be associated with elevated risk.

4.5.2 Interpretation

For over a century, it has been known that the dose-response relationship for many micronutrients is non-monotonic: at low levels benefits increase with intake until plateauing at optimal concentrations, with toxicity at higher levels, as regulatory mechanisms become overwhelmed (26, 27). In a landmark paper, Daly et al. showed that maximum NTD risk was observed in mothers who had folate deficiency (0-4.4 nmol/L). There was a dose response relationship with risk plateauing beyond maternal folate levels of 11.3 nmol/L and no apparent additional benefits beyond levels of 15.9 nmol/L (28). At the other end of the spectrum, this study observed an increased ASD risk when mothers had plasma folate levels in the top decile during the third trimester (corresponding to 60.3 nmol/L), which is well beyond the highest level recommended by WHO (45.3 nmol/L).

This study is not able to directly attribute the source of these high levels for the top decile. It is possible that adverse birth outcomes such as NTD, spontaneous abortions, stillbirths and developmental disabilities, (29-31) in a previous delivery might have prompted some pregnant women to consume higher dosages of prenatal vitamins, which could explain elevated biomarker levels. However, there were no significant difference in previous adverse pregnancy outcomes between mothers with ASD and children with neurotypical development in this sample. Upon further examination of EMR-based medication history for mothers of children with ASD, none of them were prescribed megadoses of prenatal vitamins.

Mandatory folic acid fortification was instituted in the U.S. in January 1998 and the BBC began enrolling mothers in October 1998. Considering that BBC is a post-fortification study, it is possible that women with high folic acid intake also consumed folic acid from multiple sources including fortified foods, possibly creating an accumulation of folic acid, as observed in a recent

NHANES study (32). In this sample, mothers who consumed more supplements were also more likely to have consumed fortified foods (e.g. pasta, bread, cereal), suggesting that the combination of dietary folic acid along with supplement intake might have potentially resulted in elevated levels, although correlations between supplement intake and biomarker levels were not extremely high.

This study notes that the highest ASD risk (HR 13.7, 95% CI 6.5, 28.9) was observed in children of mothers with both plasma folate and B12 elevated (≥90th percentile). In addition, a significant interaction was observed between plasma folate and B12 ($p<0.001$) suggesting possible perturbation in one-carbon metabolism, which intimately involves both micronutrients.

One possible theory suggests that elevated maternal folate levels in the past few decades have altered natural selection, increasing the survival rates of those with the *MTHFR* C677T polymorphism by reducing miscarriage rates (15). It is also possible that genetic variation may interfere with folic acid absorption or metabolism to folate precluding the benefits of folic acid and promoting accumulation in maternal blood. In this study, there was no relationship between the *MTHFR* C677T variant, plasma folate and ASD risk. However, the study did not exhaustively examine variation in this gene, or other genes in the one-carbon cycle. Also, due to the small number of mothers with TT genotype in this sample, the study might be underpowered to address this question.

It is not surprising that some nutrients are tightly regulated within a narrow range, given that both deficiency and excess can induce abnormal brain development (33). The concentration of nutrients can have an impact on the brain development with a nutrient that

promotes normal development in one concentration may be toxic at another (33). Similarly, the timing of supplementation or deficiency can also have a role to play in brain function (33, 34). Pregnant women have superior absorption of folic acid and B12 compared to non-pregnant counterparts (35) and excrete minimal folate in urine during the third trimester (36) – all of which could have lead to increased plasma levels. Considering the elevated maternal levels in addition to fetus's ability to actively absorb micronutrients, it is likely that some offspring may have accumulated high levels of these micronutrients. The fetal brain is vulnerable to nutritional insults especially during the third trimester, when several neurological processes including synaptogenesis increase in cortical volume and cortical connectivity between different regions, myelination and pruning are occurring at a rapid rate (33, 37-40).

Human and animal studies have shown that sub-optimal intake of folic acid during pregnancy can induce persistent changes in the offspring's genome, thereby influencing physiological outcomes (41-45). With regards to neurocognitive development, increase in maternal folate during gestation in animal models alters gene expression in cerebral and cerebellar hemispheres (41, 42). Specifically, key developmental genes involved in neural pathways, gamma-Aminobutyic acid (GABA), dopamine-serotonin and synaptic plasticity demonstrated altered expression and impairment in many of the pathways are linked to ASD (42, 46, 47). Prolonged exposure to high folic acid has been shown to alter offspring's behavior, including greater anxiety-like behavior, ultrasonic vocalizations in pups (linked to autism in mouse models) and hyperactivity (42). B12 plays an important role in DNA methylation, in addition to being integrally involved in myelination, (48) cellular growth and differentiation (49). Yet, there is a dearth of research on the role of B12 status on developing brains (19). A

cross-sectional study showed that maternal B12 measured at parturition was inversely associated with DNA methylation of insulin-like growth factor (IGF-2) in cord blood (50).

4.5.3 Limitations and Strengths of the study

Case and neurotypical development classification was based on EMR, rather than using adjudication based on research-reliable gold standard diagnostic assessments such as the Autism Diagnostic Interview-Revised (ADI-R) or the Autism Diagnostic Observation Schedule (ADOS) (51). This approach enables consideration of all children with available administrative data, but may misclassify some children as ASD who have other developmental or behavioral problems. The study findings were consistent even when restricting cases to those with multiple visits indicating an ASD diagnostic code, including by a specialist. Further, if such misclassification of atypical, but non-ASD children exists in the sample, the results would imply that elevated maternal plasma folate and B12 levels could have implications in other developmental disabilities beyond ASD.

Maternal dietary intake data during preconception and pregnancy might have provided additional perspectives on the study results and lack of this information is a limitation. Maternal plasma folate and B12 were measured 24-72 hours after delivery; this may reflect maternal folate and B12 levels only during the third trimester and may not reflect early pregnancy status. Despite plasma folate being a marker of recent intake and more susceptible to variations in diet, it is well correlated with red blood cell folate and thus is a reliable marker (52). Although we had information on the frequency of maternal multivitamin supplement intake, lack of information on specific dosage is a limitation. A study based on NHANES data suggests that a majority of pregnant women consume ≥800 µg of folic acid when using

supplements and we assume this to be true of women in this cohort as well (53). We adjusted for well-recognized ASD risk factors, but there is a possibility of residual confounding. Finally, the study population consists mainly of urban low-income minority women and extrapolation of study findings to other populations should be made with caution.

While the study suggests that very low or very high maternal folate and B12 measured at delivery may be associated with ASD in offspring, further research in the following areas will be beneficial, beginning with replication of these findings in independent cohorts with more vigorous ASD phenotyping, understanding the mechanism connecting elevated folate, B12 to neurocognitive development and examining the source of elevated biomarkers. Further, more research is needed to understand if the window of exposure affects the impact of folic acid supplementation.

4.6 Conclusion

The etiology of ASD is complex and this study provides a new perspective but not a definitive explanation. This hypothesis-generating study does not question the importance of consuming adequate amounts of folic acid and B12 during pregnancy; the results confirm the protective effects of adequate vitamin supplementation for ASD risk, as observed in previous studies. Rather, it raises questions about whether excessive amounts of maternal folate and B12 may be harmful if a “U-shaped” risk curve is confirmed, as has been observed for other health-related risk (54). This result suggests further investigation of this potentially U-shaped risk in other cohorts and underscores the critical importance of identifying optimum maternal levels of folate and B12 for fetal/child neurodevelopment.

4.7 References

1. Liptak GS, Benzoni LB, Mruzek DW, Nolan KW, Thingvoll MA, Wade CM, et al. Disparities in diagnosis and access to health services for children with autism: data from the National Survey of Children's Health. J Dev Behav Pediatr. 2008;29(3):152-60.
2. Schmidt RJ, Hansen RL, Hartiala J, Allayee H, Schmidt LC, Tancredi DJ, et al. Prenatal vitamins, one-carbon metabolism gene variants, and risk for autism. Epidemiology. 2011;22(4):476-85.
3. DeVilbiss EA, Gardner RM, Newschaffer CJ, Lee BK. Maternal folate status as a risk factor for autism spectrum disorders: a review of existing evidence. The British journal of nutrition. 2015:1-10.
4. Developmental Disabilities Monitoring Network Surveillance Year Principal I, Centers for Disease C, Prevention. Prevalence of autism spectrum disorder among children aged 8 years - autism and developmental disabilities monitoring network, 11 sites, United States, 2010. MMWR Surveill Summ. 2014;63(2):1-21.
5. Xu G, Jing J, Bowers K, Liu B, Bao W. Maternal diabetes and the risk of autism spectrum disorders in the offspring: a systematic review and meta-analysis. J Autism Dev Disord. 2014;44(4):766-75.
6. Gardener H, Spiegelman D, Buka SL. Prenatal risk factors for autism: comprehensive meta-analysis. Br J Psychiatry. 2009;195(1):7-14.
7. Crider KS, Yang TP, Berry RJ, Bailey LB. Folate and DNA methylation: a review of molecular mechanisms and the evidence for folate's role. Adv Nutr. 2012;3(1):21-38.
8. Christensen KE, Mikael LG, Leung KY, Levesque N, Deng L, Wu Q, et al. High folic acid consumption leads to pseudo-MTHFR deficiency, altered lipid metabolism, and liver injury in mice. The American journal of clinical nutrition. 2015;101(3):646-58.
9. Choumenkovitch SF, Selhub J, Wilson PW, Rader JI, Rosenberg IH, Jacques PF. Folic acid intake from fortification in United States exceeds predictions. J Nutr. 2002;132(9):2792-8.
10. Pfeiffer CM, Hughes JP, Lacher DA, Bailey RL, Berry RJ, Zhang M, et al. Estimation of trends in serum and RBC folate in the U.S. population from pre- to postfortification using assay-adjusted data from the NHANES 1988-2010. J Nutr. 2012;142(5):886-93.
11. Pfeiffer CM, Sternberg MR, Fazili Z, Yetley EA, Lacher DA, Bailey RL, et al. Unmetabolized folic acid is detected in nearly all serum samples from US children, adolescents, and adults. J Nutr. 2015;145(3):520-31.
12. Wang G, Hu FB, Mistry KB, Zhang C, Ren F, Huo Y, et al. Association Between Maternal Prepregnancy Body Mass Index and Plasma Folate Concentrations With Child Metabolic Health. JAMA Pediatr. 2016;170(8):e160845.
13. Suren P, Roth C, Bresnahan M, Haugen M, Hornig M, Hirtz D, et al. Association between maternal use of folic acid supplements and risk of autism spectrum disorders in children. JAMA. 2013;309(6):570-7.
14. Beard CM, Panser LA, Katusic SK. Is excess folic acid supplementation a risk factor for autism? Med Hypotheses. 2011;77(1):15-7.
15. Rogers EJ. Has enhanced folate status during pregnancy altered natural selection and possibly Autism prevalence? A closer look at a possible link. Med Hypotheses. 2008;71(3):406-10.

16. King CR. A novel embryological theory of autism causation involving endogenous biochemicals capable of initiating cellular gene transcription: a possible link between twelve autism risk factors and the autism 'epidemic'. Med Hypotheses. 2011;76(5):653-60.
17. Braun JM, Froehlich T, Kalkbrenner A, Pfeiffer CM, Fazili Z, Yolton K, et al. Brief report: are autistic-behaviors in children related to prenatal vitamin use and maternal whole blood folate concentrations? J Autism Dev Disord. 2014;44(10):2602-7.
18. Steenweg-de Graaff J, Ghassabian A, Jaddoe VW, Tiemeier H, Roza SJ. Folate concentrations during pregnancy and autistic traits in the offspring. The Generation R Study. Eur J Public Health. 2015;25(3):431-3.
19. Zhang Y, Hodgson NW, Trivedi MS, Abdolmaleky HM, Fournier M, Cuenod M, et al. Decreased Brain Levels of Vitamin B12 in Aging, Autism and Schizophrenia. PLoS One. 2016;11(1):e0146797.
20. Wang X, Zuckerman B, Pearson C, Kaufman G, Chen C, Wang G, et al. Maternal cigarette smoking, metabolic gene polymorphism, and infant birth weight. Jama. 2002;287(2):195-202.
21. Li M, Fallin MD, Riley A, Landa R, Walker SO, Silverstein M, et al. The Association of Maternal Obesity and Diabetes With Autism and Other Developmental Disabilities. Pediatrics. 2016;137(2):1-10.
22. Schmidt RJ, Tancredi DJ, Ozonoff S, Hansen RL, Hartiala J, Allayee H, et al. Maternal periconceptional folic acid intake and risk of autism spectrum disorders and developmental delay in the CHARGE (CHildhood Autism Risks from Genetics and Environment) case-control study. Am J Clin Nutr. 2012;96(1):80-9.
23. Arendt JF, Nexo E. Unexpected high plasma cobalamin : proposal for a diagnostic strategy. Clin Chem Lab Med. 2013;51(3):489-96.
24. Tamura T, Picciano MF. Folate and human reproduction. The American journal of clinical nutrition. 2006;83(5):993-1016.
25. Milman N, Byg KE, Bergholt T, Eriksen L, Hvas AM. Cobalamin status during normal pregnancy and postpartum: a longitudinal study comprising 406 Danish women. Eur J Haematol. 2006;76(6):521-5.
26. Raubenheimer D, Lee KP, Simpson SJ. Does Bertrand's rule apply to macronutrients? Proceedings Biological sciences / The Royal Society. 2005;272(1579):2429-34.
27. Barua S, Kuizon S, Ted Brown W, Junaid MA. High Gestational Folic Acid Supplementation Alters Expression of Imprinted and Candidate Autism Susceptibility Genes in a sex-Specific Manner in Mouse Offspring. J Mol Neurosci. 2015.
28. Daly LE, Kirke PN, Molloy A, Weir DG, Scott JM. Folate levels and neural tube defects. Implications for prevention. Jama. 1995;274(21):1698-702.
29. Wilson RD, Genetics C, Wilson RD, Audibert F, Brock JA, Carroll J, et al. Pre-conception Folic Acid and Multivitamin Supplementation for the Primary and Secondary Prevention of Neural Tube Defects and Other Folic Acid-Sensitive Congenital Anomalies. J Obstet Gynaecol Can. 2015;37(6):534-52.
30. Zahran KM, Adb Elaal DEM, Kamel HS, Samy EI, Ismail AM, Abbas AM. A combination treatment of folic acid, aspirin, doxycycline and progesterone for women with recurrent early pregnancy loss; hospital based study. Middle East Fertility Society Journal. 2016;21(1):22-6.

31. Czeizel AE, Puho E. Maternal use of nutritional supplements during the first month of pregnancy and decreased risk of Down's syndrome: case-control study. Nutrition. 2005;21(6):698-704; discussion 74.
32. Orozco AM, Yeung LF, Guo J, Carriquiry A, Berry RJ. Characteristics of U.S. Adults with Usual Daily Folic Acid Intake above the Tolerable Upper Intake Level: National Health and Nutrition Examination Survey, 2003-2010. Nutrients. 2016;8(4):195.
33. Georgieff MK. Nutrition and the developing brain: nutrient priorities and measurement. Am J Clin Nutr. 2007;85(2):614S-20S.
34. Fuglestad AJ, Rao R, Georgieff MK. The role of nutrition in cognitive development [Available from: http://hirezz.com/satvenandmer.com/pdf/dha/Nutrition and Cognitive Development .pdf.
35. Hellegers A, Okuda K, Nesbitt RE, Jr., Smith DW, Chow BF. Vitamin B12 absorption in pregnancy and in the newborn. The American journal of clinical nutrition. 1957;5(3):327-31.
36. West AA, Yan J, Perry CA, Jiang X, Malysheva OV, Caudill MA. Folate-status response to a controlled folate intake in nonpregnant, pregnant, and lactating women. The American journal of clinical nutrition. 2012;96(4):789-800.
37. Mahoney AD, Minter B, Burch K, Stapel-Wax J. Autism spectrum disorders and prematurity: a review across gestational age subgroups. Adv Neonatal Care. 2013;13(4):247-51.
38. Tau GZ, Peterson BS. Normal development of brain circuits. Neuropsychopharmacology. 2010;35(1):147-68.
39. Cusick SE, Georgieff MK. The Role of Nutrition in Brain Development: The Golden Opportunity of the "First 1000 Days". J Pediatr. 2016;175:16-21.
40. Georgieff MK, Brunette KE, Tran PV. Early life nutrition and neural plasticity. Dev Psychopathol. 2015;27(2):411-23.
41. Barua S, Kuizon S, Brown WT, Junaid MA. DNA Methylation Profiling at Single-Base Resolution Reveals Gestational Folic Acid Supplementation Influences the Epigenome of Mouse Offspring Cerebellum. Front Neurosci. 2016;10:168.
42. Barua S, Chadman KK, Kuizon S, Buenaventura D, Stapley NW, Ruocco F, et al. Increasing maternal or post-weaning folic acid alters gene expression and moderately changes behavior in the offspring. PLoS One. 2014;9(7):e101674.
43. Ly A, Ishiguro L, Kim D, Im D, Kim SE, Sohn KJ, et al. Maternal folic acid supplementation modulates DNA methylation and gene expression in the rat offspring in a gestation period-dependent and organ-specific manner. J Nutr Biochem. 2016;33:103-10.
44. Friso S, Choi SW. Gene-nutrient interactions and DNA methylation. J Nutr. 2002;132(8 Suppl):2382S-7S.
45. Haggarty P, Hoad G, Campbell DM, Horgan GW, Piyathilake C, McNeill G. Folate in pregnancy and imprinted gene and repeat element methylation in the offspring. The American journal of clinical nutrition. 2013;97(1):94-9.
46. Bourgeron T. From the genetic architecture to synaptic plasticity in autism spectrum disorder. Nat Rev Neurosci. 2015;16(9):551-63.
47. Robertson CE, Ratai EM, Kanwisher N. Reduced GABAergic Action in the Autistic Brain. Curr Biol. 2016;26(1):80-5.
48. Black MM. Effects of vitamin B12 and folate deficiency on brain development in children. Food Nutr Bull. 2008;29(2 Suppl):S126-31.

49. McCullough LE, Miller EE, Mendez MA, Murtha AP, Murphy SK, Hoyo C. Maternal B vitamins: effects on offspring weight and DNA methylation at genomically imprinted domains. Clin Epigenetics. 2016;8:8.
50. Ba Y, Yu H, Liu F, Geng X, Zhu C, Zhu Q, et al. Relationship of folate, vitamin B12 and methylation of insulin-like growth factor-II in maternal and cord blood. Eur J Clin Nutr. 2011;65(4):480-5.
51. Falkmer T, Anderson K, Falkmer M, Horlin C. Diagnostic procedures in autism spectrum disorders: a systematic literature review. Eur Child Adolesc Psychiatry. 2013;22(6):329-40.
52. Galloway M, Rushworth L. Red cell or serum folate? Results from the National Pathology Alliance benchmarking review. J Clin Pathol. 2003;56(12):924-6.
53. Branum AM, Bailey R, Singer BJ. Dietary supplement use and folate status during pregnancy in the United States. J Nutr. 2013;143(4):486-92.
54. Aaltonen J, Ojala T, Laitinen K, Piirainen TJ, Poussa TA, Isolauri E. Evidence of infant blood pressure programming by maternal nutrition during pregnancy: a prospective randomized controlled intervention study. J Pediatr. 2008;152(1):79-84, e1-2.

Table 4-1 Maternal and offspring characteristics by offspring case status

Characteristics	Neurotypical (n=1,171)	ASD (n=86)
Mothers		
Age at birth (years), mean (SD)	28.3 (6.6)	30.9 (6.5)
Parity (%)		
0	501 (42.8)	31 (36.1)
1	337 (28.8)	37 (43.0)
2 or more	326 (27.8)	18 (20.9)
Missing	7 (0.6)	0 (0.0)
Mother's education (%)		
High School or less	754 (64.4)	48 (55.8)
Some college or more	415 (35.3)	37 (43.0)
Missing	4 (0.3)	1 (1.2)
Maternal BMI (SD)	26.3 (6.2)	28.1 (7.6)
Diabetes (%)		
No	1056 (90.2)	70 (81.4)
Gestational diabetes mellitus	67 (5.7)	8 (9.3)
Diabetes mellitus	48 (4.1)	7 (8.1)
Missing	0 (0.0)	1 (1.2)
Smoking during & 3 months prior to pregnancy (%)		
No	1000 (85.4)	69 (80.2)
Yes	164 (14.0)	15 (17.4)
Missing	7 (0.6)	2 (2.3)
Maternal plasma folate (%)		
<14.7 nmol/L (<10th percentile)	118 (10.1)	7 (8.1)
≥14.7 to <60.3 nmol/L (10-90 percentile)	942 (80.4)	65 (75.6)
≥60.3 nmol/L (≥90th percentile)	111 (9.5)	14 (16.3)

Maternal plasma vitamin B12 (%)		
<247.0 pmol/L (<10th percentile)	119 (10.2)	6 (7.0)
≥247.0 to <536.8 pmol/L 10-90 percentile)	945 (80.7)	62 (72.1)
≥536.8 pmol/L (≥90th percentile)	107 (9.1)	18 (20.9)
Maternal plasma homocysteine (%)		
<11.7 µmol/L (<90th percentile)	1050 (89.7)	80 (93.0)
≥11.7 µmol/L (≥90th percentile)	121 (10.3)	6 (7.0)
Maternal supplement intake (3rd trimester) (%)		
<2 times/week	116 (9.9)	15 (17.4)
3 – 5 times/week	447 (38.2)	19 (22.1)
>5 times/week	608 (51.9)	50 (60.5)
Maternal *MTHFR* genotype		
CC	742 (63.4)	52 (60.5)
CT	323 (27.6)	24 (27.9)
TT	65 (5.6)	7 (8.1)
Missing	41 (3.5)	3 (3.5)
Offspring		
Gender (%)		
Male	525 (44.8)	63 (73.3)
Female	646 (55.2)	23 (26.7)
Gestational age (%)		
Term	911 (77.8)	57 (66.3)
Late preterm (34-36 weeks)	161 (13.8)	13 (15.1)
Early preterm (<34 weeks)	99 (8.5)	16 (18.6)
Year of birth (%)		
1998-2006	526 (44.9)	38 (44.2)
2007-2013	645 (55.1)	48 (55.8)

[a]The Boston Birth Cohort uses a rolling enrolment and the study sample were enrolled between 1998 and 2013, and have been followed up from birth up to the last visit recorded in the EMR

BMI, body mass index; *MTHFR*, methylene tetrahydrofolate reductase

Table 4-2 Maternal self-reported multivitamin intake during preconception and 1st, 2nd and 3rd trimesters and ASD risk in offspring

		Unadjusted	Adjusted[a]
Maternal Supplement intake	**N[b]**	**HR (95% CI)**	**HR (95% CI)**
Preconception			
Yes	54	0.4 (0.1, 1.8)	0.5 (0.1, 2.1)
No	1087	1.0 (Reference)	1.0 (Reference)
First Trimester			
≤2 times/week	164	2.1 (1.1, 4.0)	3.4 (1.6, 7.2)
3-5 times/week	457	1.0 (Reference)	1.0 (Reference)
>5 times/week	628	1.9 (1.1, 3.1)	2.3 (1.2, 3.9)
Second Trimester			
≤2 times/week	115	2.6 (1.3, 5.3)	3.8 (1.8, 8.0)
3-5 times/week	466	1.0 (Reference)	1.0 (Reference)
>5 times/week	674	1.8 (1.1, 3.0)	2.1 (1.2, 3.6)
Third Trimester			
≤2 times/week	131	2.7 (1.4, 5.3)	3.5 (1.7, 7.4)
3-5 times/week	466	1.0 (Reference)	1.0 (Reference)
>5 times/week	660	1.8 (1.1, 3.1)	2.1 (1.2, 3.6)

[a]Adjusted for maternal characteristics: age, education, parity, BMI, smoking status, diabetes status, race and *MTHFR* genotype; offspring characteristics: gestational age, sex and year of birth

[b] N may be different for preconception and each trimester periods due to missing data

BMI, body mass index; *MTHFR*, methylene tetrahydrofolate reductase

Table 4-3 Maternal plasma folate, vitamin B12 concentrations in samples obtained 24-72 hours after delivery and risk of ASD in offspring

		Unadjusted	Adjusted[a]
Maternal folate - WHO cutpoints	**n**	**HR (95% CI)**	**HR (95% CI)**
<13.5 nmol/L	103	0.7 (0.3, 1.6)	1.1 (0.5, 2.8)
≥13.5 nmol/L to ≤45.3 nmol/L	852	1.0 (Reference)	1.0 (Reference)
>45.3 nmol/L (corresponds to 76th percentile)	302	1.2 (0.8, 2.0)	1.5 (0.9, 2.5)
Maternal vitamin B12			
<200 pmol/L	35	1.7 (0.6, 4.8)	1.9 (0.7, 5.3)
≥200 pmol/L to ≤600 pmol/L	1136	1.0 (Reference)	1.0 (Reference)
>600 pmol/L (corresponds to 93.1th percentile)	86	2.7 (1.4, 4.9)	3.0 (1.6, 5.7)
Maternal Folate – Extreme Deciles			
<14.7 nmol/L (<10th percentile)	125	0.7 (0.3, 1.5)	1.2 (0.5, 2.8)
>14.7 to <60.3 (≥10th to <90th, middle 80th percentile)	1007	1.0 (Reference)	1.0 (Reference)
≥60.3 nmol/L (≥90th percentile)	125	1.8 (1.0, 3.2)	2.5 (1.3, 4.6)
Vitamin B12 – Extreme Deciles			

<247.0pmol/L (<10th percentile)	125	0.7 (0.3, 1.6)	0.7 (0.3, 1.7)
≥247.0 to <536.8 (≥10th to <90th, middle 80th percentile)	1007	1.0 (Reference)	1.0 (Reference)
≥536.8pmol/L (≥90th percentile)	125	2.6 (1.6, 4.5)	2.5 (1.4, 4.5)
Joint effects of folate & vitamin B12 Percentiles[b]			
Folate & vitamin B12 (10-90 percentile)	815	1.0 (Reference)	1.0 (Reference)
Folate (<10th percentile) & vitamin B12 (10-90 percentile)	104	0.6 (0.2, 1.5)	0.8 (0.3, 2.2)
Folate (≥90th percentile) & vitamin B12 (10-90 percentile)	88	0.6 (0.2, 1.8)	0.8 (0.2, 2.6)
Vitamin B12 (<10th percentile) & Folate (10-90 percentile)	94	0.6 (0.2, 1.6)	0.5 (0.2, 1.6)
Vitamin B12 (≥90th percentile) & Folate (10-90 percentile)	98	1.2 (0.6, 2.7)	1.1 (0.5, 2.4)
Either Folate & vitamin B12 (<10 percentile)[c]	229	0.6 (0.3, 1.1)	0.8 (0.4, 1.5)
Both Folate & vitamin B12 (<10 percentile)	21	1.1 (0.3, 4.5)	2.4 (0.5, 10.4)
Either Folate or vitamin B12 (≥90 percentile)[d]	223	1.6 (0.9, 2.5)	1.8 (1.1, 3.1)
Both Folate & vitamin B12 (≥90 percentile)	27	6.3 (3.3, 12.1)	13.7 (6.5, 28.9)
Maternal Homocysteine			
≥11.7 µmol/L (≥90 percentile)	127	0.5 (0.2, 1.1)	0.5 (0.2, 1.4)

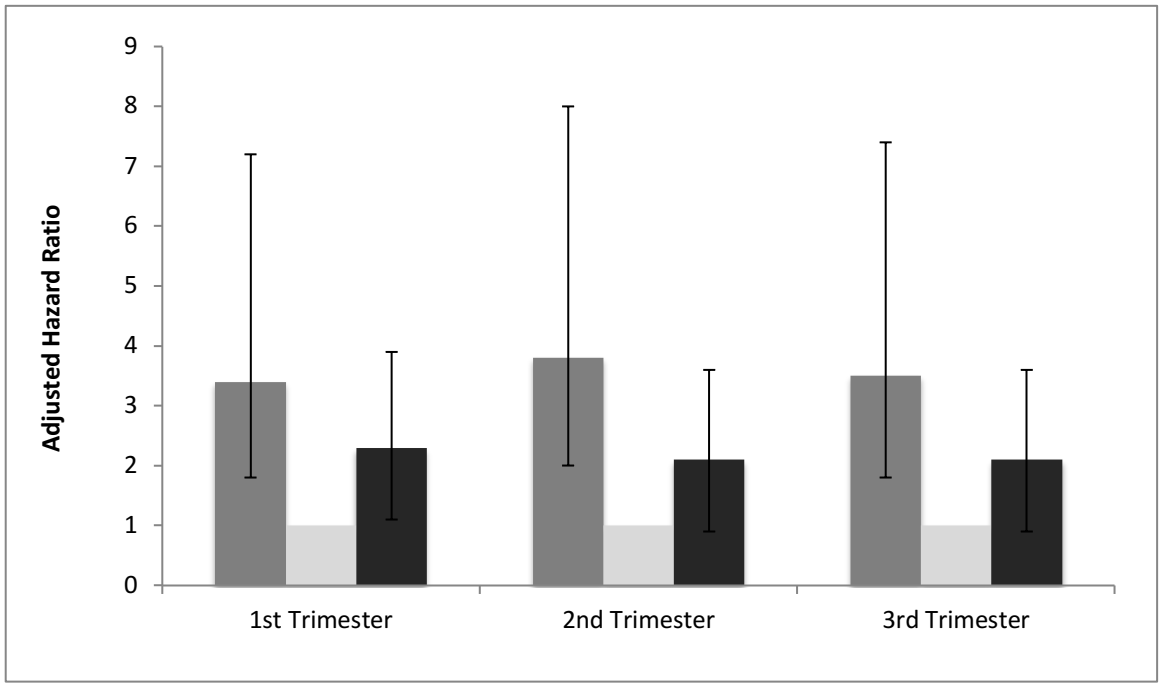
[a] Adjusted for maternal characteristics: age, education, parity, BMI, smoking status, diabetes status, race and *MTHFR* genotype; offspring characteristics: gestational age, sex and year of birth

[b] There was interaction between maternal plasma folate and vitamin B12 (P<0.01)

[c] Either folate or vitamin B12 <10th percentile compares the risk of having a ASD child in mothers who had at least one of the biomarkers <10th percentile versus those who had both of these biomarkers in the middle 80th percentile

[d] Either folate or vitamin B12 ≥90th percentile compares the risk of having a ASD child in mothers who had at least one of the biomarkers ≥90th percentile versus those who had both of these biomarkers in the middle 80th percentile

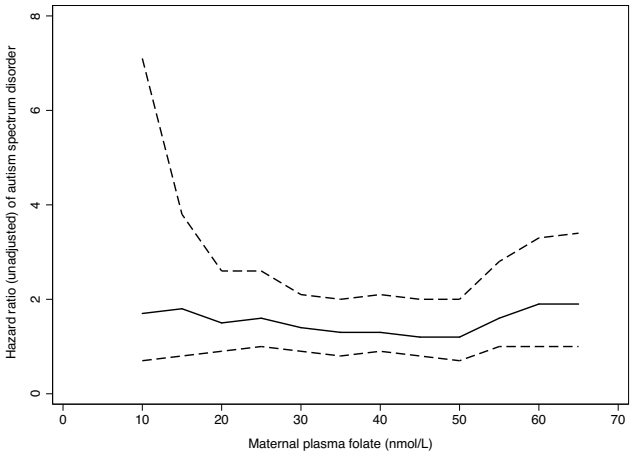
Figure 4-1 Maternal self-reported multivitamin supplement intake and ASD risk in offspring



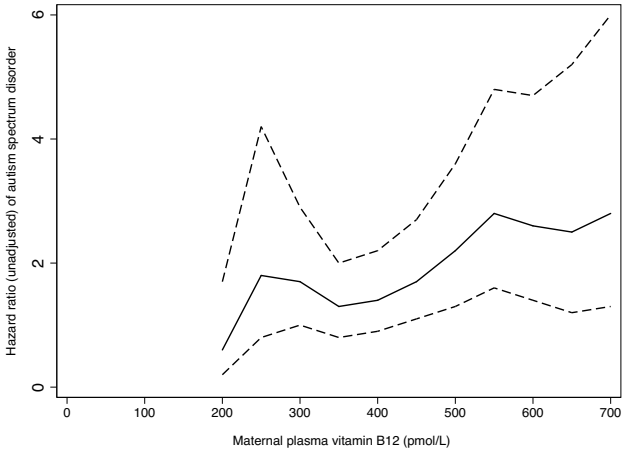
Adjusted for maternal characteristics: age, education, parity, BMI, smoking status, diabetes status, race and *MTHFR* genotype; offspring characteristics: gestational age, sex and year of birth

Figure 4-2 Association between maternal folate and vitamin B12 concentrations and risk of ASD in offspring

A



B



Unadjusted association between maternal plasma folate (panel A) and plasma vitamin B12 (panel B) levels at different cut-off points and risk of ASD in offspring. The unadjusted HR for plasma folate (panel A) was truncated at 65 nmol/L due to the small sample size beyond the specified cutoff point (unadjusted HR for mothers whose plasma folate ≥65 nmol/L was 1.9, 95% CI 1.0, 3.4; n=113). The unadjusted HR for plasma vitamin B12 (panel B) was truncated at 700 pmol/L due to declining sample sizes beyond the specified cutoff point (unadjusted HR for `mothers whose plasma vitamin B12 ≥700 pmol/L was 2.8, 95% CI, 1.3, 6.0; n=45).

Supplemental table 4-1: ICD-9-CM code based definitions for ASD and neurotypical development in the Boston Birth Cohort, 2003 - 2013

	ICD-9-CM codes	N
ASD case definition		61
Inclusion criteria (any one of these codes):	299.0, 299.01, 299.8, 299.81, 299.9, 299.91	
Definition for children with neurotypical development		601
Exclusion criteria (any one of these codes):		
ADHD	314.0 - 314.9	
Conduct Disorder	312.0 - 312.9	
Emotional disturbances of childhood or adolescence including Oppositional Defiant Disorder	313.0 - 313.9	
Developmental Delay	315.0 - 315.9	
Intellectual Disability	317 – 319	
Congenital Anomalies	740 - 759.9	

Supplemental table 4-2: Comparison between the Boston Birth Cohort study sample[a] and NHANES sample (2009-2010) for women of reproductive age with regard to plasma/serum folate levels

	BBC	NHANES
Sample size	N=1,257	N=1,971
Study population	Women that just delivered babies	95% of the participants are women of reproductive age but not pregnant
Age group	14 – 47 years	15 – 50 years
Race/ethnicity	Blacks – 59.4% White – 4.8% Hispanics – 25.6% Others – 10.3%	Blacks – 18.3% White – 42.4% Hispanics & Mex. Am – 32.2% Others – 7.2%
Biomarker measured	Plasma folate	Serum folate
Assay	Chemiluminescent immunoassay	Microbiological assay
Mean	36.1 nmol/L	37.7 nmol/L
Median	31.3 nmol/L	34 nmol/L
25^{th} percentile	21.9 nmol/L	23.8 nmol/L
75^{th} percentile	44.7 nmol/L	47.9 nmol.L
Range	4.5 -135.9 nmol/L	9.3 – 175 nmol/L

[a] The Boston Birth Cohort uses a rolling enrollment and the study sample were enrolled between 1998 and 2013, and have been followed up from birth up to the last visit recorded in the EMR

Supplemental table 4-3: Comparison between the Boston Birth Cohort[a] study sample and NHANES sample (2011-2012) data for women of reproductive age with regard to plasma/serum vitamin B12 levels

	BBC	NHANES
Sample size	N=1,257	N=1,879
Study population	Women that just delivered babies	95% of the participants are women of reproductive age but not pregnant
Age group	14 – 47 years	15 – 50 years
Race/ethnicity	Blacks – 59.4% White – 4.8% Hispanics – 25.6% Others – 10.3%	Blacks – 26.2% White – 31.6% Hispanics & Mex. Am – 22.6% Others – 19.6%
Biomarker measured	Plasma vitamin B12	Serum vitamin B12
Assay	ACCESS Immunoassay	Electrochemiluminescence immunoassay
Mean	380.0 pmol/L	433.9 pmol/L
Median	359.3 pmol/L	380.8 pmol/L
25^{th} percentile	295.4 pmol/L	281.9 pmol/L
75^{th} percentile	439.5 pmol/L	519.6 pmol/L
Range	59.8 – 750.0 pmol/L	79.7 – 4140.2 pmol/L

[a] The Boston Birth Cohort uses a rolling enrollment and the study sample were enrolled between 1998 and 2013, and have been followed up from birth up to the last visit recorded in the EMR

Supplemental table 4-4: Hazard ratios for unadjusted and adjusted plasma folate concentrations at different cutpoints in the Boston Birth Cohort[a] (N=1,257)

		Unadjusted			Adjusted [b]		
Plasma folate levels (nmol/L)	**n**	HR	95% CI	p value	HR	95% CI	P value
<10	35	Ref			Ref		
≥10	1222	1.7	0.4 - 7.1	0.44	0.8	0.2 - 3.2	0.76
<15	138	Ref			Ref		
≥15	1119	1.8	0.8 - 3.8	0.16	1.1	0.5 - 2.5	0.80
<20	263	Ref			Ref		
≥20	994	1.5	0.9 - 2.6	0.15	1.2	0.6 - 2.1	0.60
<25	409	Ref			Ref		
≥25	848	1.6	1.0 - 2.6	0.06	1.3	0.8 - 2.1	0.38
<30	587	Ref			Ref		
≥30	670	1.4	0.9 - 2.1	0.14	1.1	0.7 - 1.8	0.60
<35	732	Ref			Ref		
≥35	525	1.3	0.8 - 2.0	0.25	1.2	0.8 - 1.9	0.41
<40	857	Ref			Ref		
≥40	400	1.3	0.9 - 2.1	0.20	1.3	0.8 - 2.2	0.25
<45	948	Ref			Ref		
≥45	309	1.2	0.8 - 2.0	0.38	1.3	0.8 - 2.1	0.34
<50	1029	Ref			Ref		
≥50	228	1.2	0.7 - 2.0	0.58	1.3	0.8 - 2.3	0.32
<55	1089	Ref			Ref		
≥55	168	1.6	1.0 - 2.8	0.07	1.8	1.0 - 3.2	0.04
<60	1131	Ref			Ref		
≥60	126	1.9	1.0 - 3.3	0.04	2.4	1.3 - 4.4	0.007
<65	1144	Ref			Ref		
≥65	113	1.9	1.0 - 3.4	0.04	2.4	1.3 - 4.6	0.007

[a] The Boston Birth Cohort uses a rolling enrollment and the study sample were enrolled between 1998 and 2013, and have been followed up from birth up to the last visit recorded in the EMR

[b] Adjusted for maternal characteristics: age, education, parity, BMI, smoking status, diabetes status, race and *MTHFR* genotype; offspring characteristics: gestational age, sex and year of birth

Supplemental table 4-5: Hazard ratios for unadjusted and adjusted plasma vitamin B12 concentrations at different cutpoints in the Boston Birth Cohort[a] (N=1,257)

		Unadjusted			Adjusted[b]		
Plasma vitamin B12 levels (pmol/L)	**n**	HR	95% CI	p value	HR	95% CI	p value
<200	35	Ref			Ref		
≥200	1222	0.6	0.2 - 1.7	0.37	0.6	0.2 - 1.7	0.37
<250	133	Ref			Ref		
≥250	1124	1.8	0.8 - 4.2	0.16	1.8	0.7 - 4.4	0.23
<300	343	Ref			Ref		
≥300	914	1.7	1.0 – 2.9	0.06	1.7	0.9 - 3.1	0.09
<350	581	Ref			Ref		
≥350	676	1.3	0.8 - 2.0	0.22	1.1	0.7 - 1.8	0.66
<400	794	Ref			Ref		
≥400	463	1.4	0.9 - 2.2	0.12	1.2	0.7 - 1.9	0.56
<450	974	Ref			Ref		
≥450	283	1.7	1.1 - 2.7	0.02	1.6	1.0 - 2.7	0.07
<500	1084	Ref			Ref		
≥500	173	2.2	1.3 - 3.6	0.003	1.9	1.1 - 3.3	0.02
<550	1143	Ref			Ref		
≥550	114	2.8	1.6 - 4.8	<0.001	3.0	1.7 - 5.3	<0.001
<600	1171	Ref			Ref		
≥600	86	2.6`	1.4 - 4.7	0.002	2.6	1.4 - 5.0	0.004
<650	1199	Ref			Ref		
≥650	58	2.5	1.2 - 5.2	0.01	2.8	1.3 - 6.0	0.009
<700	1212	Ref			Ref		
≥700	45	2.8	1.3 - 6.0	0.009	3.5	1.5 - 7.8	0.003

[a] The Boston Birth Cohort uses a rolling enrollment and the study sample were enrolled between 1998 and 2013, and have been followed up from birth up to the last visit recorded in the EMR

[b] Adjusted for maternal characteristics: age, education, parity, BMI, smoking status, diabetes status, race and *MTHFR* genotype; offspring characteristics: gestational age, sex and year of birth

Supplemental table 4-6: Maternal self-reported multivitamin supplement intake during 1st, 2nd and 3rd trimesters and corresponding median maternal plasma folate and B12 levels measured 24-72 hours after delivery in the Boston Birth Cohort [a] (N=1,257)

		Plasma Folate (nmol/L)		Plasma B12 (pmol/L)	
Maternal Supplement intake	N [b]	Median	IQR	Median	IQR
First Trimester					
<2 times/wk	164	29.2	19.3 – 44.1	364.6	298.5 – 438.4
3-5 times/wk	457	31.2	22.3 – 44.6	357.4	288.6 – 440.5
>5 times/wk	628	32.4	22.4 – 44.9	359.1	296.6 – 439.1
Missing	8	28.2	18.6 – 56.8	321.3	277.6 – 426.6
Second Trimester					
<2 times/wk	115	25.3	16.7 – 44.1	345.1	275.4 – 437.8
3-5 times/wk	466	31.6	22.4 – 45.0	358.7	291.1 – 441.1
>5 times/wk	674	32.2	22.5 – 44.4	360.9	297.8 – 439.6
Missing	2	31.5	15.6 – 47.4	419.3	341.8 – 496.7
Third Trimester					
<2 times/wk	131	24.5	16.4 – 38.3	351.1	289.1 – 437.4
3-5 times/wk	466	32.0	22.5 – 45.1	358.7	291.6 – 443.9
>5 times/wk	660	32.3	22.5 – 44.7	360.3	297.1 – 439.1

[a] The Boston Birth Cohort uses a rolling enrollment and the study sample were enrolled between 1998 and 2013, and have been followed up from birth up to the last visit recorded in the EMR

[b] N may be different for preconception and each trimester periods due to missing data

Supplemental table 4-7: Maternal self-reported supplement intake for 1st, 2nd, and 3rd trimesters stratified by maternal parity

Parity	n	Frequency of supplement intake, n (%)			P value
		≤ 2x/wk	3-5x/wk	≥5x/wk	
First trimester					0.025
Nulliparous	531	57 (10.7)	187 (35.2)	287 (54.1)	
Multiparous	716	107 (14.9)	270 (37.7)	339 (47.4)	
Second trimester					0.08
Nulliparous	534	39 (7.3)	194 (36.3)	301 (56.4)	
Multiparous	719	76 (10.6)	272 (37.8)	371 (51.6)	
Third trimester					0.015
Nulliparous	534	43 (8.1)	190 (35.6)	301 (56.4)	
Multiparous	721	88 (12.2)	276 (38.3)	357 (49.5)	

Supplemental table 4-8: Maternal *MTHFR* genotype stratified by offspring status (ASD vs. Neurotypical) in the Boston Birth Cohort [a] (N=1,257)

	Offspring status		
Maternal *MTHFR* genotype	**ASD, n (%)**	**Neurotypical, n (%)**	**p value**
CC	742 (63.4)	52 (60.5)	0.79
CT	323 (27.6)	24 (27.9)	
TT	65 (5.6)	7 (8.1)	
Missing	41 (3.5)	3 (3.5)	

[a] The Boston Birth Cohort uses a rolling enrollment and the study sample were enrolled between 1998 and 2013, and have been followed up from birth up to the last visit recorded in the EMR

Supplemental table 4-9: Maternal *MTHFR* genotype stratified by maternal folate, vitamin B12 levels (both middle 80% vs. both elevated, ≥90th percentile vs. folate only elevated, ≥90th percentile) in the Boston Birth Cohort [a] (N=1,257)

Maternal *MTHFR* genotype	Folate + B12 (middle, ≥10 to <90 percentile)	Folate + B12 (both elevated, ≥ 90 percentile)	Folate only elevated, ≥ 90 percentile)	p value
CC	508 (62.3)	21 (77.8)	60 (61.2)	0.51
CT	230 (28.2)	5 (18.5)	27 (27.6)	
TT	49 (6.0)	1 (3.7)	9 (9.2)	
Missing	28 (3.4)	0 (0.0)	2 (2.0)	

[a] The Boston Birth Cohort uses a rolling enrollment and the study sample were enrolled between 1998 and 2013, and have been followed up from birth up to the last visit recorded in the EMR

Supplemental table 4-10: Mean maternal folate (nmol/L) by maternal *MTHFR* genotype in the Boston Birth Cohort [a] (N=1,257)

	Maternal folate (nmol/L)	
Maternal *MTHFR* genotype	**n**	**Geometric Mean (SD)**
CC	794	30.8 (29.6 - 32.1)
CT	347	30.1 (28.4 - 32.0)
TT	72	34.2 (30.3 - 38.6)
Missing	44	30.3 (25.5 - 35.9)

[a] The Boston Birth Cohort uses a rolling enrollment and the study sample were enrolled between 1998 and 2013, and have been followed up from birth up to the last visit recorded in the EMR

Supplemental table 4-11: Sensitivity Analyses by restricting cases (n=61) and controls (n=601) to highest confidence in the Boston Birth Cohort [a] (N=662)

		Unadjusted			Adjusted [b]		
Maternal plasma folate – WHO cutpoints	n	HR	95% CI	p value	HR	95% CI	p value
<13.5 nmol/L	52	0.4	0.09 - 1.5	0.15	0.8	0.2 - 3.5	0.78
≥13.5 to ≤45.3 nmol/L	428	Ref			Ref		
>45.3 nmol/L (corresponds to 76th percentile)	182	1.1	0.7 – 1.9	0.67	1.4	0.8 - 2.6	0.29
Maternal plasma vitamin B12							
<200 pmol/L	25	0.9	0.2 - 3.5	0.84	1.2	0.3 – 5.0	0.85
≥200 & ≤600 pmol/L	594	Ref			Ref		
>600 pmol/L (corresponds to 93.1th percentile)	43	3.2	1.6 - 6.2	0.001	4.7	2.1 - 10.3	<0.001
Maternal Folate – Extreme Deciles							
<14.7 nmol/L (<10th percentile)	62	0.5	0.1 - 1.5	0.21	1.2	0.4 - 4.2	0.73
≥14.7 to <60.3 (≥10th to <90th, middle 80th percentile)	522	Ref			Ref		
≥60.3 nmol/L (≥90th percentile)	78	1.9	1.0 - 3.6	0.05	2.4	1.2 - 5.0	0.02
Vitamin B12 – Extreme Deciles							
<247.0 pmol/L (<10th percentile)	68	0.3	0.1 - 1.3	0.11	0.4	0.1 - 1.9	0.27
≥247.0 to <536.8 pmol/L (≥10th to <90th, percentile)	530	Ref			Ref		
≥536.8 pmol/L (≥90th percentile)	64	3.5	1.9 - 6.1	<0.001	3.9	2.0 - 7.7	<0.001
Joint effects of folate & vitamin B12 [c]							
Folate & vitamin B12 (≥10th to <90th, middle 80th percentile)	428	Ref			Ref		
Folate (<10th) & vitamin B12 (middle 80th percentile)	47	0.6	0.2 - 2.1	0.47	1.3	0.4 - 4.4	0.66
Folate (≥90th) & vitamin B12 (middle 80th	55	0.7	0.2 - 2.3	0.59	1.0	0.3 - 3.5	0.97

percentile)							
Vitamin B12 (<10th) & Folate (middle 80th percentile)	48	0.4	0.1 - 1.8	0.25	0.6	0.1 - 2.6	0.49
Vitamin B12 (≥90th) & Folate (middle 80th percentile)	46	1.9	0.9 - 4.4	0.11	2.1	0.8 - 5.4	0.11
Both Folate & vitamin B12 (<10th percentile)	5	Estimates could not be calculated because of small cell size					
Either Folate & vitamin B12 (<10th percentile) [d]	115	0.4	0.2 – 1.1	0.08	0.8	0.3 – 2.2	0.68
Folate & vitamin B12 (≥90th percentile)	18	6.6	3.2 - 13.8	<0.001	17.1	6.7 – 43.9	<0.001
Either folate or vitamin B12 (≥90th percentile) [e]	124	1.9	1.1 - 3.4	0.02	2.8	1.5 - 5.2	0.002

[a] The Boston Birth Cohort uses a rolling enrollment and the study sample were enrolled between 1998 and 2013, and have been followed up from birth up to the last visit recorded in the EMR

[b] Adjusted for maternal characteristics: age, education, parity, BMI, smoking status, diabetes status, race and *MTHFR* genotype; offspring characteristics: gestational age, sex and year of birth

[c] There was significant interaction between maternal plasma folate and vitamin B12 (p value - 0.017)

[d] Either folate or vitamin B12 <10th compares the risk of having a ASD child in mothers who had at least one of the biomarkers <10th vs. those who had both of these biomarkers in the middle 80th percentile

[e] Either folate or vitamin B12 ≥90th compares the risk of having a ASD child in mothers who had at least one of the biomarkers ≥90th vs. those who had both of these biomarkers in the middle 80th percentile

Supplemental Table 4-13: Maternal plasma folate, vitamin B12 levels in samples obtained 24-72 hours after delivery and risk of ASD in offspring in the in the Boston Birth Cohort [a] (N=1,257) (using Propensity Score Matching)

Maternal folate	**OR (95% CI) [b, c]**	**p value**
<90th percentile	Ref	
≥90th percentile (≥60.3 nmol/L)	3.4 (1.4 – 8.3)	0.006
Maternal vitamin B12		
<90th percentile	Ref	
≥90th percentile (≥536.8 pmol/L)	6.8 (2.7 – 17.9)	<0.001

Maternal Vitamin B12	**OR (95% CI) [b, c]**	**p value**
<90th percentile	Ref	
≥90th percentile (≥536.8 pmol/L)	3.2 (1.5 – 7.0)	0.003
Maternal Folate		
<90th percentile	Ref	
≥90th percentile (≥60.3 nmol/L)	3.0 (1.2 – 7.6)	0.02

[a] The Boston Birth Cohort uses a rolling enrollment and the study sample were enrolled between 1998 and 2013, and have been followed up from birth up to the last visit recorded in the EMR

[b] Adjusted for maternal characteristics: age, education, parity, BMI, smoking status, diabetes status, race and *MTHFR* genotype; offspring characteristics: gestational age, sex and year of birth

[c] OR for plasma folate and vitamin B12 after 3:1 nearest neighbor matching, without replacement

Supplemental table 4-14: Maternal plasma folate, vitamin B12 levels measured 24-72 hours after delivery and risk of ASD in offspring in the Boston Birth Cohort[a] (N=1,257) using Logistic Regression model

		Unadjusted			Adjusted [b]		
Classifying folate and vitamin B12 levels based on deficiency, normal and excess							
Maternal folate - WHO cutpoints	**n**	**OR**	**95% CI**	**p value**	**OR**	**95% CI**	**p value**
<13.5 nmol/L	103	0.9	0.4 - 2.1	0.77	1.3	0.5 - 3.2	0.63
≥13.5 nmol/L to ≤45.3 nmol/L	852	Ref			Ref		
>45.3 nmol/L (corresponds to 76th percentile)	302	1.2	0.7 - 2.0	0.42	1.5	0.8 - 2.6	0.19
Maternal vitamin B12							
<200 pmol/L	35	2.0	0.7 - 5.7	0.22	2.0	0.6 - 6.6	0.23
≥200 pmol/L to ≤600 pmol/L	1136	Ref			Ref		
>600 pmol/L (corresponds to 93.1th percentile)	86	2.5	1.3 - 4.8	0.007	3.1	1.5 - 6.5	0.002
Maternal Folate – Extreme Deciles							
<14.7 nmol/L (<10th percentile)	125	0.9	0.4 - 1.9	0.71	1.4	0.6 - 3.4	0.42
>14.7 to <60.3 (≥10th to <90th, middle 80th percentile)	1007	Ref			Ref		
≥60.3 nmol/L (≥90th percentile)	125	1.8	1.0 - 3.4	0.05	2.3	1.1 - 4.7	0.02
Vitamin B12 – Extreme Deciles							
<247.0 pmol/L (<10th percentile)	125	0.8	0.3 - 1.8	0.55	0.7	0.3 - 2.0	0.50
≥247.0 to <536.8 (≥10th to <90th, middle 80th percentile)	1007	Ref			Ref		
≥536.8 pmol/L (≥90th percentile)	125	2.6	1.5 - 4.5	0.001	2.7	1.4 - 5.2	0.003
Joint effects of folate & vitamin B12 Deciles[c]							
Folate & vitamin B12 (≥10th to <90th, middle 80th percentile)	815	Ref			Ref		
Folate (<10th percentile) & vitamin B12 (middle 80th percentile)	104	0.7	0.3 - 1.8	0.48	1.0	0.4 - 2.7	0.98
Folate (≥90th percentile) & vitamin B12 (middle 80th percentile)	88	0.5	0.2 - 1.6	0.25	0.7	0.2 - 2.3	0.54

Vitamin B12 (<10th percentile) & Folate (middle 80th percentile)	94	0.6	0.2 - 1.8	0.38	0.5	0.1 - 1.7	0.28
Vitamin B12 (≥90th percentile) & Folate (middle 80th percentile)	98	1.1	0.5 - 2.5	0.85	1.0	0.4 - 2.5	0.995
Either Folate & vitamin B12 (< 10th percentile)[d]	229	0.7	0.4 – 1.4	0.32	0.8	0.4 – 1.8	0.66
Both Folate & vitamin B12 (< 10th percentile)	21	1.5	0.3 – 6.5	0.60	2.6	0.5 – 13.0	0.25
Either Folate or vitamin B12 (≥90th percentile)[e]	223	1.5	0.9 - 2.5	0.16	1.8	1.0 - 3.2	0.05
Both Folate & vitamin B12 (≥90th percentile)	27	9.7	4.3 - 21.9	<0.001	18.0	6.9 - 46.9	<0.001

[a] The Boston Birth Cohort uses a rolling enrollment and the study sample were enrolled between 1998 and 2013, and have been followed up from birth up to the last visit recorded in the EMR

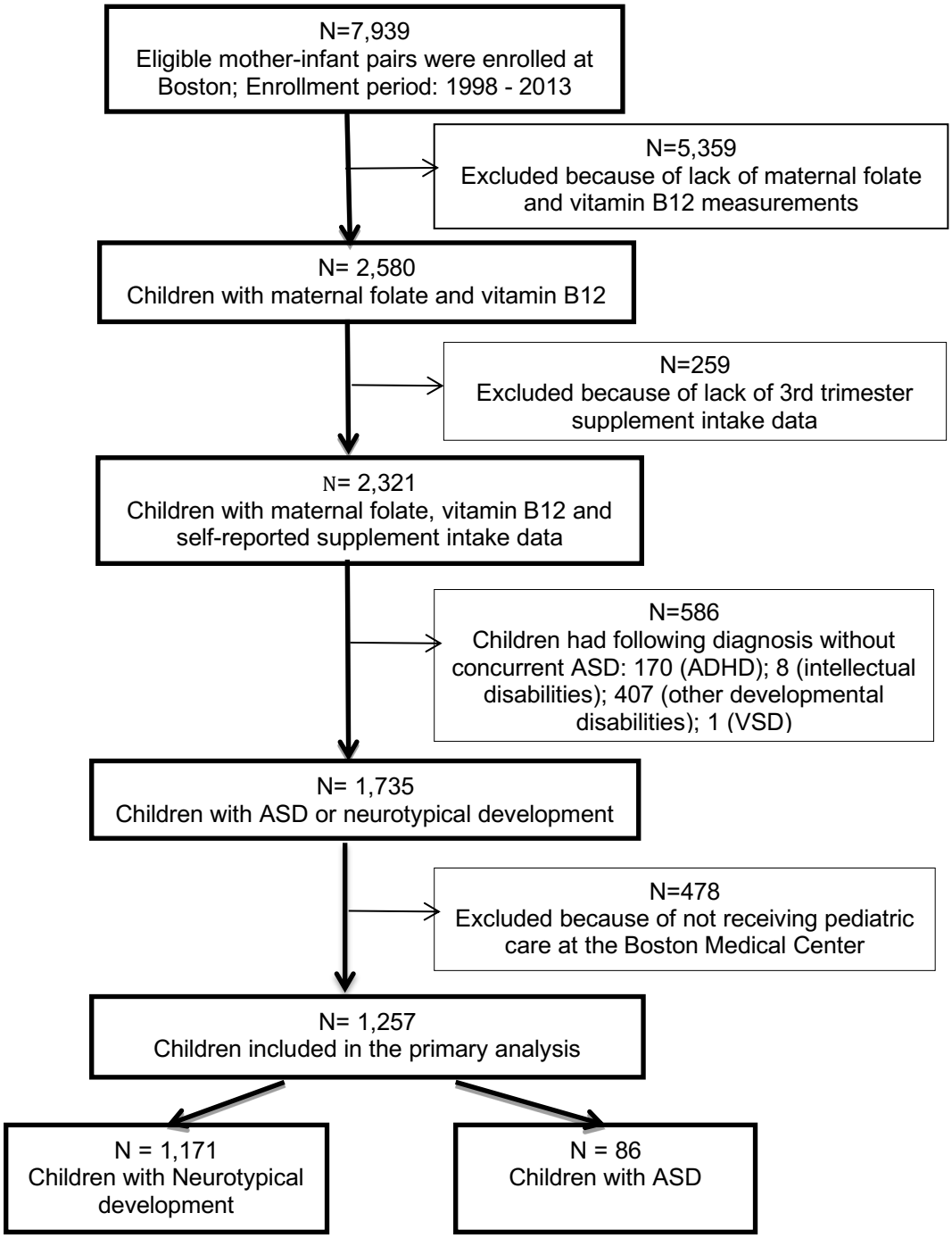
[b] Adjusted for maternal characteristics: age, education, parity, BMI, smoking status, diabetes status, race and *MTHFR* genotype; offspring characteristics: gestational age, sex and year of birth

[c] There was significant interaction between maternal plasma folate and vitamin B12 (p<0.001)

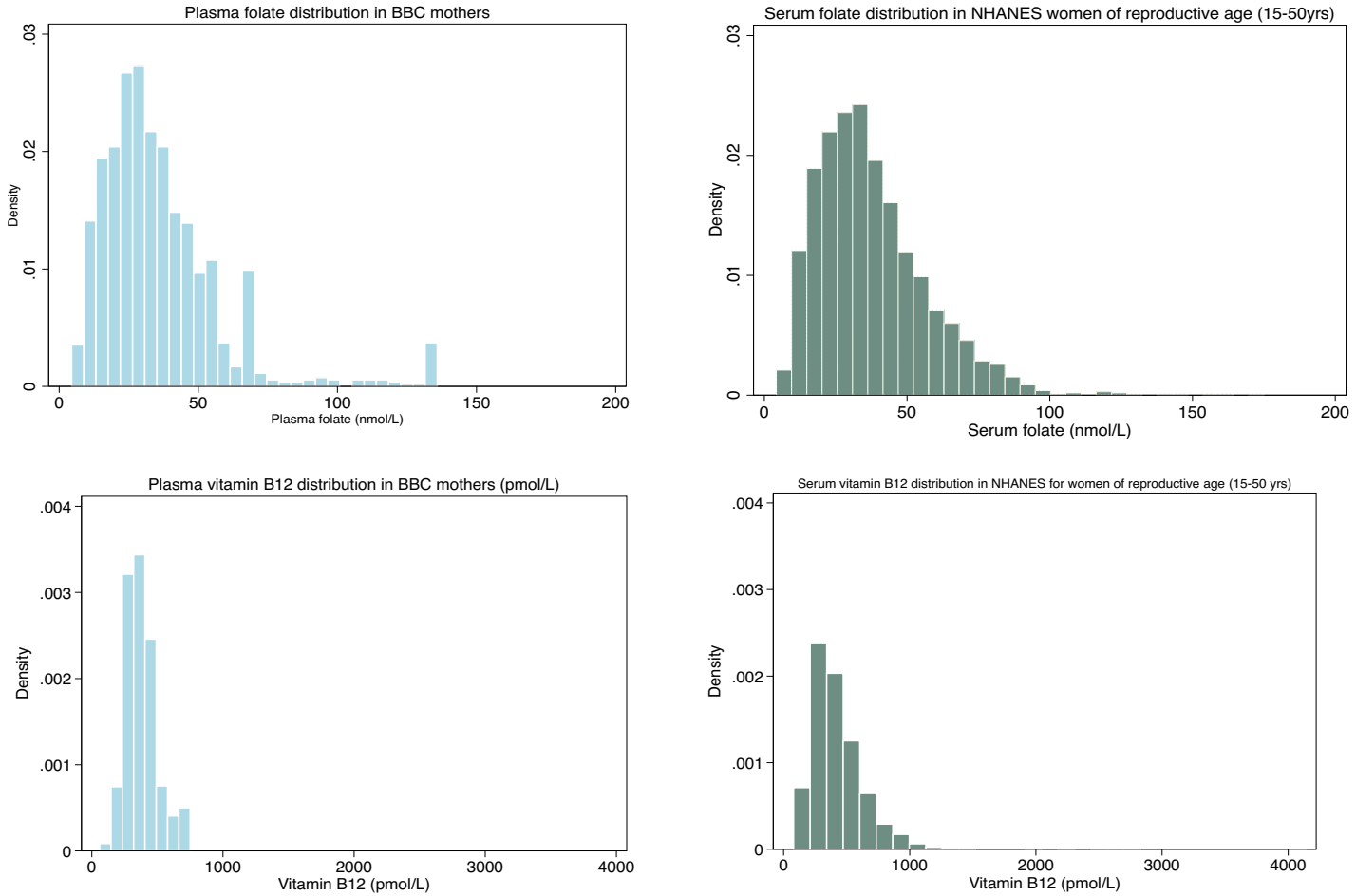
[d] Either folate or vitamin B12 <10th percentile compares the risk of having a ASD child in mothers who had at least one of the biomarkers <10th percentile vs. those who had both of these biomarkers in the middle 80th percentile

[e] Either folate or vitamin B12 ≥90th percentile compares the risk of having a ASD child in mothers who had at least one of the biomarkers ≥90th percentile vs. those who had both of these biomarkers in the middle 80th percentile

Supplemental figure 4-1: Flow chart of initial enrollment and postnatal follow-up of the Boston Birth Cohort and the sample included in the analyses

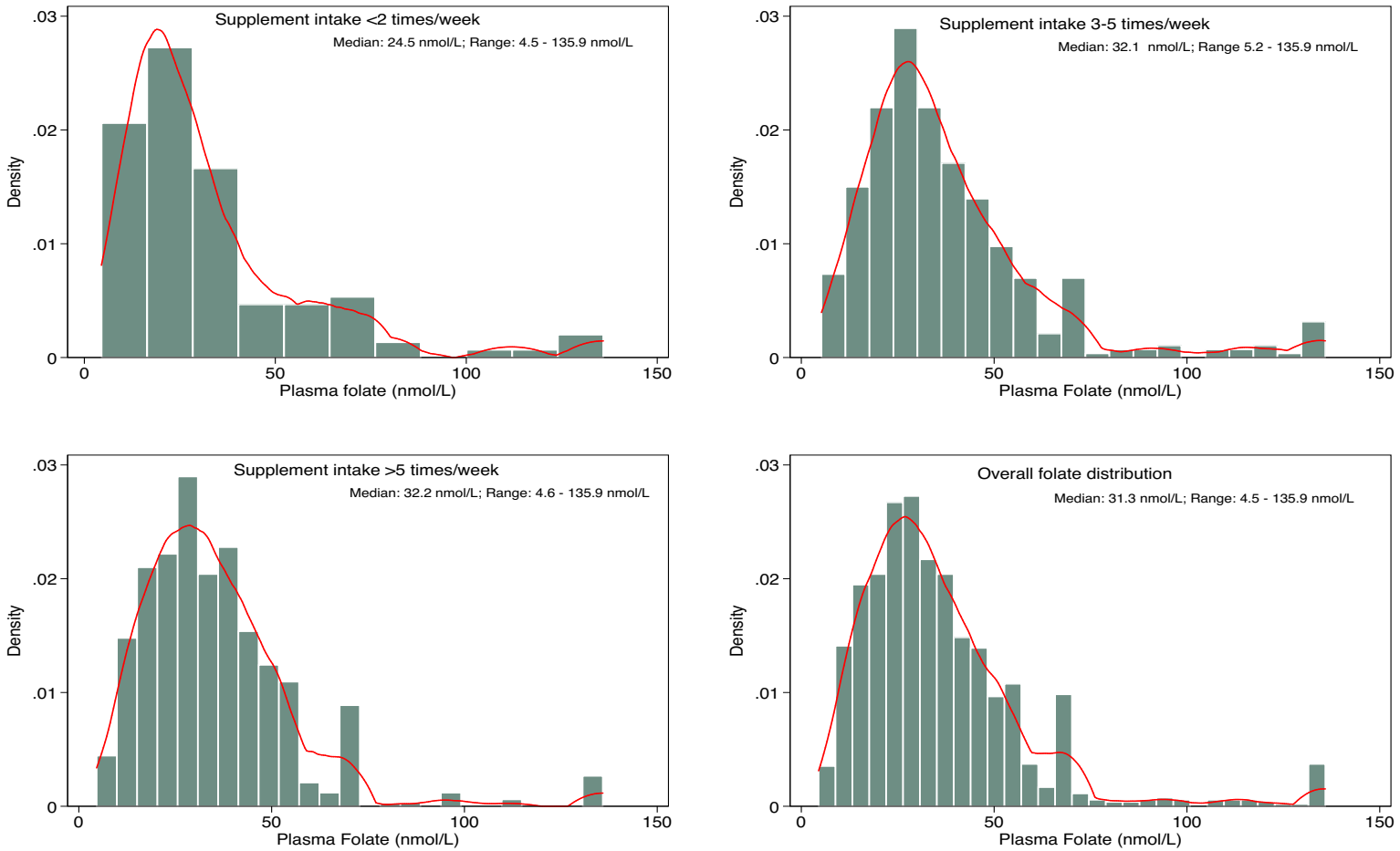


Supplemental figure 4-2: Comparing the distributions of Boston Birth Cohort [a] (N=1,257) mother's plasma folate and vitamin B12 levels with NHANES women of reproductive ages (15-50 years) for plasma/serum folate (2009 – 2010) (N=1,971) and plasma/serum vitamin B12 (2011-2012) (N=1,879) levels



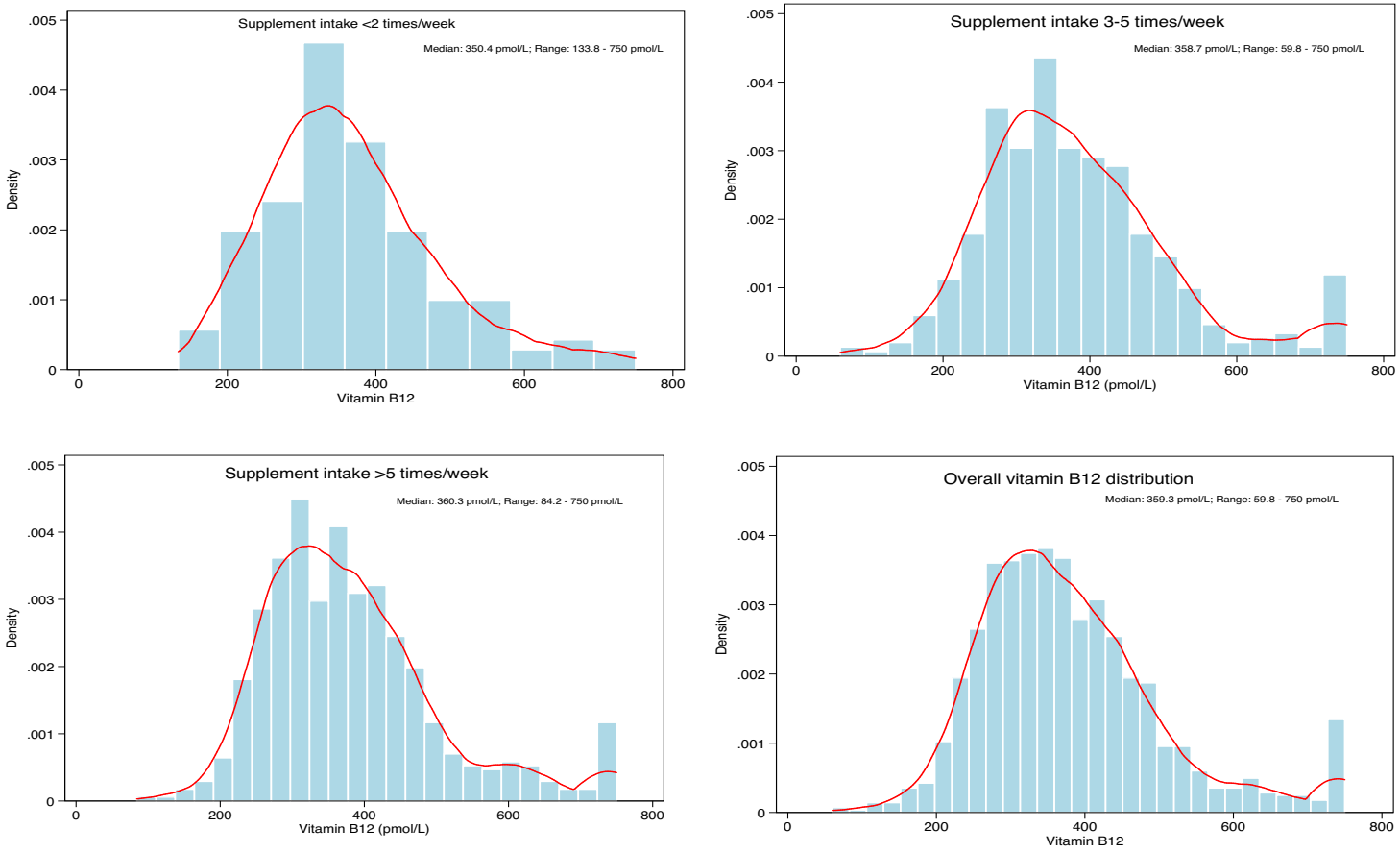
[a] The Boston Birth Cohort uses a rolling enrollment and the study sample were enrolled between 1998 and 2013, and have been followed up from birth up to the last visit recorded in the EMR

Supplemental figure 4-3: Plasma folate distribution by levels of supplement intake during third trimester in the Boston Birth Cohort[a] (N=1,257)



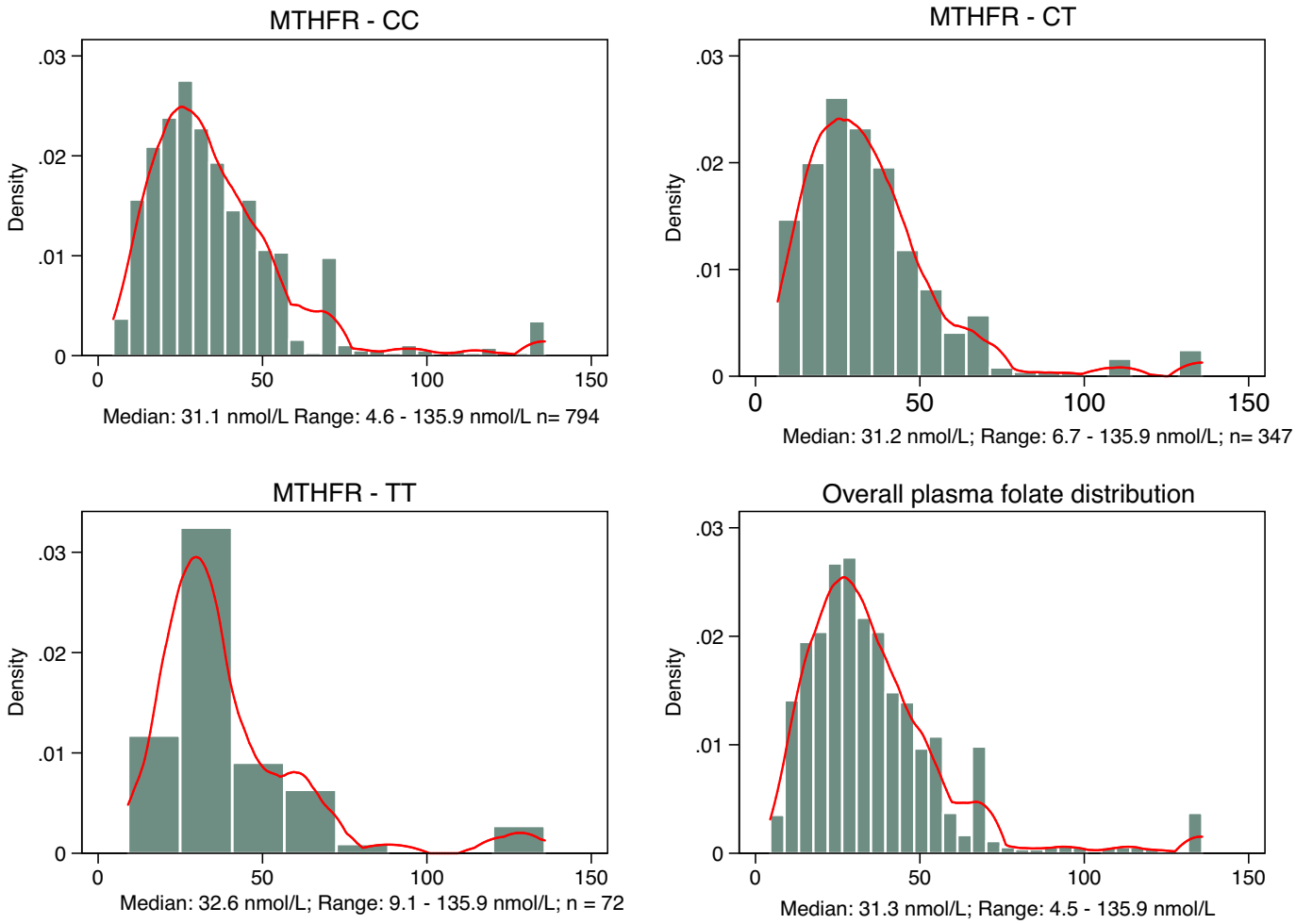
[a] The Boston Birth Cohort uses a rolling enrollment and the study sample were enrolled between 1998 and 2013, and have been followed up from birth up to the last visit recorded in the EMR

Supplemental figure 4-4: Plasma vitamin B12 distribution by levels of supplement intake during third trimester in the Boston Birth Cohort[a] (N=1,257)



[a] The Boston Birth Cohort uses a rolling enrollment and the study sample were enrolled between 1998 and 2013, and have been followed up from birth up to the last visit recorded in the EMR

Supplemental figure 4-5: Maternal plasma folate distribution by maternal *MTHFR* genotype in the Boston Birth Cohort [a] (N=1,257)



[a] The Boston Birth Cohort uses a rolling enrollment and the study sample were enrolled between 1998 and 2013, and have been followed up from birth up to the last visit recorded in the EMR

CHAPTER 5

Manuscript 2

Cord and Early Childhood Plasma Leptin, Fetal and Infancy Growth Pattern, and Development of Autism Spectrum Disorder in the Boston Birth Cohort

(This paper has received a favorable peer review and is currently under revision for publication in *Autism Research* with contributions from co-authors Barry Zuckerman, Xiumei Hong, Guoying Wang, Yuelong Ji, David Paige, Cuiling Zhang, Jessica DiBari, M. Daniele Fallin, Xiaobin Wang)

5.1 Abstract

Leptin is a pro-inflammatory cytokine that plays an important role in energy homeostasis. Emerging evidence suggests that leptin levels are altered in children with autism spectrum disorder (ASD); however this has not been studied prospectively. Rapid growth during infancy and early childhood has been implicated in ASD, but the evidence is inconsistent. Since leptin is involved in growth and is a potential risk factor for ASD, we explored the associations between 1) cord, early childhood leptin and ASD; and 2) birth weight for gestational age, early childhood weight gain and ASD. We also assessed the mediating role of leptin in the relationship between weight gain during infancy and ASD. This study was conducted in a sample of 822 subjects from the Boston Birth Cohort. ASD was defined from diagnostic codes in electronic medical records. Extremely rapid weight gain during infancy was associated with a greater ASD risk and this persisted after adjusting for potential confounders (aOR: 3.11; 95% CI: 1.37, 7.07). Similarly, children that had higher plasma leptin levels, prior to ASD diagnosis, had an increased ASD risk in both unadjusted and adjusted models (aOR: 7.87; 95% CI: 2.06, 30.04). Further, early childhood leptin indirectly mediated the relationship between rapid weight gain and ASD. No associations were found between birth weight for gestational age, cord leptin and risk of ASD. Our findings provide a basis to further explore whether the combination of early life growth pattern and a biomarker such as leptin can predict ASD earlier.

Key Words: Leptin, rapid weight gain in infancy, autism

5.2 Introduction

Autism Spectrum Disorder (ASD) is a neurodevelopmental conditions characterized by impairments in sociability and communication, as well as increased repetitive and/or restrictive behaviors and interests (1-5). ASD prevalence in the U.S. has increased dramatically since 1996 and now, 1 in 68 children are diagnosed with ASD (6, 7). The precise cause of ASD is largely unknown (8). Numerous studies have demonstrated associations with genetic, environmental, perinatal and immunological risk factors, leading to a hypothesis that ASD likely has a multifactorial pathogenesis or is a common end point of multiple causal pathways (8, 9).

Among the perinatal risk factors, preterm birth and small for gestational age (SGA) have been studied extensively in the context of ASD (8, 10-12). SGA is a proxy for intrauterine growth restriction (13) and studies have reported that SGA children are at increased risk of ASD (8, 14, 15); however, the results are inconsistent (15-17). Many children that are small at birth tend to have rapid catch-up growth during early postnatal period (18, 19). Rapid growth during first year of life has been shown to be associated with ASD (20-22), although not all studies have confirmed these findings (23, 24). While abnormal birth weight percentiles and rapid early growth have been identified as independent risk factors of ASD, the combined effect of SGA with rapid postnatal weight gain has not been explored. Most studies previously conducted on SGA infants have not accounted for the rate of postnatal growth, and vice versa. Further, the biological mechanisms behind SGA and rapid weight gain and ASD risk have not been clearly elucidated (25, 26).

Leptin is a peptide hormone that is predominantly secreted by the white adipose tissue and has been studied in the context of fetal growth and early childhood weight gain (27, 28). Studies have found that children with SGA have lower leptin levels possibly reflecting a lower fat mass as a result of intrauterine restricted fetal weight gain (29-32). Metabolically, lower cord blood leptin levels are known to predict rapid weight gain in infancy (33-36). Beyond leptin's role in prenatal and postnatal weight gain, this pleiotropic cytokine has been shown to be important in the regulation of the immune system, neurodevelopment including neuron excitability, synaptic plasticity, neural differentiation and promoting migration of neuronal lineage cells to the cortical plate (28, 37-40). Emerging evidence suggests that children (2-15 years) with ASD have significantly higher plasma leptin levels than controls (28, 40-43). Among a few studies that have researched leptin-ASD association in children, most were done after ASD diagnosis (28, 40), thus unable to assess the temporal relationship. To our knowledge, none of the studies have assessed cord and early childhood leptin levels independently and simultaneously in relation to ASD in a prospective birth cohort.

Despite studies observing cytokine involvement in ASD (41, 44-46) and the knowledge about the role of fetal and infant growth and ASD (20-22), existing studies have not evaluated the potential link between growth and leptin levels related to ASD. We set out to understand whether elevated leptin levels observed in people with ASD are related to rapid weight gain during infancy, and whether leptin has a mechanistic role in explaining the early growth-ASD relationship. Specifically, in this report we sought to explore the relationship between – 1) birth weight for gestational age, weight gain during first year of life and ASD risk; 2) cord and early childhood leptin and ASD risk; and 3) the potential of leptin mediating the relationship between

weight gain during first year and ASD risk. We analyzed the longitudinal data from the Boston Birth Cohort (BBC), a predominantly urban low-income minority population.

5.3 Methods

5.3.1 Participation and data collection procedure

As illustrated in supplemental figure 5-1, this study included 822 children from the BBC, who were recruited at birth (between 1998 and 2009), were followed prospectively until 2015 and had a median length of follow-up of 7.5 years (interquartile range: 5.2 – 9.8 years). Mothers of newborns were invited to participate in the study 24-72 hours after birth. Over 90% of those that were approached agreed to participate and were initiated into the study (47, 48). For every preterm (defined as <37 weeks of gestation) and/or low birth weight baby (defined as <2,500 g), approximately two term and normal birth weight babies (and their mothers) were enrolled in the study (48). The exclusion criterion for initial enrolment was multiple-gestation pregnancies and newborns with major birth defects. Participants and non-participants did not differ on characteristics such as infant birth weight, maternal ethnicity, or other sociodemographic characteristics (48). A sub-set of the participants were enrolled in the follow-up study that began in 2003 and children not planning to receive pediatric care at the Boston Medical Center were not included in the postnatal follow-up. There were no major differences in baseline demographic characteristics between those with and without postnatal follow-up (49).

Of 2,932 that were eligible for postnatal follow-up, 2,110 were excluded for the following reasons: 1,589 did not have at least one of the exposure variables (cord leptin, early

childhood leptin) and 521 had other competing diagnosis such as ADHD, intellectual disabilities (ID) or other developmental disabilities (DD). Electronic Medical Records (EMR) containing clinicians' primary and secondary diagnoses using ICD-9 codes were obtained for 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.

5.3.2 Identification of children with ASD

Based on EMR, children that were ever diagnosed with autism (ICD-9 code 299.00), Asperger syndrome (299.80) and/or pervasive developmental disorder not otherwise specified (299.90) were categorized as having ASD. Neurotypical children were those that were never diagnosed with ASD, ADHD, ID and other DD. When children had concurrent diagnosis, such as ASD and ADHD or ASD and ID or ASD and other DD, they were classified as having ASD. Two separate sensitivity analyses were conducted - 1) using a stringent criteria, defined as having ASD diagnosis on more than two separate occasions in the EMR and one visit to specialists such as behavioral pediatrician, pediatric neurologist or child psychologist; and 2) using a stringent control that additionally excluded children who did not have other competing diagnoses (Conduct disorder (312.0 - 312.9), Emotional disturbances of childhood or adolescence including Oppositional Defiant Disorder (313.0 - 313.9), Congenital Anomalies (740 - 759.9)).

5.3.3 Exposure variables

Birth weight for gestational age was defined as follows: SGA (<10^{th} percentile), appropriate for gestational age (AGA) (≥10^{th} – 90^{th} percentile) and large for gestational age (LGA) (>90^{th} percentile) (47). Child's length (<2 years) and height and weight were measured during the well-child visits at the Boston Medical Center. WHO reference values was used to

calculate weight-for-age *z*-scores and was defined as the change in weight-for-age *z*-scores from birth until the target time-point. Weight-for-age *z*-score was categorized into the following groups: slow (weight gain *z*-score <-0.67), on track (−0.67 to 0.67), rapid (>0.67 to 1.28), and extremely rapid (>1.28) (50).

Umbilical cord blood sample was collected at delivery and non-fasting early childhood venous blood sample were collected during subsequent follow-up visits. The median age of early childhood leptin measurement was 18.4 months (IQR: 10.3-49.2 months). All the blood samples were processed immediately after collection and plasma samples were stored in a freezer at −80°C. Only children with blood samples obtained prior to ASD diagnosis were included in the early childhood biomarker analysis. As mentioned elsewhere, plasma leptin levels were measured in duplicates using a sandwich immunoassay based on flow metric xMAP technology on Luminex 200 machines (Luminex Corp., Austin, TX). The interassay coefficient of variation was 4.5% (50). Unlikely leptin levels, defined as greater than 3 SD, were observed in 8 and 7 subjects for cord and early childhood leptin, respectively and were re-assigned a value of 3SD (51).

5.3.4 Statistical Analyses

The outcome variable was ASD and major exposure variables were 1) birth weight for gestational age and weight gain during first year of life and 2) cord blood and early childhood leptin levels. Correlation between cord and early childhood leptin was minimal (0.02). Normality of the data was assessed using Shapiro-Wilk test and both cord and early childhood leptin levels were log transformed because of the skewed distribution. Data analyses were performed to compare neurotypical children and those with ASD using chi-square tests for

categorical variables and ANOVA for continuous variables. Logistic regression models were applied to estimate the crude and adjusted associations between weight gain during first year of life, log-transformed leptin levels at birth and early childhood (X, independent variables) and ASD (Y, dependent variable). All results are presented as odds ratio. Throughout, we used 2-sided statistical tests with a significance level of 0.05. Data were analyzed using STATA version 13.0 (StataCorp, College Station, TX).

Mediation analysis: The role of leptin as a potential mediator of the association between weight gain during infancy and ASD risk was examined. KHB command in STATA was used to decompose the total effect of weight gain in infancy into natural direct and indirect effects, mediated by early childhood leptin levels (52). The total effect (defined as the effect of weight gain during infancy on ASD without the mediating variable early childhood leptin) was decomposed into direct effect (the effect of weight gain during infancy on ASD when controlling for early childhood leptin, the mediator) and indirect effect (the effect of weight gain during infancy on ASD through early childhood leptin, the mediator). The proportion of mediating effect among the total effect was calculated as indirect effect divided by the total effect.

5.3.5 Other covariates

Covariates were selected *a priori* based on the existing literature, including our own work in the BBC (47, 49, 51, 53). Following covariates were adjusted in the analysis: maternal age at delivery, smoking during pregnancy (ever smoked 3 months before pregnancy/during pregnancy vs. not smoked before pregnancy/during pregnancy), parity (not including the index pregnancy), maternal education (high school or less vs. some college or more), maternal pre-pregnancy BMI, maternal diabetes status (defined below), maternal age at delivery,

race/ethnicity, child's sex (female vs. male), gestational age at birth (defined below), year of the baby's birth (1998-2006 vs. 2007-2013), mode of feeding (defined below), age at which early childhood blood was drawn and follow-up time for each subject.

Maternal diabetes status was classified into the following: 1) no preexisting diabetes mellitus (DM) or gestational diabetes mellitus (GDM), 2) preexisting DM, and 3) GDM. Subjects were categorized as having preexisting DM if any of the following criteria were met before pregnancy: (a) diabetes diagnosis by a physician; (b) received a ICD-9 code of "250.x" or "648.0x"; (c) fasting plasma glucose level ≥126 mg/dl (7.0 mmol/l) or a casual plasma glucose≥200 mg/dl (11.1 mmol/l); (d) treatment with anti-diabetes medicines (oral medicines or insulin). Subjects were categorized as having GDM if there was no evidence of preexisting diabetes (as defined above) and met any of the following criteria: (a) physician diagnoses of GDM; (b) received a ICD code of "648.8x"; (c) fasting plasma glucose level ≥126 mg/dl (7.0 mmol/l) or casual plasma glucose≥200 mg/dl (11.1 mmol/l); (d) two or more of the following plasma glucose values in oral glucose tolerance test (OGTT) were met or exceeded: i) Fasting: 95 mg/dL (5.3 mmol/L); ii) 1 h: 180 mg/dL (10.0 mmol/L); iii) 2 h: 155 mg/dL (8.6 mmol/L); iv) 3-h 140mg/dL (7.8mmol/L); v) treatment with anti-diabetes medicines (oral medicines or insulin) (54-56).

Neonates who were delivered ≥ 37 completed weeks of gestation were categorized as term, while those delivered <34 weeks, and ≥34 but <37 weeks of gestation were considered early and late preterm, respectively. Race/ethnicity was categorized into black, white, Hispanic and Other. Data on mode of feeding was collected from mothers using a standardized questionnaire during a follow-up visit in the first few years of life. Mode of feeding was

categorized into the following: 1) formula only, 2) both formula and breastfeeding, and 3) breastfeeding only. When mothers had multiple follow-up visits, data collected during the first visit was used (57).

5.4 Results

Table 5-1 describes the characteristics of mothers and children by child's case status (neurotypical vs. ASD). As expected, previously recognized risk factors were more prevalent among children with ASD compared to neurotypical children, including male sex, advanced maternal age, preterm birth and lower birth weight. Consistent with the literature (58, 59), girls had higher cord and early childhood plasma leptin levels than boys (Supplemental table 5-1). Children that were SGA had lower cord leptin levels and were more likely to have extremely rapid weight gain (Supplemental tables 5-1 and 5-2). Children with most rapid weight gain during infancy were also likely to have higher early childhood leptin levels, but the latter did not differ by birth weight for gestational age (Supplemental table 5-1).

5.4.1 Fetal growth pattern and ASD risk

A total of 599 children had birth weight for gestational age data, of which 47 were later diagnosed with ASD (Supplemental figure 5-1). Five of these 47 ASD subjects had co-occurring ID. Compared to children with AGA, neither SGA children nor LGA children were at a greater risk of ASD, before or after the adjustment of covariates including maternal age at delivery, parity, smoking status, education, race, maternal BMI, maternal diabetes, child's sex, follow-up time and gestational age (Table 5-2).

5.4.2 Weight gain during infancy and ASD risk

A total of 573 children had weight gain during infancy data, of which 46 were later diagnosed with ASD (Supplemental figure 5-1). Five of these 46 subjects had co-occurring ID. Prenatal determinants of weight gain during infancy are presented in Supplemental table 5-2, and preterm birth was a major determinant of infant excessive weight gain. When compared to children whose growth was on track based on weight gain *z*-scores, children that had slow weight gain or rapid weight gain did not have an increased ASD risk (OR_{slow}: 1.52; 95% CI: 0.49, 4.71, OR_{rapid}: 1.33; 95% CI: 0.46, 3.86) (Table 5-2). However, extremely rapid weight gain during infancy was associated with an increased risk of ASD ($OR_{extremely\ rapid}$: 2.64; 95% CI: 1.20, 5.78). This association persisted after adjusting for covariates (child's sex, race, follow-up time and breastfeeding status) in model 3 ($aOR_{extremely\ rapid}$: 3.11; 95% CI: 1.37, 7.07). Next, we sequentially added birth weight for gestational age to model 3 covariates and observed a consistent association (Table 5-2). However, the significance attenuated after adjusting for gestational age in addition to model 3 covariates (aOR: 2.08; 95% CI: 0.84, 5.13). The association did not attenuate after adjusting for prenatal determinants such as maternal BMI, diabetes status and age at the time of delivery, in addition to covariates specified in model 3 (Table 5-2). Sensitivity analyses using stringent comparators or cases showed a consistent association between birth weight for gestational age, weight gain during infancy and risk of ASD (Supplemental tables 5-3 and 5-4).

5.4.3 Cord leptin and ASD risk

A total of 655 children had data on cord leptin levels, of which 39 were later diagnosed with ASD (Supplemental Figure 5-1). Two of these 39 ASD subjects had co-occurring ID. Prenatal and perinatal determinants of cord leptin are presented in Supplemental Table 5-1. The mean

cord leptin levels were 38.23 pg/mL in children with neurotypical development and 27.37 pg/mL in children with ASD (Table 5-1). As observed in other cohorts (59), higher cord blood leptin levels correlated with increase in gestational age. In the unadjusted model, cord leptin levels (stratified into quartiles) were not associated with the risk of ASD (Table 5-3). Similarly, no associations were observed after adjusting for covariates in models 1 and 2. The relationship between cord leptin and ASD was not altered, irrespective of whether cord leptin was assessed as a continuous or a categorical variable (Supplement table 5-5).

5.4.4 Early childhood leptin and ASD

A total of 652 children were included in the analyses, of which 36 were later diagnosed with ASD (Supplemental Figure 5-1). Four of these 36 ASD subjects had co-occurring ID. Figure 5-1 provides a distribution of early childhood leptin levels among neurotypical children and those with ASD, showing a shift in distribution towards right for children with ASD. Supplemental figure 5-2 provides a distribution of when early childhood leptin levels were measured. When compared to children that had the lowest leptin levels (quartile 1), children with highest leptin levels (quartile 4) had an increased ASD risk (OR: 5.41; 95% CI: 1.53, 19.05) (Table 5-3). The association remained significant after adjusting for covariates (model 3) including child's sex, race, child's age when leptin was measured, follow-up time and breastfeeding status (aOR: 7.87; 95% CI: 2.06, 30.04). Further adjusting for gestational age (model 4), cord leptin levels (model 5) or prenatal determinants such as maternal BMI, diabetes status and age at the time of delivery (model 6) did not attenuate the association between early childhood leptin and ASD. Analysis using early childhood plasma leptin levels as a continuous variable or categorical variable yielded consistent findings (Supplement table 5-5).

Sensitivity analyses using stringent controls or cases showed similar trends in association between cord, early childhood leptin and ASD risk (Supplemental tables 5-6 and 5-7). Results from alternative analyses using Cox proportional hazard regression for birth weight for gestational age, weight gain during infancy, cord and early childhood leptin, and ASD risk (Supplemental tables 5-8 and 5-9) were consistent in direction and statistical significance.

5.4.5 Early childhood leptin mediating the relationship between weight gain during infancy and ASD risk

A total 476 children had both weight gain during infancy data and early childhood leptin measurements (Table 5-4). In the unadjusted model that assessed the role of early childhood leptin as a mediator of the relationship between weight gain during infancy and risk of ASD, the total effect of extremely rapid weight gain was statistically significant (OR: 2.80; 95% CI: 1.07, 7.28). Both direct (OR: 2.22; 95% CI: 0.84, 5.87) and indirect effects (OR: 1.26; 95% CI: 0.95, 1.67) were non-significant. In the adjusted model, the total effect attenuated (aOR: 1.80; 95% CI: 0.55, 5.90). However, the indirect effect became significant (aOR: 1.56; 95% CI: 1.01, 2.42), suggesting an indirect-only mediation (60) and that the early childhood leptin potentially mediates 76.03% of the total relationship between extreme rapid weight gain and ASD, after adjusting for confounders. Other weight gain categories such as slow and rapid weight gain were not significantly associated with ASD and adjusting for early childhood leptin levels did not alter their association with ASD.

5.5 Discussion

In this prospective cohort study, our results showed that extremely rapid weight gain during infancy and elevated early childhood leptin levels measured prior to ASD diagnosis were

associated with an increased ASD risk in childhood. Our findings are in line with several studies that have reported that rapid weight gain during infancy is a potential indicator of early autism risk (21, 22, 25, 26, 61). We extend the previous findings and suggest that the association between rapid weight gain and ASD is potentially mediated, at least indirectly, by early childhood plasma leptin. To our knowledge, this is the first study to assess the interrelationships between birth weight, infancy weight gain and leptin in the context of ASD in a prospective birth cohort.

Conceptual Framework of ASD risk factors from prenatal to early childhood:

Epidemiological studies and animal models have consistently showed that an adverse in-utero environment may lead to altered programming of tissue structure and function, predisposing to later behavioral problems, learning difficulties, abnormal or delayed cognitive development and other conditions (62, 63). Sub-optimal prenatal environmental influences could induce permanent fetal adaptations that are beneficial for short-term survival, but increases the vulnerability to later pathogenic environmental stimuli (64, 65). As illustrated in Figure 5-2, based on the findings by us and others, postnatal influences such as extremely rapid weight gain and elevated leptin levels may not be isolated stand-alone occurrences during the first year of life; but could rather be a compensatory event to an adverse prenatal condition or deviation in biological mechanism (23, 50, 66). In support of this argument, we observed that children that were exposed to maternal diabetes or overweight/obesity during pregnancy and those that were SGA or early preterm, were more likely to have extremely rapid weight gain during infancy. Further, in line with the existing evidence (67), low concentrations of cord blood leptin were associated with rapid weight gain during the first year of life suggesting that cord

leptin could serve as a signal for catch-up growth. Taken together, it can be inferred that the incongruent prenatal (e.g. SGA) and postnatal milieu (rapid catch up growth) associated with endocrinologic alterations (early childhood leptin levels) may have a negative impact on the brain architecture and circuits, which could predispose an individual to adverse neurobehavioral outcomes (62, 68). Given this context, we further elaborate our findings and discuss how they compare to the previous studies and provide possible explanations.

Many studies, in addition to ours, reported an inconsistent association between fetal growth and ASD (15-17, 69). Langridge et al. demonstrated that the percentage of optimal birth weight, a measure of fetal growth (70), was not associated with ASD, especially among those with intellectual disability (69). Glasson et al. also showed no association between SGA and ASD (16), while Schnedel et al., noted that the relationship was observed only in girls and not in boys (17). Similarly, Larrson et al. observed that the association between fetal growth and ASD attenuated after adjusting for other covariates (15). In contrast, a few studies showed that SGA children had elevated risk of ASD (8, 14, 71). A possible explanation for the lack of association between fetal growth and ASD is likely due to the heterogeneity in the SGA group related to the timing of onset. Fetal growth restriction may have different clinical manifestation and sequelae depending on whether the onset of growth restriction is early or late during gestation (72, 73). In our study, considering SGA as a homogenous group could have possibly blurred the association between fetal growth and ASD. We do not have data in the current study to tease apart this association, but can be explored further in future studies. Other potential reasons including methodological differences, lack of control for confounding factors, and sample size variations could explain some of the inconsistencies (14, 17).

Consistent with our findings, several studies have shown that children who are later diagnosed with ASD have accelerated weight gain during infancy and early childhood (21, 22, 26, 74). This accelerated weight gain may not be a distinct morphological feature, but is suggestive of a broader autistic phenotype characterized by rapid increase in head circumference, height and weight (22, 26, 74-77). In support of this hypothesis, studies have shown that head circumference is well correlated with weight and height in ASD children(20, 26, 74). Rapid increase in head circumference in children with ASD is one of the most consistent findings that many studies have demonstrated (20, 22, 23, 26, 61, 76, 77). Although we did not analyze head circumferences due to incomplete data, weight has been shown to be the strongest predictor of head circumference during most of infancy (74). Taken together, our findings support the existing evidence that extremely rapid weight gain during infancy is associated with ASD, possibly indicative of an overall growth dysfunction. There are many speculations about why rapid weight gain is observed in children with ASD. Studies have posited that an abnormality in factors (such as metabolism, growth or neurotrophic factors and hormone levels) may predispose an individual to overall accelerated growth as well as ASD (21, 22, 74, 76).

Our study showed that early childhood leptin levels were altered in children with ASD. While prior studies assessing this relationship were mainly cross-sectional, our prospective study for the first time showed that elevated leptin levels are observed even prior to ASD diagnosis. Considering the role of leptin in neurocognition, elevated leptin and associated leptin resistance during the critical periods of postnatal brain development may have permanent adverse implications (78, 79). While the mechanism behind leptin resistance is still being

understood, it is believed to involve reduced transport of leptin to the brain, poor negative feedback mechanism, endoplasmic reticulum stress and an intracellular leptin signaling system that is saturable (79-81).

Similar to other studies (36, 82), we noted that low cord leptin levels closely reflected birth weight and also predicted the greatest weight gain during infancy. However, cord blood leptin was not associated with ASD. This finding may be intriguing especially in the context that early childhood leptin is associated with ASD; however, our results are consistent with the existing evidence that cord and early childhood leptin may have different roles to play (80, 83).

Cord blood leptin is derived primarily from the fetal tissue and is reflective of fetal adiposity (30, 84-86). While leptin is detectable in the fetus at around 18 weeks, rapid increase in leptin levels are observed after 34 weeks, in tandem with increase in fetal adipose tissues (85, 87). Perinatal and neonatal periods are considered to be a window of maximum leptin sensitivity with normal neonates having two to three times higher leptin when compared to adults (37, 78, 88, 89). Neonatal leptin has a different physiological response and promotes hyperphagia and swallowing activity in newborn and may not inhibit growth, food intake or energy expenditure (65, 90-92). However, leptin sensitivity declines with age (80, 93).

After closure of the critical window, higher leptin does not protect against adiposity and some children even develop leptin tolerance (80). Thus, leptin, once positively associated with birth weight and less adiposity during early childhood (27, 59, 80) no longer possesses the same effect – demonstrating that the effect of leptin in perinatal period is distinct from that of later life (94). One study that longitudinally measured cord and early childhood leptin showed that while high cord blood leptin was initially shown to be protective against adiposity, it was

subsequently associated with weight gain and adiposity at age 7 (80). These age-specific effects of leptin have been linked to developmental changes in leptin receptor expression – which are widely expressed in the central nervous system starting from mid-gestation (95). While these findings are related to adiposity, it is plausible to believe that leptin's role may be similar with neurocognitive outcomes.

Leptin as a mediator: After establishing independent associations between ASD and 1) extremely rapid weight gain during infancy, and 2) early childhood leptin, we showed that children with extremely rapid weight gain during infancy had elevated leptin levels. In support of this, animal models that have shown that rapid catch-up growth in early childhood is associated with leptin resistance and this occurs independent of postnatal diet induced obesity (64, 96, 97). It has been hypothesized that proinflammatory cytokines may mediate the relationship between rapid postnatal growth and ASD (68). As a proof of concept, our study was able to demonstrate the mediating effect of leptin in the association between extremely rapid weight gain and ASD. However, in our dataset, cord leptin did not possess any mediating effects unlike early childhood leptin.

Mechanism of leptin in ASD: Inflammation is a possible mechanism through which leptin may impact the psychopathology of ASD. Leptin, a pro-inflammatory cytokine may play a role in the pathophysiology of conditions such as schizophrenia (98, 99) and ASD (100, 101). A variety of independent studies have linked cytokine dysregulation to ASD (100). Cytokines act as immune mediators and their imbalance during development and throughout life can adversely impact neural activity and mediate behavioral aspect of the disorder (100). Inflammatory cytokines are implicated in higher neurological functions such as memory and

cognition, in addition to being involved in brain development, synaptic functioning including processes of differentiation, migration, proliferation and impairments in behavior (102). Thus, abnormal inflammatory activity and imbalance of cytokines during development can adversely impact neural activity and could contribute to behavioral and neurological dysfunction in ASD (100, 102).

Altered leptin levels can also impact brain structure and function. For example, leptin levels are increased at the site of inflammation in the post-mortem brain tissue (40). Leptin deficient and leptin resistant state is associated with lower brain weight, protein content, reduction in brain myelin, neuronal soma size and several synaptic proteins. Reduced brain weight is observed in animals that lacked leptin signaling (92). A study conducted on autopsy tissues showed that there is a marked increase in leptin levels in anterior cingulate gyrus among those that had ASD (103). In a small subset of patients, genetic correlation was observed between ASD and leptin coding (104). Leptin is involved in long-term potentiation and long-term depression (105) and dysregulation of this function is implicated in ASD (105). Further, leptin is also known to suppress serotonin synthesis, which is reported in ASD, possibly suggesting another biological pathway through which leptin can be involved in ASD (78).

In our earlier report in the BBC, we showed that maternal obesity and diabetes was associated with increased risk of ASD in offspring (49). Our results, along with consistent findings across diverse populations (106-109) raised the possibility of early metabolic dysfunction in the development of ASD. Studies have posited that early life manipulations of leptin in animal models alter susceptibility to subsequent obesity and metabolic disorders (65, 82). Periods of hypo- or hyperleptinemia may induce metabolic adaptations, which could be the

basis of developmental programming (65). In this context, the role of leptin as a potential mediator of the developmental programming of ASD (94) may be a novel proposition for ASD, but requires further investigation. Additional research is warranted on the role of other hormones with leptin opposing action, so as to better understand the metabolic milieu involved in ASD. Similarly, future studies should also examine variants in leptin and leptin receptors to better understand the biological pathways of leptin in ASD.

5.5.1 Limitations and Strengths

Although our study stemmed from a rigorously designed prospective birth cohort, the findings may be tempered due to some limitations. First, case and neurotypical development classification of children was based on EMR data and it is possible that there may be outcome misclassification. However, this misclassification may not be differential given the prospective study design. Second, the relatively small number of cases in our prospective cohort design could have resulted in wide confidence intervals and imprecise estimates. Third, although our models accounted for breastfeeding vs. formula feeding, more research is needed to examine the role of perinatal nutrition and its influences on weight gain, early childhood leptin and ASD. Fourth, we could not directly assess fat mass at the time of leptin measurement and this could have resulted in some residual confounding. Fifth, plasma leptin levels follow a circadian rhythm (81) and may be impacted by fasting status; although, the timing of plasma sample collection for leptin measurements was random, the distribution was comparable between ASD and neurotypical groups. Finally, our study population consisted mainly of urban low-income

minority populations that were also at high risk for conditions such as SGA and preterm births and thus, the results may not be generalizable to the U.S. population.

Despite these limitations, our study has a number of strengths. This is one of the first longitudinal studies that addressed leptin levels at birth and in early childhood in the context of ASD. Infant weight gain was assessed as part of well-child visit during first year of life, when child's ASD status was not known. By using a sample of children that have weight gain data as well as data on cord and early childhood leptin, we were uniquely poised to examine the inter-relationship of these variables in the development of ASD.

5.6 Conclusion

In the BBC, we showed that extremely rapid weight gain during infancy and elevated leptin levels during early childhood were independently associated with greater ASD risk and early childhood plasma leptin levels at least indirectly mediated the relationship between early childhood weight gain and ASD. Furthermore, the prenatal and postnatal risk factors for ASD are interrelated and act along a continuum from prenatal to postnatal periods. An important implication of these findings is that in addition to prenatal factors, pathogenic processes underlying ASD likely continue during the postnatal period, including infancy and possibly extending to early childhood (26). Even though accelerated weight gain in early life, combined with elevated plasma leptin may not be a unique biomarker for ASD, our preliminary findings provide a basis from which to further explore the relationship between prenatal events, infancy rapid weight gain, leptin and ASD under a life course framework (23). Additional research is needed to understand if a combination of prenatal and early childhood anthropometric, biological, genetic variables and behavioral signs together can accurately predict ASD sooner. If

proven to be useful by future studies, this will provide an opportunity to start intervention earlier thereby potentially halting or mitigating the progression towards ASD (22, 23, 110).

5.7 References

1. Leitner Y. The co-occurrence of autism and attention deficit hyperactivity disorder in children - what do we know? Frontiers in human neuroscience. 2014;8:268.
2. Murray MJ. Attention-deficit/Hyperactivity Disorder in the context of Autism spectrum disorders. Curr Psychiatry Rep. 2010;12(5):382-8.
3. Schaevitz L, Berger-Sweeney J, Ricceri L. One-carbon metabolism in neurodevelopmental disorders: using broad-based nutraceutics to treat cognitive deficits in complex spectrum disorders. Neurosci Biobehav Rev. 2014;46 Pt 2:270-84.
4. Jeste SS. Neurodevelopmental behavioral and cognitive disorders. Continuum. 2015;21(3 Behavioral Neurology and Neuropsychiatry):690-714.
5. Zhubi A, Cook EH, Guidotti A, Grayson DR. Epigenetic mechanisms in autism spectrum disorder. International review of neurobiology. 2014;115:203-44.
6. Krakowiak P, Walker CK, Bremer AA, Baker AS, Ozonoff S, Hansen RL, et al. Maternal metabolic conditions and risk for autism and other neurodevelopmental disorders. Pediatrics. 2012;129(5):e1121-8.
7. Van Naarden Braun K, Christensen D, Doernberg N, Schieve L, Rice C, Wiggins L, et al. Trends in the prevalence of autism spectrum disorder, cerebral palsy, hearing loss, intellectual disability, and vision impairment, metropolitan atlanta, 1991-2010. PLoS One. 2015;10(4):e0124120.
8. Moore GS, Kneitel AW, Walker CK, Gilbert WM, Xing G. Autism risk in small- and large-for-gestational-age infants. American journal of obstetrics and gynecology. 2012;206(4):314 e1-9.
9. Limperopoulos C. Autism spectrum disorders in survivors of extreme prematurity. Clin Perinatol. 2009;36(4):791-805, vi.
10. Schieve LA, Tian LH, Baio J, Rankin K, Rosenberg D, Wiggins L, et al. Population attributable fractions for three perinatal risk factors for autism spectrum disorders, 2002 and 2008 autism and developmental disabilities monitoring network. Ann Epidemiol. 2014;24(4):260-6.
11. Joseph RM, Korzeniewski SJ, Allred EN, O'Shea TM, Heeren T, Frazier JA, et al. Extremely low gestational age and very low birthweight for gestational age are risk factors for autism spectrum disorder in a large cohort study of 10-year-old children born at 23-27 weeks' gestation. American journal of obstetrics and gynecology. 2017;216(3):304 e1- e16.
12. Padilla N, Eklof E, Martensson GE, Bolte S, Lagercrantz H, Aden U. Poor Brain Growth in Extremely Preterm Neonates Long Before the Onset of Autism Spectrum Disorder Symptoms. Cereb Cortex. 2017;27(2):1245-52.
13. Hunter DS, Hazel SJ, Kind KL, Owens JA, Pitcher JB, Gatford KL. Programming the brain: Common outcomes and gaps in knowledge from animal studies of IUGR. Physiol Behav. 2016;164(Pt A):233-48.
14. Lampi KM, Lehtonen L, Tran PL, Suominen A, Lehti V, Banerjee PN, et al. Risk of autism spectrum disorders in low birth weight and small for gestational age infants. J Pediatr. 2012;161(5):830-6.

15. Larsson HJ, Eaton WW, Madsen KM, Vestergaard M, Olesen AV, Agerbo E, et al. Risk factors for autism: perinatal factors, parental psychiatric history, and socioeconomic status. American journal of epidemiology. 2005;161(10):916-25; discussion 26-8.
16. Glasson EJ, Bower C, Petterson B, de Klerk N, Chaney G, Hallmayer JF. Perinatal factors and the development of autism: a population study. Arch Gen Psychiatry. 2004;61(6):618-27.
17. Schendel D, Bhasin TK. Birth weight and gestational age characteristics of children with autism, including a comparison with other developmental disabilities. Pediatrics. 2008;121(6):1155-64.
18. Castanys-Munoz E, Kennedy K, Castaneda-Gutierrez E, Forsyth S, Godfrey KM, Koletzko B, et al. Systematic review indicates postnatal growth in term infants born small-for-gestational-age being associated with later neurocognitive and metabolic outcomes. Acta Paediatr. 2017;106(8):1230-8.
19. Ong KK. Catch-up growth in small for gestational age babies: good or bad? Curr Opin Endocrinol Diabetes Obes. 2007;14(1):30-4.
20. Dementieva YA, Vance DD, Donnelly SL, Elston LA, Wolpert CM, Ravan SA, et al. Accelerated head growth in early development of individuals with autism. Pediatr Neurol. 2005;32(2):102-8.
21. Torrey EF, Dhavale D, Lawlor JP, Yolken RH. Autism and head circumference in the first year of life. Biol Psychiatry. 2004;56(11):892-4.
22. Dissanayake C, Bui QM, Huggins R, Loesch DZ. Growth in stature and head circumference in high-functioning autism and Asperger disorder during the first 3 years of life. Dev Psychopathol. 2006;18(2):381-93.
23. Courchesne E, Carper R, Akshoomoff N. Evidence of brain overgrowth in the first year of life in autism. Jama. 2003;290(3):337-44.
24. Rommelse NN, Peters CT, Oosterling IJ, Visser JC, Bons D, van Steijn DJ, et al. A pilot study of abnormal growth in autism spectrum disorders and other childhood psychiatric disorders. J Autism Dev Disord. 2011;41(1):44-54.
25. Suren P, Stoltenberg C, Bresnahan M, Hirtz D, Lie KK, Lipkin WI, et al. Early growth patterns in children with autism. Epidemiology. 2013;24(5):660-70.
26. Sacco R, Militerni R, Frolli A, Bravaccio C, Gritti A, Elia M, et al. Clinical, morphological, and biochemical correlates of head circumference in autism. Biol Psychiatry. 2007;62(9):1038-47.
27. Karakosta P, Chatzi L, Plana E, Margioris A, Castanas E, Kogevinas M. Leptin levels in cord blood and anthropometric measures at birth: a systematic review and meta-analysis. Paediatr Perinat Epidemiol. 2011;25(2):150-63.
28. Blardi P, de Lalla A, Ceccatelli L, Vanessa G, Auteri A, Hayek J. Variations of plasma leptin and adiponectin levels in autistic patients. Neurosci Lett. 2010;479(1):54-7.
29. Pighetti M, Tommaselli GA, D'Elia A, Di Carlo C, Mariano A, Di Carlo A, et al. Maternal serum and umbilical cord blood leptin concentrations with fetal growth restriction. Obstet Gynecol. 2003;102(3):535-43.
30. Catov JM, Patrick TE, Powers RW, Ness RB, Harger G, Roberts JM. Maternal leptin across pregnancy in women with small-for-gestational-age infants. American journal of obstetrics and gynecology. 2007;196(6):558 e1-8.

31. Harigaya A, Nagashima K, Nako Y, Morikawa A. Relationship between concentration of serum leptin and fetal growth. J Clin Endocrinol Metab. 1997;82(10):3281-4.
32. Koistinen HA, Koivisto VA, Andersson S, Karonen SL, Kontula K, Oksanen L, et al. Leptin concentration in cord blood correlates with intrauterine growth. J Clin Endocrinol Metab. 1997;82(10):3328-30.
33. Perng W, Oken E, Roumeliotaki T, Sood D, Siskos AP, Chalkiadaki G, et al. Leptin, acylcarnitine metabolites and development of adiposity in the Rhea mother-child cohort in Crete, Greece. Obes Sci Pract. 2016;2(4):471-6.
34. Kettaneh A, Heude B, Romon M, Oppert JM, Borys JM, Balkau B, et al. High plasma leptin predicts an increase in subcutaneous adiposity in children and adults. Eur J Clin Nutr. 2007;61(6):719-26.
35. Li S, Liu R, Arguelles L, Wang G, Zhang J, Shen X, et al. Adiposity trajectory and its associations with plasma adipokine levels in children and adolescents-A prospective cohort study. Obesity (Silver Spring). 2016;24(2):408-16.
36. Ong KK, Ahmed ML, Sherriff A, Woods KA, Watts A, Golding J, et al. Cord blood leptin is associated with size at birth and predicts infancy weight gain in humans. ALSPAC Study Team. Avon Longitudinal Study of Pregnancy and Childhood. J Clin Endocrinol Metab. 1999;84(3):1145-8.
37. Paz-Filho GJ, Babikian T, Asarnow R, Delibasi T, Esposito K, Erol HK, et al. Leptin replacement improves cognitive development. PloS one. 2008;3(8):e3098.
38. Harvey J. Leptin regulation of neuronal excitability and cognitive function. Curr Opin Pharmacol. 2007;7(6):643-7.
39. Harvey J, Solovyova N, Irving A. Leptin and its role in hippocampal synaptic plasticity. Prog Lipid Res. 2006;45(5):369-78.
40. Ashwood P, Kwong C, Hansen R, Hertz-Picciotto I, Croen L, Krakowiak P, et al. Brief report: plasma leptin levels are elevated in autism: association with early onset phenotype? J Autism Dev Disord. 2008;38(1):169-75.
41. Rodrigues DH, Rocha NP, Sousa LF, Barbosa IG, Kummer A, Teixeira AL. Changes in adipokine levels in autism spectrum disorders. Neuropsychobiology. 2014;69(1):6-10.
42. Al-Zaid FS, Alhader AA, Al-Ayadhi LY. Altered ghrelin levels in boys with autism: a novel finding associated with hormonal dysregulation. Sci Rep. 2014;4:6478.
43. Essa M.M., Braidy N., Al-Sharbati M.M., Al-Farsi YM, Ali A, Waly M.I., et al. Elevated plasma leptin levels in autisic children of Sultanate of Oman. International Journal of Biological & Medical Research. 2011;2(3):803-5.
44. Masi A, Glozier N, Dale R, Guastella AJ. The Immune System, Cytokines, and Biomarkers in Autism Spectrum Disorder. Neurosci Bull. 2017;33(2):194-204.
45. Krakowiak P, Goines PE, Tancredi DJ, Ashwood P, Hansen RL, Hertz-Picciotto I, et al. Neonatal Cytokine Profiles Associated With Autism Spectrum Disorder. Biol Psychiatry. 2017;81(5):442-51.
46. Xu N, Li X, Zhong Y. Inflammatory cytokines: potential biomarkers of immunologic dysfunction in autism spectrum disorders. Mediators Inflamm. 2015;2015:531518.
47. Wang G, Divall S, Radovick S, Paige D, Ning Y, Chen Z, et al. Preterm birth and random plasma insulin levels at birth and in early childhood. Jama. 2014;311(6):587-96.

48. Wang X, Zuckerman B, Pearson C, Kaufman G, Chen C, Wang G, et al. Maternal cigarette smoking, metabolic gene polymorphism, and infant birth weight. Jama. 2002;287(2):195-202.
49. Li M, Fallin MD, Riley A, Landa R, Walker SO, Silverstein M, et al. The Association of Maternal Obesity and Diabetes With Autism and Other Developmental Disabilities. Pediatrics. 2016;137(2):1-10.
50. Wang G, Johnson S, Gong Y, Polk S, Divall S, Radovick S, et al. Weight Gain in Infancy and Overweight or Obesity in Childhood across the Gestational Spectrum: a Prospective Birth Cohort Study. Sci Rep. 2016;6:29867.
51. Wang G, Hu FB, Mistry KB, Zhang C, Ren F, Huo Y, et al. Association Between Maternal Prepregnancy Body Mass Index and Plasma Folate Concentrations With Child Metabolic Health. JAMA Pediatr. 2016;170(8):e160845.
52. Breen R, Karlson KB, Holm A. Total, Direct, and Indirect effects in logit and probit models. Sociological Methods & Research. 2013:1-23.
53. Raghavan R, Riley AW, Volk H, Caruso D, Hironaka L, Sices L, et al. Maternal Multivitamin Intake, Plasma Folate and Vitamin B12 Levels and Autism Spectrum Disorder Risk in Offspring. Paediatric and perinatal epidemiology. 2017.
54. American Diabetes A. 2. Classification and Diagnosis of Diabetes. Diabetes Care. 2016;39 Suppl 1:S13-22.
55. American Diabetes A. Gestational diabetes mellitus. Diabetes Care. 2003;26 Suppl 1:S103-5.
56. Organization WH. The ICD-9 classification of mental and behavioural disorders: Clinical descriptions and diagnostic guidelines: World Health Organization; [Available from: http://www.icd9data.com/2015/Volume1/default.htm.
57. Hong X, Wang G, Liu X, Kumar R, Tsai HJ, Arguelles L, et al. Gene polymorphisms, breast-feeding, and development of food sensitization in early childhood. J Allergy Clin Immunol. 2011;128(2):374-81 e2.
58. Tome MA, Lage M, Camina JP, Garcia-Mayor RV, Dieguez C, Casanueva FF. Sex-based differences in serum leptin concentrations from umbilical cord blood at delivery. Eur J Endocrinol. 1997;137(6):655-8.
59. Mantzoros CS, Rifas-Shiman SL, Williams CJ, Fargnoli JL, Kelesidis T, Gillman MW. Cord blood leptin and adiponectin as predictors of adiposity in children at 3 years of age: a prospective cohort study. Pediatrics. 2009;123(2):682-9.
60. Zhao X, Lynch JGJ, Chen Q. Reconsidering Baron and Kenny: Myths and Truths about Mediation Analysis. The Journal of Consumer Research. 2010;37(2):197-206.
61. Chawarska K, Campbell D, Chen L, Shic F, Klin A, Chang J. Early generalized overgrowth in boys with autism. Arch Gen Psychiatry. 2011;68(10):1021-31.
62. Van den Bergh BR. Developmental programming of early brain and behaviour development and mental health: a conceptual framework. Dev Med Child Neurol. 2011;53 Suppl 4:19-23.
63. Vickers MH. Developmental programming and adult obesity: the role of leptin. Curr Opin Endocrinol Diabetes Obes. 2007;14(1):17-22.
64. Krechowec SO, Vickers M, Gertler A, Breier BH. Prenatal influences on leptin sensitivity and susceptibility to diet-induced obesity. J Endocrinol. 2006;189(2):355-63.

65. Vickers MH, Sloboda DM. Leptin as mediator of the effects of developmental programming. Best Pract Res Clin Endocrinol Metab. 2012;26(5):677-87.
66. Karaolis-Danckert N, Buyken AE, Kulig M, Kroke A, Forster J, Kamin W, et al. How pre- and postnatal risk factors modify the effect of rapid weight gain in infancy and early childhood on subsequent fat mass development: results from the Multicenter Allergy Study 90. The American journal of clinical nutrition. 2008;87(5):1356-64.
67. Ong KK, Ahmed ML, Emmett PM, Preece MA, Dunger DB. Association between postnatal catch-up growth and obesity in childhood: prospective cohort study. BMJ. 2000;320(7240):967-71.
68. Pylipow M, Spector LG, Puumala SE, Boys C, Cohen J, Georgieff MK. Early postnatal weight gain, intellectual performance, and body mass index at 7 years of age in term infants with intrauterine growth restriction. J Pediatr. 2009;154(2):201-6.
69. Langridge AT, Glasson EJ, Nassar N, Jacoby P, Pennell C, Hagan R, et al. Maternal conditions and perinatal characteristics associated with autism spectrum disorder and intellectual disability. PloS one. 2013;8(1):e50963.
70. Blair EM, Liu Y, de Klerk NH, Lawrence DM. Optimal fetal growth for the Caucasian singleton and assessment of appropriateness of fetal growth: an analysis of a total population perinatal database. BMC Pediatr. 2005;5(1):13.
71. Maimburg RD, Vaeth M. Perinatal risk factors and infantile autism. Acta Psychiatr Scand. 2006;114(4):257-64.
72. Dall'Asta A, Brunelli V, Prefumo F, Frusca T, Lees CC. Early onset fetal growth restriction. Matern Health Neonatol Perinatol. 2017;3:2.
73. Savchev S, Figueras F, Sanz-Cortes M, Cruz-Lemini M, Triunfo S, Botet F, et al. Evaluation of an optimal gestational age cut-off for the definition of early- and late-onset fetal growth restriction. Fetal Diagn Ther. 2014;36(2):99-105.
74. Mraz KD, Green J, Dumont-Mathieu T, Makin S, Fein D. Correlates of head circumference growth in infants later diagnosed with autism spectrum disorders. J Child Neurol. 2007;22(6):700-13.
75. van Daalen E, Swinkels SH, Dietz C, van Engeland H, Buitelaar JK. Body length and head growth in the first year of life in autism. Pediatr Neurol. 2007;37(5):324-30.
76. Fukumoto A, Hashimoto T, Ito H, Nishimura M, Tsuda Y, Miyazaki M, et al. Growth of head circumference in autistic infants during the first year of life. J Autism Dev Disord. 2008;38(3):411-8.
77. Sacco R, Gabriele S, Persico AM. Head circumference and brain size in autism spectrum disorder: A systematic review and meta-analysis. Psychiatry Res. 2015;234(2):239-51.
78. Valleau JC, Sullivan EL. The impact of leptin on perinatal development and psychopathology. J Chem Neuroanat. 2014;61-62:221-32.
79. Glavas MM, Kirigiti MA, Xiao XQ, Enriori PJ, Fisher SK, Evans AE, et al. Early overnutrition results in early-onset arcuate leptin resistance and increased sensitivity to high-fat diet. Endocrinology. 2010;151(4):1598-610.
80. Boeke CE, Mantzoros CS, Hughes MD, S LR-S, Villamor E, Zera CA, et al. Differential associations of leptin with adiposity across early childhood. Obesity (Silver Spring). 2013;21(7):1430-7.

81. Mantzoros CS, Magkos F, Brinkoetter M, Sienkiewicz E, Dardeno TA, Kim SY, et al. Leptin in human physiology and pathophysiology. Am J Physiol Endocrinol Metab. 2011;301(4):E567-84.
82. Dulloo AG. Thrifty energy metabolism in catch-up growth trajectories to insulin and leptin resistance. Best Pract Res Clin Endocrinol Metab. 2008;22(1):155-71.
83. Zhang M, Cheng H, Zhao X, Hou D, Yan Y, Cianflone K, et al. Leptin and Leptin-to-Adiponectin Ratio Predict Adiposity Gain in Nonobese Children over a Six-Year Period. Child Obes. 2017;13(3):213-21.
84. Hauguel-de Mouzon S, Lepercq J, Catalano P. The known and unknown of leptin in pregnancy. Am J Obstet Gynecol. 2006;194(6):1537-45.
85. Mellati AA, Mazloomzadeh S, Anjomshoaa A, Alipour M, Karimi F, Mazloomi S, et al. Multiple correlations between cord blood leptin concentration and indices of neonatal growth. Arch Med Res. 2010;41(1):26-32.
86. Tessier DR, Ferraro ZM, Gruslin A. Role of leptin in pregnancy: consequences of maternal obesity. Placenta. 2013;34(3):205-11.
87. Grisaru-Granovsky S, Samueloff A, Elstein D. The role of leptin in fetal growth: a short review from conception to delivery. Eur J Obstet Gynecol Reprod Biol. 2008;136(2):146-50.
88. Bouret SG. Nutritional programming of hypothalamic development: critical periods and windows of opportunity. Int J Obes Suppl. 2012;2(Suppl 2):S19-24.
89. Bouret SG. Organizational actions of metabolic hormones. Front Neuroendocrinol. 2013;34(1):18-26.
90. Alexe DM, Syridou G, Petridou ET. Determinants of early life leptin levels and later life degenerative outcomes. Clin Med Res. 2006;4(4):326-35.
91. El-Haddad MA, Desai M, Gayle D, Ross MG. In utero development of fetal thirst and appetite: potential for programming. J Soc Gynecol Investig. 2004;11(3):123-30.
92. Bouret SG, Simerly RB. Minireview: Leptin and development of hypothalamic feeding circuits. Endocrinology. 2004;145(6):2621-6.
93. Levin BE, Dunn-Meynell AA, Banks WA. Obesity-prone rats have normal blood-brain barrier transport but defective central leptin signaling before obesity onset. Am J Physiol Regul Integr Comp Physiol. 2004;286(1):R143-50.
94. Cottrell EC, Cripps RL, Duncan JS, Barrett P, Mercer JG, Herwig A, et al. Developmental changes in hypothalamic leptin receptor: relationship with the postnatal leptin surge and energy balance neuropeptides in the postnatal rat. Am J Physiol Regul Integr Comp Physiol. 2009;296(3):R631-9.
95. Cottrell EC, Mercer JG, Ozanne SE. Postnatal development of hypothalamic leptin receptors. Vitam Horm. 2010;82:201-17.
96. Coupe B, Grit I, Hulin P, Randuineau G, Parnet P. Postnatal growth after intrauterine growth restriction alters central leptin signal and energy homeostasis. PloS one. 2012;7(1):e30616.
97. Yura S, Itoh H, Sagawa N, Yamamoto H, Masuzaki H, Nakao K, et al. Role of premature leptin surge in obesity resulting from intrauterine undernutrition. Cell Metab. 2005;1(6):371-8.
98. Stubbs B, Wang AK, Vancampfort D, Miller BJ. Are leptin levels increased among people with schizophrenia versus controls? A systematic review and comparative meta-analysis. Psychoneuroendocrinology. 2016;63:144-54.

99. Haupt DW, Luber A, Maeda J, Melson AK, Schweiger JA, Newcomer JW. Plasma leptin and adiposity during antipsychotic treatment of schizophrenia. Neuropsychopharmacology. 2005;30(1):184-91.
100. Goines PE, Ashwood P. Cytokine dysregulation in autism spectrum disorders (ASD): possible role of the environment. Neurotoxicol Teratol. 2013;36:67-81.
101. Ashwood P, Krakowiak P, Hertz-Picciotto I, Hansen R, Pessah I, Van de Water J. Elevated plasma cytokines in autism spectrum disorders provide evidence of immune dysfunction and are associated with impaired behavioral outcome. Brain Behav Immun. 2011;25(1):40-5.
102. Tonhajzerova I, Ondrejka I, Mestanik M, Mikolka P, Hrtanek I, Mestanikova A, et al. Inflammatory Activity in Autism Spectrum Disorder. Adv Exp Med Biol. 2015;861:93-8.
103. Vargas DL, Nascimbene C, Krishnan C, Zimmerman AW, Pardo CA. Neuroglial activation and neuroinflammation in the brain of patients with autism. Ann Neurol. 2005;57(1):67-81.
104. Bochukova EG, Huang N, Keogh J, Henning E, Purmann C, Blaszczyk K, et al. Large, rare chromosomal deletions associated with severe early-onset obesity. Nature. 2010;463(7281):666-70.
105. Bliss TV, Collingridge GL, Morris RG. Synaptic plasticity in health and disease: introduction and overview. Philos Trans R Soc Lond B Biol Sci. 2014;369(1633):20130129.
106. Li YM, Ou JJ, Liu L, Zhang D, Zhao JP, Tang SY. Association Between Maternal Obesity and Autism Spectrum Disorder in Offspring: A Meta-analysis. J Autism Dev Disord. 2016;46(1):95-102.
107. Xu G, Jing J, Bowers K, Liu B, Bao W. Maternal diabetes and the risk of autism spectrum disorders in the offspring: a systematic review and meta-analysis. J Autism Dev Disord. 2014;44(4):766-75.
108. Xiang AH, Wang X, Martinez MP, Walthall JC, Curry ES, Page K, et al. Association of maternal diabetes with autism in offspring. JAMA. 2015;313(14):1425-34.
109. Nahum Sacks K, Friger M, Shoham-Vardi I, Abokaf H, Spiegel E, Sergienko R, et al. Prenatal exposure to gestational diabetes mellitus as an independent risk factor for long-term neuropsychiatric morbidity of the offspring. American journal of obstetrics and gynecology. 2016;215(3):380 e1-7.
110. Allely CS, Gillberg C, Wilson P. Neurobiological abnormalities in the first few years of life in individuals later diagnosed with autism spectrum disorder: a review of recent data. Behav Neurol. 2014;2014:210780.

Appendix

Table 5-1 Maternal and child characteristics by child's status (neurotypical vs. ASD)

	Neurotypical (n=769)	ASD (n=53)	p value
Characteristics			
Mothers			
Age at birth (yrs), mean (SD)	28.21 (6.51)	30.35 (6.28)	0.02
Parity (%)			0.92
0	331 (41.74)	21 (39.62)	
1 or more	447 (58.13)	32 (60.38)	
Missing	1 (0.13)	0 (0.0)	
Mother's education (%)			0.43
High School or less	498 (64.76)	31 (58.49)	
Some college or more	266 (34.59)	21 (39.62)	
Missing	5 (0.65)	1 (1.89)	
Maternal BMI (%)			0.16
Underweight (<18.5) + Normal Weight (≥18.5-<25)	371 (48.24)	19 (35.85)	
Overweight (25-29.9)	235 (30.56)	18 (33.96)	
Obesity (≥30)	163 (21.20)	16 (30.19)	
Diabetes mellitus (%)			0.19
No	688 (89.47)	45 (84.91)	
Gestational	53 (6.89)	3 (5.66)	
Pre-gestational diabetes	27 (3.51)	5 (9.43)	
Missing	1 (0.13)	0 (0.0)	
Smoking during & 3 months prior to pregnancy (%)			0.17
No	659 (85.70)	41 (77.36)	
Yes	105 (13.65)	12 (22.64)	
Missing	5 (0.65)	0 (0.00)	
Offspring			
Sex (%)			<0.001
Male	327 (42.52)	39 (73.58)	
Female	442 (57.48)	14 (26.42)	
Race-ethnicity (%)			0.91
Black	434 (56.44)	32 (60.38)	
White	46 (5.98)	4 (7.55)	
Hispanic	185 (24.06)	11 (20.75)	
Other	99 (12.87)	6 (11.32)	
Missing	5 (0.65)	0 (0.0)	

Gestational age (%)			0.007
Term	579 (75.29)	32 (60.38)	
Late preterm (≥34 - <37 weeks)	126 (16.38)	10 (18.87)	
Early preterm (<34 weeks)	64 (8.32)	11 (20.75)	
Birthweight (g)	3009.07 (714.09)	2782.26 (886.18)	0.03
Year of birth (%)			0.70
1998-2006	400 (52.02)	29 (54.72)	
2007-2013	369 (46.98)	24 (45.28)	
Birth weight for gestational age (%)[a,b]			0.73
Appropriate for gestational age (AGA)	442 (80.07)	36 (76.60)	
Small for gestational age (SGA)	59 (10.69)	5 (10.64)	
Large for gestational age (LGA)	51 (9.24)	6 (12.77)	
Weight gain during infancy (%)[c, d]			0.06
On target	178 (33.78)	9 (19.57)	
Slow	65 (12.33)	5 (10.87)	
Rapid weight gain	89 (16.89)	6 (13.04)	
Extremely rapid weight gain	195 (37.00)	26 (56.52)	
Cord blood leptin (SD)[e]	38.23 (34.01)	27.37 (19.94)	0.05
Early childhood leptin (SD)[f]	4.22 (5.60)	5.88 (5.68)	0.08
Mode of feeding (%)			0.62
Formula	177 (23.02)	10 (18.87)	
Both	537 (69.83)	37 (69.81)	
Breastfeeding	49 (6.37)	5 (9.43)	
Missing	6 (0.78)	1 (1.89)	

[a] Fetal growth defined as AGA (≥10^{th} – 90^{th} percentile); SGA (<10^{th} percentile), and LGA (>90^{th} percentile)

[b] n =599 (Neurotypical n=552; ASD n=47)

[c] Weight gain *z*-scores during the first year of life were defined as the change in weight-for-age *z*-scores from birth until the target time-point and was categorized into the following groups: slow (weight gain *z*-score <-0.67), on track (−0.67 to 0.67), rapid (>0.67 to 1.28), and extremely rapid (>1.28)

[d] n=573 (Neurotypical n=527; ASD n=46)

[e] n=655 (Neurotypical n=616; ASD n=39)

[f] n=652 (Neurotypical n=616; ASD n=36)

Table 5-2 Association between birth weight for gestational age, weight gain during infancy and ASD risk in children

	Total n	**ASD n**	OR	95% CI	p value
Birth weight for gestational age[a]					
Unadjusted					
AGA	478	36	Ref		
SGA	64	5	1.04	0.39, 2.76	0.94
LGA	57	6	1.44	0.58, 3.59	0.43
Model 1					
AGA	478	36	Ref		
SGA	64	5	0.81	0.28, 2.34	0.70
LGA	57	6	1.23	0.44, 3.47	0.69
Model 2					
AGA	478	36	Ref		
SGA	64	5	0.86	0.29, 2.54	0.79
LGA	57	6	1.37	0.48, 3.91	0.55
Weight gain during infancy[b]					
Unadjusted					
On target	187	9	Ref		
Slow	70	5	1.52	0.49, 4.71	0.47
Rapid weight gain	95	6	1.33	0.46, 3.86	0.60
Extremely rapid weight gain	221	26	2.64	1.20, 5.78	0.02
Model 3					
On target	187	9	Ref		
Slow	70	5	1.71	0.53, 5.52	0.37
Rapid weight gain	95	6	1.39	0.43, 4.44	0.58
Extremely rapid weight gain	221	26	3.11	1.37, 7.07	0.007
Model 4					
On target	187	9	Ref		
Slow	70	5	1.55	0.46, 5.24	0.48
Rapid weight gain	95	6	1.50	0.46, 4.88	0.50
Extremely rapid weight gain	221	26	3.33	1.44, 7.72	0.005
Model 5					
On target	187	9	Ref		
Slow	70	5	1.74	0.54, 5.66	0.36
Rapid weight gain	95	6	1.32	0.41, 4.24	0.64
Extremely rapid weight gain	221	26	2.08	0.84, 5.13	0.11
Model 6					
On target	187	9	Ref		

Slow	70	5	1.40	0.40, 4.86	0.59
Over growth	95	6	1.22	0.36, 4.14	0.75
Extremely rapid weight gain	221	26	3.33	1.40, 7.89	0.006

[a] In-utero growth defined as Appropriate for gestational age (≥10th – 90th percentile); Small for gestational age (<10th percentile), and Large for gestational age (>90th percentile)
[b] Weight gain during infancy defined as the change in weight-for-age *z*-scores from birth until the target time-point and was categorized into the following groups: slow (weight gain *z*-score <-0.67), on track (−0.67 to 0.67), rapid (>0.67 to 1.28), and extremely rapid (>1.28)

Model 1: Adjusted for maternal age at delivery, parity, smoking, education, maternal BMI, maternal diabetes status, race, child's sex and follow-up time
Model 2: Adjusted for Model 1 + gestational age
Model 3: Adjusted for child's sex, race, follow-up time and breastfeeding status
Model 4: Adjusted for Model 3 + birth weight for gestational age
Model 5: Adjusted for Model 3 + gestational age
Model 6: Adjusted for Model 3 + maternal age, maternal diabetes status, maternal BMI

Table 5-3 Association between cord, early childhood plasma leptin levels and ASD risk in children

	Total n	ASD n	OR	95% CI	p value
Cord leptin					
Unadjusted					
Q1	164	10	Ref		
Q2	163	11	1.11	0.46, 2.70	0.81
Q3	164	11	1.11	0.46, 2.68	0.82
Q4	164	7	0.69	0.25, 1.85	0.46
Model 1					
Q1	164	10	Ref		
Q2	163	11	1.44	0.53, 3.87	0.48
Q3	164	11	1.76	0.66, 4.69	0.26
Q4	164	7	0.96	0.31, 2.94	0.94
Model 2					
Q1	164	10	Ref		
Q2	163	11	2.01	0.68, 5.92	0.21
Q3	164	11	2.74	0.90, 8.31	0.08
Q4	164	7	1.40	0.42, 4.66	0.58
Early childhood leptin					
Unadjusted					
Q1	163	3	Ref		
Q2	163	8	2.75	0.72, 10.57	0.14
Q3	163	10	3.49	0.94, 12.91	0.06
Q4	163	15	5.41	1.53, 19.05	0.009
Model 3					
Q1	163	3	Ref		
Q2	163	8	3.32	0.83, 13.37	0.09
Q3	163	10	4.61	1.17, 18.22	0.03
Q4	163	15	7.87	2.06, 30.04	0.003
Model 4					
Q1	163	3	Ref		
Q2	163	8	3.30	0.81, 13.41	0.10
Q3	163	10	4.93	1.23, 19.77	0.02
Q4	163	15	7.89	2.05, 30.44	0.003
Model 5					
Q1	163	3	Ref		
Q2	163	8	3.59	0.68, 18.96	0.13
Q3	163	10	4.18	0.79, 22.19	0.09
Q4	163	15	8.41	1.69, 41.81	0.009

Model 6					
Q1	163	3	Ref		
Q2	163	8	3.43	0.84, 13.94	0.09
Q3	163	10	5.09	1.28, 20.21	0.02
Q4	163	15	7.46	1.93, 28.82	0.004

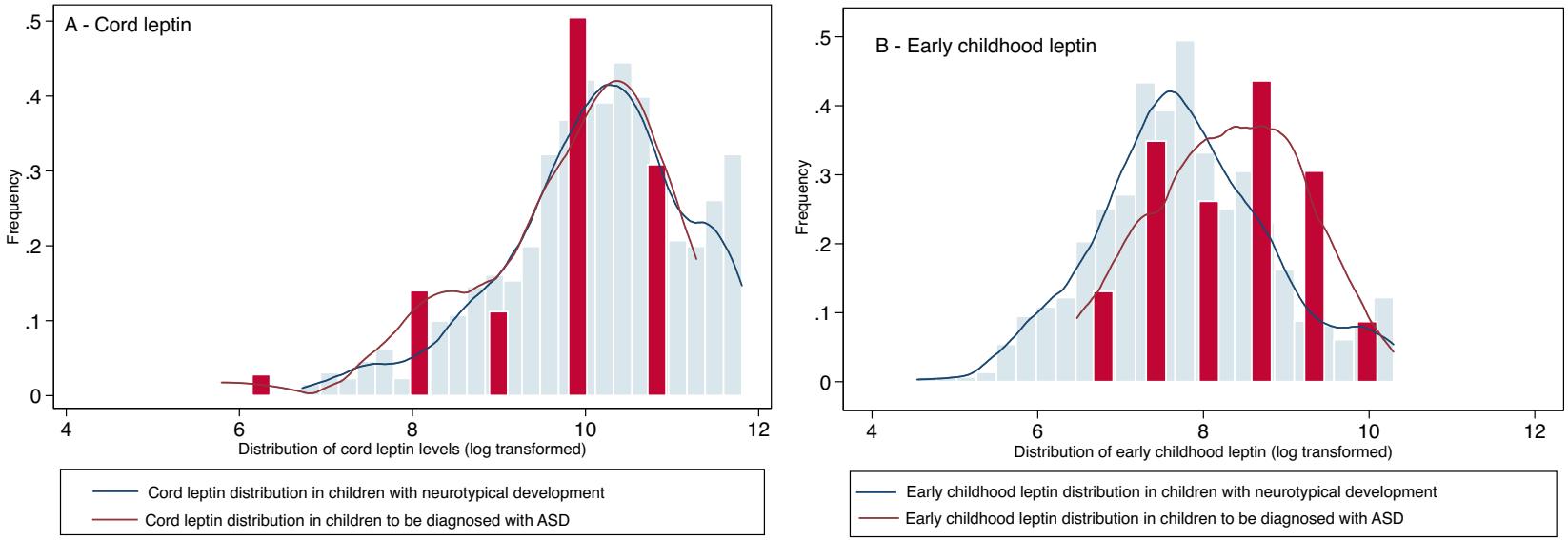
Model 1: Adjusted for maternal characteristics such as maternal age at delivery, parity, smoking, maternal BMI, maternal diabetes status, education, race, child's sex and follow-up time
Model 2: Adjusted for Model 1 + gestational age
Model 3: Adjusted for child's sex, race, age of leptin measurement, follow-up time and breastfeeding status
Model 4: Adjusted for Model 3 + gestational age
Model 5: Adjusted for Model 3 + cord leptin levels
Model 6: Adjusted for Model 3 + maternal age, maternal BMI, maternal diabetes status

Table 5-4 Mediation analysis – Leptin as a mediator in the relationship between weight gain during first year of life and ASD risk

	Total Effect, OR (95% CI)	Direct Effect, OR (95% CI)	Indirect effect, OR (95% CI)	Percentage mediated by early childhood leptin (%)
Unadjusted (Total N=476; ASD=32)				
On track	Ref			
Slow	1.92 (0.52, 7.11)	1.93 (0.52, 7.16)	0.99 (0.81, 1.22)	
Rapid weight gain	1.39 (0.38, 5.11)	1.17 (0.32, 4.32)	1.19 (0.93, 1.52)	
Extreme rapid weight gain	2.80 (1.08, 7.28)	2.22 (0.84, 5.87)	1.26 (0.95, 1.67)	
Model 1: Adjusted				
On track	Ref			
Slow	1.80 (0.44, 7.47)	1.81 (0.44, 7.49)	1.00 (0.72, 1.39)	
Rapid weight gain	1.06 (0.23, 4.85)	0.82 (0.18, 3.78)	1.29 (0.89, 1.87)	
Extreme rapid weight gain	1.80 (0.55, 5.90)	1.15 (0.34, 3.88)	1.56 (1.01, 2.42)	76.03

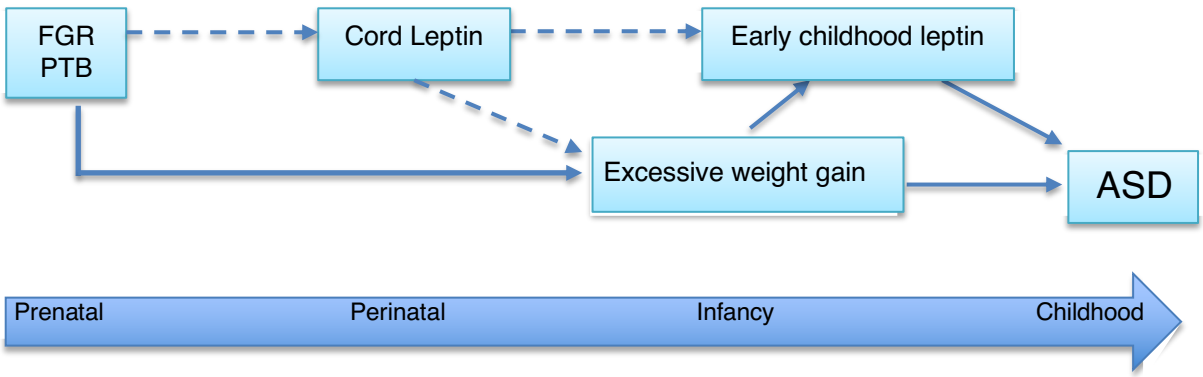
Model 1: Adjusted for child's sex, race, breast-feeding category, age of leptin measurement, follow-up time and gestational age
A causal inference framework was used to estimate ORs and 95% CI for total, direct and indirect effects. A logistic regression model was fit using categorical exposure (weight gain during infancy) and continuous mediator (leptin)

Figure 5-1 Distribution of cord and early childhood plasma leptin levels (log transformed) in children categorized by ASD status



Panel A: Distribution of cord leptin levels (log transformed) in neurotypical children and those to be diagnosed with ASD
Panel B: Distribution of early childhood leptin levels (log transformed) in neurotypical children and those to be diagnosed with ASD

Figure 5-2 Illustration of prenatal, perinatal and early childhood factors of ASD in a life course framework



Conceptual model characterizing the relation between *in utero* growth / preterm birth, cord and early childhood leptin, weight gain during infancy, and risk of ASD in childhood
FGR – Fetal Growth Rate
PTB – Preterm birth
Solid line depicts established associations and dotted line depicts potential association

Supplemental Table 5-1: Prenatal and Perinatal determinants of cord blood and early childhood leptin

	Mean cord leptin (pg/ml)	P value	Mean early childhood leptin (pg/ml)	P value
Maternal factors				
BMI				
<25	33.27	0.002	3.93	0.02
≥25 to <30	39.62		3.92	
≥30	44.64		5.40	
Diabetes				
No	35.77	<0.001	4.30	0.75
Yes	52.50		4.08	
Smoking				
No	38.87	0.02	4.27	0.80
Yes	30.33		4.12	
Race				
Black	40.32	0.05	4.69	0.05
White	27.71		2.27	
Hispanic	34.21		4.15	
Other	37.53		3.64	
Child factors				
IUGR[a]				
AGA	39.08	<0.001	3.97	0.14
SGA	20.52		3.86	
LGA	59.15		5.50	
Weight gain during infancy[b]				
On target			3.10	<0.001
Slow			2.93	

Rapid			4.20	
Extremely rapid			5.20	
Gestational Age				
<34 weeks	12.94	<0.001	3.51	0.50
≥34 to <37 weeks	28.83		4.10	
≥37 weeks	41.37		4.40	
Sex				
Girls	42.44	<0.001	4.83	0.004
Boys	31.62		3.56	
Breast feeding[b]				
Bottle only			4.43	0.94
Both bottle and breast feeding			4.24	
Only breast feeding			4.28	

[a]AGA: Appropriate for gestational age, SGA: Small for gestational age, LGA: Large for gestational age

[b]Cord blood leptin levels were not calculated for postnatal variables (breastfeeding and weight gain during first year of life)

Supplemental Table 5-2: Determinants of weight gain pattern during infancy

	On track	Slow growth	Rapid weight gain	Extreme rapid weight gain	p value
Gestational age (%)					<0.001
Term	177 (39.86)	66 (14.86)	87 (19.59)	114 (25.68)	
Late preterm	10 (12.35)	4 (4.94)	7 (8.64)	60 (74.07)	
Early preterm	0 (0.0)	0 (0.0)	1 (2.08)	47 (97.92)	
Birth weight for gestational age[a] (%)					<0.001
AGA	152 (33.12)	45 (9.80)	81 (17.65)	181 (39.43)	
SGA	12 (19.35)	3 (4.84)	13 (20.97)	34 (54.84)	
LGA	23 (44.23)	22 (42.31)	1 (1.92)	6 (11.54)	
Maternal BMI (%)					0.01
<25	92 (34.98)	26 (9.89)	37 (14.07)	108 (41.06)	
≥25 to <30	67 (37.43)	20 (11.17)	30 (16.76)	62 (34.64)	
≥30	28 (21.37)	24 (18.32)	28 (21.37)	51 (38.93)	
Maternal diabetes (%)					0.03
No GDM/DM	174 (34.05)	57 (11.15)	81 (15.85)	199 (38.94)	
GDM/DM	13 (20.97)	13 (20.97)	14 (22.58)	22 (35.48)	
Maternal obesity + Diabetes (%)					0.001
No Obesity & Diabetes	150 (36.95)	41 (10.10)	59 (14.53)	156 (38.42)	
Obesity or Diabetes	33 (23.40)	21 (14.89)	30 (21.28)	57 (40.43)	
Obesity & Diabetes	4 (15.38)	8 (30.77)	6 (23.08)	8 (30.77)	

[a]AGA: Appropriate for gestational age, SGA: Small for gestational age, LGA: Large for gestational age

Supplemental Table 5-3: Sensitivity Analyses on the association between birth weight for gestational age, rapid weight gain during first year of life and subsequent ASD risk in BBC using stringent controls[a]

			Unadjusted			Adjusted		
	Total n	ASD n	OR	95% CI	p value	OR	95% CI	p value
Model 1: Birth weight for gestational age[b]								
AGA	276	36	Ref			Ref		
SGA	39	5	0.98	0.36, 2.67	0.97	1.22	0.39, 3.75	0.79
LGA	35	6	1.38	0.54, 3.55	0.51	1.28	0.39, 4.20	0.69
Model 2: Birth weight for gestational age[b]								
AGA	276	36	Ref			Ref		
SGA	39	5	0.98	0.36, 2.67	0.97	1.29	0.41, 4.06	0.66
LGA	35	6	1.38	0.54, 3.55	0.51	1.47	0.44, 4.94	0.53
Model 3: Weight gain during infancy[c]								
On target	102	9	Ref			Ref		
Slow	44	5	1.32	0.42, 4.21	0.63	1.43	0.42, 4.87	0.56
Rapid weight gain	51	6	1.38	0.46, 4.11	0.57	1.73	0.51, 5.86	0.38
Extremely rapid weight gain	133	26	2.51	1.12, 5.63	0.03	3.21	1.36, 7.60	0.008
Model 4: Weight gain during infancy [c]								
On target	102	9	Ref			Ref		
Slow	44	5	1.32	0.42, 4.21	0.63	1.21	0.33, 4.41	0.77
Rapid weight gain	51	6	1.38	0.46, 4.11	0.57	1.93	0.55, 6.78	0.31
Extremely rapid weight gain	133	26	2.51	1.12, 5.63	0.03	3.51	1.44, 8.56	0.006
Model 5: Weight gain during infancy [c]								
On target	102	9	Ref			Ref		
Slow	44	5	1.32	0.42, 4.21	0.63	1.45	0.43, 4.94	0.55
Rapid weight gain	51	6	1.38	0.46, 4.11	0.57	1.60	0.47, 5.44	0.45
Extremely rapid weight gain	133	26	2.51	1.12, 5.63	0.03	2.08	0.81, 5.36	0.13
Model 6: Weight gain during infancy [c]								
On target	102	9	Ref			Ref		
Slow	44	5	1.32	0.42, 4.21	0.63	1.01	0.26, 3.96	0.97
Rapid weight gain	51	6	1.38	0.46, 4.11	0.57	1.79	0.49, 6.60	0.38
Extremely rapid weight gain	133	26	2.51	1.12, 5.63	0.03	3.59	1.42, 9.07	0.007

[a] Stringent control defined as exclusion of children with any of the following conditions – Conduct disorder (312.0 - 312.9), Emotional disturbances of childhood or adolescence including Oppositional Defiant Disorder (313.0 - 313.9), Congenital Anomalies (740 - 759.9)
[b] Birth weight for gestational age defined as appropriate for gestational age (AGA) (≥10^{th} – 90^{th} percentile); Small for gestational age (SGA) (<10^{th} percentile), and Large for gestational age (LGA) (>90^{th} percentile)
[c] Weight gain during infancy defined as the change in weight-for-age *z*-scores from birth until the target time-point and was categorized into the following groups: slow (weight gain *z*-score <-0.67), on track (−0.67 to 0.67), rapid (>0.67 to 1.28), and extremely rapid (>1.28)

Model 1: Adjusted for maternal age at delivery, parity, smoking, education, maternal BMI, maternal diabetes status, race, child's sex and follow-up time
Model 2: Adjusted for Model 1 + gestational age
Model 3: Adjusted for child's sex, race, follow-up time and breastfeeding status
Model 4: Adjusted for Model 3 + birth weight for gestational age pattern
Model 5: Adjusted for Model 3 + gestational age
Model 6: Adjusted for Model 3 + maternal age, maternal BMI, maternal diabetes status

Supplemental Table 5-4: Sensitivity Analysis on the association between birth weight for gestational age, rapid weight gain during first year of life and subsequent ASD risk in the BBC using cases that were diagnosed at least twice and by a specialist

			Unadjusted			Adjusted		
	Total n	ASD n	OR	95% CI	p value	OR	95% CI	p value
Birth weight for gestational age[a]								
Model 1								
AGA	476	29	Ref			Ref		
SGA	64	3	0.76	0.22, 2.56	0.66	0.58	0.15, 2.15	0.41
LGA	56	4	1.19	0.40, 3.51	0.76	0.90	0.26, 3.14	0.86
Model 2								
AGA	476	29	Ref			Ref		
SGA	64	3	0.76	0.22, 2.56	0.66	0.62	0.16, 2.36	0.48
LGA	56	4	1.19	0.40, 3.51	0.76	1.00	0.28, 3.56	1.00
Weight gain during infancy[b]								
Model 3								
On target	187	7	Ref			Ref		
Slow	69	3	1.17	0.29, 4.65	0.83	1.37	0.33, 5.68	0.66
Rapid weight gain	95	5	1.43	0.44, 4.63	0.55	1.40	0.39, 5.08	0.61
Extremely rapid weight gain	219	20	2.58	1.07, 6.26	0.04	3.03	1.21, 7.57	0.02
Model 4								
On target	187	7	Ref			Ref		
Slow	69	3	1.17	0.29, 4.65	0.83	1.44	0.34, 6.05	0.62
Rapid weight gain	95	5	1.43	0.44, 4.63	0.55	1.37	0.38, 5.01	0.63
Extremely rapid weight gain	219	20	2.58	1.07, 6.26	0.04	2.99	1.20, 7.50	0.02
Model 5								
On target	187	7	Ref			Ref		
Slow	69	3	1.17	0.29, 4.65	0.83	1.41	0.34, 5.89	0.64
Rapid weight gain	95	5	1.43	0.44, 4.63	0.55	1.30	0.36, 4.76	0.69
Extremely rapid weight gain	219	20	2.58	1.07, 6.26	0.04	1.68	0.60, 4.71	0.32
Model 6								
On target	187	7	Ref			Ref		
Slow	69	3	1.17	0.29, 4.65	0.83	1.16	0.27, 4.93	0.84
Rapid weight gain	95	5	1.43	0.44, 4.63	0.55	1.02	0.26, 3.95	0.98
Extremely rapid weight gain	219	20	2.58	1.07, 6.26	0.04	3.29	1.28, 8.49	0.01

[a] Birth weight for gestational age was defined as appropriate for gestational age (AGA) (≥10th – 90th percentile); Small for gestational age (SGA) (<10th percentile), and Large for gestational age (LGA) (>90th percentile)
[b] Weight gain during infancy defined as the change in weight-for-age *z*-scores from birth until the target time-point and was categorized into the following groups: slow (weight gain *z*-score <-0.67), on track (−0.67 to 0.67), rapid (>0.67 to 1.28), and extremely rapid (>1.28)

Model 1: Adjusted for maternal age at delivery, parity, smoking, education, maternal BMI, maternal diabetes status, race, child's sex and follow-up time
Model 2: Adjusted for Model 1 + gestational age
Model 3: Adjusted for child's sex, race, follow-up time and breastfeeding status
Model 4: Adjusted for Model 3 + birth weight for gestational age pattern
Model 5: Adjusted for Model 3 + gestational age
Model 6: Adjusted for Model 3 + maternal age, maternal BMI and maternal diabetes status

Supplemental Table 5-5: Association between cord blood, early childhood leptin and ASD (categorized as continuous and categorical variables)

			Unadjusted			Adjusted		
	Total n	ASD n	OR	95% CI	p value	OR	95% CI	p value
Cord leptin [a]								
Leptin, continuous	655	39	0.77	0.58, 1.03	0.08	0.90	0.66, 1.24	0.52
Tertiles								
Q1	216	14	Ref			Ref		
Q2	216	15	1.08	0.51, 2.29	0.85	1.55	0.67, 3.57	0.31
Q3	223	10	0.68	0.29, 1.56	0.36	0.88	0.35, 2.24	0.79
Quintiles								
Q1	131	10	Ref			Ref		
Q2	131	6	0.58	0.20, 1.65	0.31	0.82	0.26, 2.62	0.74
Q3	131	12	1.22	0.51, 2.93	0.66	2.14	0.79, 5.80	0.14
Q4	131	8	0.79	0.30, 2.06	0.63	1.02	0.35, 2.98	0.98
Q5	131	3	0.28	0.08, 1.06	0.06	0.45	0.10, 1.96	0.29
Early childhood leptin [b]								
Leptin, continuous	652	36	1.58	1.15, 2.17	0.005	1.80	1.25, 2.60	0.002
Tertiles								
Q1	215	7	Ref			Ref		
Q2	215	8	1.15	0.41, 3.22	0.79	1.22	0.41, 3.61	0.72
Q3	222	21	3.10	1.29, 7.46	0.01	3.66	1.43, 9.36	0.007
Quintiles								
Q1	130	3	Ref			Ref		
Q2	131	4	1.33	0.29, 6.08	0.71	1.45	0.31, 6.92	0.64
Q3	130	7	2.41	0.61, 9.53	0.21	2.85	0.67, 12.01	0.16
Q4	131	9	3.12	0.83, 11.81	0.09	3.62	0.89, 14.68	0.07
Q5	130	13	4.70	1.31, 16.92	0.02	6.62	1.69, 26.00	0.007

[a] Model 1: Adjusted for maternal characteristics such as age at delivery, parity, smoking, education, race, child's gender, maternal BMI, maternal diabetes, follow-up time

[b] Model 2: Adjusted for child's sex, race, follow-up time, breast feeding and age of measurement

Supplemental Table 5-6: Sensitivity Analysis on the association between cord, early childhood leptin and subsequent ASD risk in BBC using stringent controls [a]

			Unadjusted			Adjusted		
Cord Leptin								
Model 1	**Total n**	**ASD n**	OR	95% CI	p value	OR	95% CI	p value
Leptin (continuous)	372	39	0.77	0.57, 1.03	0.08	0.99	0.70, 1.39	0.95
Model 2								
Leptin (continuous)	372	39	0.77	0.57, 1.03	0.08	1.00	0.67, 1.50	0.99
Early childhood leptin								
Model 3								
Leptin (continuous)	384	36	1.58	1.15, 2.18	0.005	1.83	1.23, 2.72	0.003
Model 4								
Leptin (continuous)	384	36	1.58	1.15, 2.18	0.005	1.86	1.24, 2.79	0.003
Model 5								
Leptin (continuous)	384	36	1.58	1.15, 2.18	0.005	1.69	1.03, 2.76	0.04
Model 6								
Leptin (continuous)	384	36	1.58	1.15, 2.18	0.005	1.79	1.19, 2.70	0.005

[a] Stringent control defined as exclusion of children with any of the following conditions – ADHD (314.0 - 314.9), Conduct disorder (312.0 - 312.9), Emotional disturbances of childhood or adolescence including Oppositional Defiant Disorder (313.0 - 313.9), Developmental Delay (315.0 - 315.9), Intellectual Disability (317 – 319), Congenital Anomalies (740 - 759.9)

Model 1: Adjusted for maternal characteristics such as maternal age at delivery, parity, smoking, maternal BMI, maternal diabetes status, education, race, child's sex and follow-up time
Model 2: Adjusted for Model 1 + gestational age
Model 3: Adjusted for child's sex, race, age of leptin measurement, follow-up time and breastfeeding status
Model 4: Adjusted for Model 3 + gestational age
Model 5: Adjusted for Model 3 + cord leptin levels
Model 6: Adjusted for Model 3 + maternal age, maternal BMI and maternal diabetes status

Supplemental Table 5-7: Sensitivity Analysis on the association between cord, early childhood leptin and ASD risk in the BBC (in children that were diagnosed at least twice and by a specialist)

			Unadjusted			Adjusted		
Cord Leptin								
Model 1	Total n	ASD n	OR	95% CI	p value	OR	95% CI	p value
Leptin (continuous)	653	31	0.77	0.56, 1.06	0.11	0.87	0.62, 1.22	0.42
Model 2								
Leptin (continuous)	653	31	0.77	0.56, 1.06	0.11	0.96	0.64, 1.43	0.83
Early childhood leptin								
Model 3								
Leptin (continuous)	654	26	1.40	0.97, 2.02	0.08	1.49	0.99, 2.26	0.06
Model 4								
Leptin (continuous)	654	26	1.40	0.97, 2.02	0.08	1.50	0.98, 2.28	0.06
Model 5								
Leptin (continuous)	654	26	1.40	0.97, 2.02	0.08	1.38	0.85, 2.24	0.20
Model 6								
Leptin (continuous)	654	26	1.40	0.97, 2.02	0.08	1.41	0.93, 2.14	0.10

Model 1: Adjusted for maternal characteristics such as maternal age at delivery, parity, smoking, maternal BMI, maternal diabetes status, education, race, child's sex and follow-up time
Model 2: Adjusted for Model 1 + gestational age
Model 3: Adjusted for child's sex, race, age of leptin measurement, follow-up time and breastfeeding status
Model 4: Adjusted for Model 3 + gestational age
Model 5: Adjusted for Model 3 + cord leptin levels
Model 6: Adjusted for Model 3 + maternal age, maternal BMI, maternal diabetes status

Supplemental Table 5-8: Association between birth weight for gestational age, Weight gain during infancy and ASD risk in children in the BBC (using Cox proportional regression hazard model)

	Total n	ASD n	HR	95% CI	p value
Birth weight for gestational age[a]					
Unadjusted					
AGA	478	36	Ref		
SGA	64	5	0.99	0.39, 2.53	0.99
LGA	57	6	1.39	0.58, 3.30	0.46
Model 1					
AGA	478	36	Ref		
SGA	64	5	0.77	0.29, 2.05	0.60
LGA	57	6	1.15	0.45, 2.95	0.77
Model 2					
AGA	478	36	Ref		
SGA	64	5	0.84	0.31, 2.25	0.73
LGA	57	6	1.26	0.49, 3.26	0.63
Weight gain during infancy[b]					
Unadjusted					
On target	187	9	Ref		
Slow	70	5	1.48	0.50, 4.42	0.48
Rapid weight gain	95	6	1.30	0.46, 3.66	0.62
Extremely rapid weight gain	221	26	2.35	1.10, 5.04	0.03
Model 3					
On target	187	9	Ref		
Slow	70	5	1.49	0.49, 4.50	0.48
Rapid weight gain	95	6	1.32	0.44, 3.98	0.62
Extremely rapid weight gain	221	26	2.63	1.21, 5.71	0.01
Model 4					
On target	187	9	Ref		
Slow	70	5	1.35	0.43, 4.28	0.61
Rapid weight gain	95	6	1.41	0.46, 4.31	0.55
Extremely rapid weight gain	221	26	2.80	1.27, 6.16	0.01
Model 5					
On target	187	9	Ref		
Slow	70	5	1.54	0.51, 4.64	0.45
Rapid weight gain	95	6	1.28	0.43, 3.87	0.66
Extremely rapid weight gain	221	26	1.97	0.84, 4.60	0.12
Model 6					
On target	187	9	Ref		

Slow	70	5	1.27	0.42, 3.85	0.67
Rapid weight gain	95	6	1.04	0.33, 3.23	0.95
Extremely rapid weight gain	221	26	2.67	1.22, 5.83	0.014

[a] Birth weight for gestational age was defined as appropriate for gestational age (AGA) (≥10th – 90th percentile); Small for gestational age (SGA) (<10th percentile), and Large for gestational age (LGA) (>90th percentile)

[b] Weight gain during infancy defined as the change in weight-for-age *z*-scores from birth until the target time-point and was categorized into the following groups: slow (weight gain *z*-score <-0.67), on track (−0.67 to 0.67), rapid (>0.67 to 1.28), and extremely rapid (>1.28)

Model 1: Adjusted for maternal age at delivery, parity, smoking, education, maternal BMI, maternal diabetes status, race, child's sex and follow-up time
Model 2: Adjusted for Model 1 + gestational age
Model 3: Adjusted for child's sex, race, follow-up time and breastfeeding status
Model 4: Adjusted for Model 3 + birth weight for gestational age
Model 5: Adjusted for Model 3 + gestational age
Model 6: Adjusted for Model 3 + maternal age, maternal diabetes status, maternal BMI

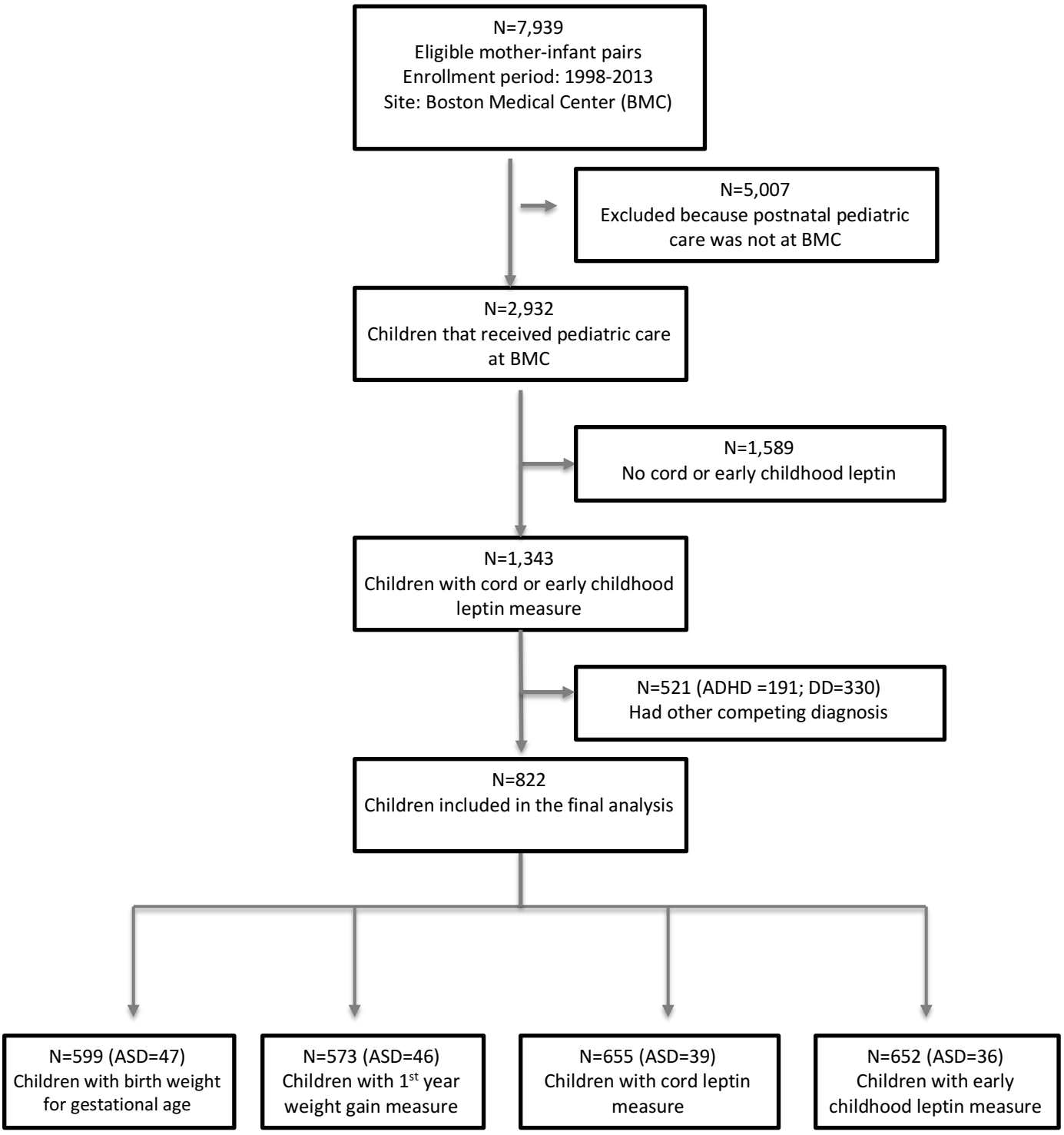
Supplemental Table 5-9: Association between cord, early childhood plasma leptin levels and ASD risk in children in the BBC (using Cox proportional regression hazard model)

	Total n	ASD n	OR	95% CI	p value
Cord leptin					
Unadjusted					
Q1	164	10	Ref		
Q2	163	11	1.12	0.48, 2.65	0.79
Q3	164	11	1.09	0.46, 2.57	0.84
Q4	164	7	0.69	0.26, 1.81	0.45
Model 1					
Q1	164	10	Ref		
Q2	163	11	1.50	0.61, 3.66	0.38
Q3	164	11	1.61	0.65, 3.97	0.30
Q4	164	7	1.09	0.38, 3.09	0.88
Model 2					
Q1	164	10	Ref		
Q2	163	11	2.11	0.79, 5.60	0.14
Q3	164	11	2.53	0.90, 7.14	0.08
Q4	164	7	1.62	0.53, 4.99	0.40
Early childhood leptin					
Unadjusted					
Q1	163	3	Ref		
Q2	163	8	2.77	0.73, 10.44	0.13
Q3	163	10	3.50	0.96, 12.72	0.06
Q4	163	15	4.56	1.32, 15.81	0.02
Model 3					
Q1	163	3	Ref		
Q2	163	8	3.32	0.87, 12.66	0.08
Q3	163	10	4.10	1.10, 15.26	0.04
Q4	163	15	6.19	1.74, 22.00	0.005
Model 4					
Q1	163	3	Ref		
Q2	163	8	3.17	0.83, 12.11	0.09
Q3	163	10	4.35	1.16, 16.32	0.03
Q4	163	15	6.26	1.76, 22.24	0.005
Model 5					
Q1	163	3	Ref		
Q2	163	8	3.84	0.76, 19.36	0.10
Q3	163	10	4.35	0.85, 22.24	0.08
Q4	163	15	6.67	1.41, 31.45	0.02

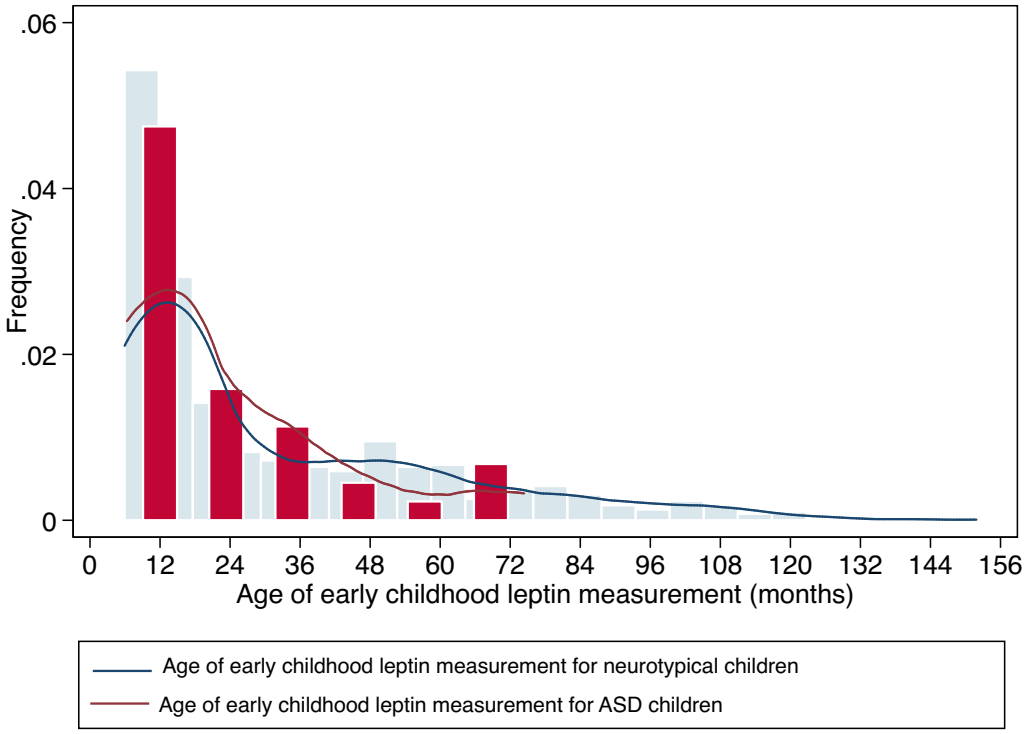
Model 6					
Q1	163	3	Ref		
Q2	163	8	3.42	0.90, 13.02	0.07
Q3	163	10	4.48	1.21, 16.68	0.03
Q4	163	15	5.61	1.58, 19.98	0.008

Model 1: Adjusted for maternal characteristics such as maternal age at delivery, parity, smoking, maternal BMI, maternal diabetes status, education, race, child's sex and follow-up time
Model 2: Adjusted for Model 1 + gestational age
Model 3: Adjusted for child's sex, race, age of leptin measurement, follow-up time and breastfeeding status
Model 4: Adjusted for Model 3 + gestational age
Model 5: Adjusted for Model 3 + cord leptin levels
Model 6: Adjusted for Model 3 + maternal age, maternal BMI, maternal diabetes status

Supplemental Figure 5-1: Flowchart of initial enrollment and postnatal follow-up of the BBC and the Sample Included in the analysis



Supplemental Figure 5-2: Distribution of age of early childhood leptin measurement



CHAPTER 6

Manuscript 3

Cord and Early Childhood Plasma Adiponectin Levels and Autism Risk:

A Prospective Birth Cohort Study

This paper is currently under peer-review by the *Journal of Autism and Developmental Disorders* with contributions from co-authors M. Daniele Fallin, Xiumei Hong, Guoying Wang, Yuelong Ji, Elizabeth A. Stuart, David Paige, Xiaobin Wang

6.1 Abstract

Emerging research suggests that adiponectin, a cytokine produced by adipose tissue, may be implicated in ASD. In this prospective birth cohort study, we assessed the association between cord, early childhood plasma adiponectin and the risk of developing ASD. ASD was defined based on ICD codes of physician diagnosis in the Electronic Medical Record. Cord adiponectin levels were inversely associated with ASD risk (aOR: 0.50; 95% CI: 0.33, 0.77), independent of preterm birth, early childhood adiponectin and other known ASD risk factors. Early childhood adiponectin, assessed prior to ASD diagnosis was associated with lower risk of ASD, which attenuated after adjusting for cord adiponectin, indicating the relative importance of cord adiponectin in ASD risk. Further research is warranted to confirm our findings and elucidate biological mechanisms.

Key words: Autism, adiponectin, preterm birth, cytokines

6.2 Introduction

Autism Spectrum Disorder (ASD) is a neurodevelopmental condition characterized by impairments in social interaction and communication, and by the presence of restrictive, repetitive behaviors and interests (1, 2). Recent epidemiological studies suggest that approximately 1% of children are diagnosed with ASD (3, 4). While genetic, environmental, and prenatal/perinatal factors have been implicated in ASD (5, 6), the etiology of the disorder remains largely unknown and no convincing biomarkers of ASD have been identified (1, 2, 7). Disturbances in immunoinflammatory factors and adipocytokines have been observed in subjects with ASD (7, 8). Aberrant immune activity during vulnerable periods of neurodevelopment could potentially play a role in neural dysfunction associated with ASD (9). Emerging evidence suggests that immune dysfunction and neuroinflammation may be a common thread across the individual ASD risk factors (10).

Beyond its energy storing capabilities, adipose tissue has emerged as an endocrine organ that orchestrates inflammatory response through the production of adipocytokines (11-13). The family of adipocytokines includes a variety of highly active molecules including interleukin (IL)-6, tumor necrosis factor (TNF)-α, leptin, adiponectin, resistin and vistafin, all of which are known to play a critical role in the regulation of inflammatory responses (11, 12). Adiponectin is the most abundant adipocytokine, mainly produced by brown and white adipose tissues (11).

The role of adiponectin in ASD deserves attention for at least two important reasons. First, as an anti-inflammatory cytokine, adiponectin functions as a mediator of inflammatory response and has a protective role against metabolic disturbances (12, 14, 15). Emerging

evidence shows that adiponectin is implicated in neurological conditions (16-19). A few studies have reported altered adiponectin levels in children with ASD (15, 20, 21), but this has not been studied prospectively. Limited sample size, lack of adjustment of numerous confounders, and cross-sectional study designs have precluded elucidation of the role of adiponectin in the development of ASD.

Second, preterm birth is an established risk factor for ASD (22-27). Lower serum adiponectin levels are noted in mothers who had preterm labor and delivery (12) as well as in children born preterm when compared to their term counterparts (28-35). While the role of adiponectin in prematurity is well characterized (28-35), existing research has not jointly looked at adiponectin and gestational age at birth in relation to ASD risk.

To fill these gaps, we conducted a study to longitudinally assess the association between plasma adiponectin, measured in cord blood at birth and early childhood venous blood, and subsequent ASD risk. In addition, we set out to understand whether preterm birth and cord adiponectin had joint effects on ASD risk. We sought to clarify these questions by analyzing longitudinal data from the Boston Birth Cohort (BBC), a predominantly urban low-income minority birth cohort, enriched with preterm births.

6.3 Methods

6.3.1 Participants and data collection procedure

This study included 847 mother-child pairs from the BBC, of which 792 children were considered neurotypical and 55 received an ASD diagnosis. Supplemental Figure 6-1 outlines the enrollment, postnatal follow-up, inclusions and exclusions. Between 1998 and 2009, mothers who delivered a singleton live birth at the Boston Medical Center (BMC), were invited

to participate in this study. After obtaining informed consent, mothers were interviewed 24-72 hours after delivery using a standardized postpartum questionnaire. Exclusion criteria for the initial enrollment were multiple-gestation pregnancies, chromosomal abnormalities, major birth defects and preterm deliveries as a result of maternal trauma. Children that continued to receive pediatric care at the BMC were included in this study, and they were followed-up until 2015 (Supplemental Figure 6-1). The study was approved by the Institutional Review Boards of the Johns Hopkins Bloomberg School of Public Health and the Boston University Medical Center.

6.3.2 Exposure measures

Plasma adiponectin in the children was measured at two time points: (1) Umbilical cord blood sample, collected at delivery, and (2) early childhood venous blood, collected during a pediatric visit (median time of measurement: 19.03 months). Plasma samples were stored in a freezer at −80°C. Adiponectin was measured using an immunoassay (ELISA) and had an inter-assay variation of <5.8% (36). The assays were run according to the manufacturer's recommendation. Children that had adiponectin assessed after ASD diagnosis were excluded from the analysis. Adiponectin levels that were greater than 3SD above the mean were re-assigned the value of 3 SD.

Gestational age at birth was characterized based on the first day of the last menstrual period data and early ultrasound data (37, 38). Children with gestational age ≥ 37 completed weeks of gestation were categorized as full-term and those <37 weeks were grouped into late- (≥ 34 to <37 weeks) and early-preterm (<34 weeks).

6.3.3 Outcome measure

Electronic Medical Records (EMR) data were used to identify children with ASD. Children were classified as an ASD case if they were ever diagnosed with autism (ICD-9 code 299.00), Asperger syndrome (299.80) and/or pervasive developmental disorder not otherwise specified (299.90). Children that were never diagnosed with ASD, ADHD (314.0 - 314.9), intellectual disabilities (317 – 319) or other developmental disabilities (315.0 - 315.9) were classified as 'neurotypical.' When children with ASD also had ADHD, intellectual disabilities, or other developmental disabilities, they were still categorized as ASD. Two separate sensitivity analyses were performed using the following criteria: 1) a stringent ASD definition that included only those that were diagnosed with ASD on two separate occasions, and had at least one visit with a specialist such as a developmental behavioral pediatrician, pediatric neurologist or child psychologist; and 2) a stringent definition for neurotypical children which excluded those with competing diagnoses such as ADHD (314.0 - 314.9), intellectual disabilities (317 – 319), other developmental disabilities (315.0 - 315.9), Conduct Disorder (312.0 - 312.9), emotional disturbances of childhood or adolescence including Oppositional Defiant Disorder (313.0 - 313.9) and Congenital Anomalies (740 - 759.9).

6.3.4 Covariates

Based on the existing literature and our earlier work in the BBC, we selected the covariates for adjustment *a priori* (36, 38-40), including maternal pre-pregnancy BMI, maternal diabetes status, maternal race/ethnicity, maternal age at the time of delivery, smoking during pregnancy (ever smoked 3 months before pregnancy/during pregnancy vs. not smoked during preconception/ pregnancy), parity (not including the index pregnancy), maternal education (high school or less vs. some college or more), child's sex (female vs. male), year of baby's birth

(1998-2006 vs. 2007-2013), mode of feeding (formula only, both formula and breast feeding and breastfeeding only), age at which early childhood blood was drawn and follow up time for each participant. Maternal pre-pregnancy weight and height were collected using a standardized questionnaire 2-3 days after delivery, which was used to calculate maternal BMI, defined as weight in kilograms divided by height in meters squared. Mother's diabetes status was categorized into the following groups: 1) normal (without a pregestational or gestational diabetes diagnosis); 2) gestational diabetes (ever diagnosed with diabetes mellitus complicating pregnancy); and 3) pregestational diabetes (ever diagnosed with diabetes). Race/ethnicity was categorized into black, white, Hispanic and Other. Plasma insulin and leptin, in cord blood and in venous blood during childhood, were measured using a sandwich immunoassay and the interassay coefficient of variation was 4.0% and 4.5%, respectively (41). Adiponectin/leptin ratio was calculated and was log-transformed. Based on the WHO reference values, weight-for-age *z*-score was used to calculate first year weight gain and was categorized into slow (weight gain *z*-score <-0.67), on track (−0.67 to 0.67), rapid (>0.67 to 1.28), and extremely rapid (>1.28) (41).

6.3.4 Statistical Analyses

Distributional assumptions such as normality of cord and plasma adiponectin levels were assessed using the Shapiro-Wilk test. Since adiponectin distributions were skewed, they were log-transformed for both cord and early childhood adiponectin levels. Preliminary data analysis was conducted to compare neurotypical and ASD children using a chi-squared test for categorical variables and ANOVA for continuous variables. Logistic regression was used to assess the relationship between log-transformed cord and early childhood plasma adiponectin levels (independent variables) and ASD (dependent variable), with adjustment for potential

confounders listed above. The results are presented as odds ratio. Baron and Kenny mediation analysis was used to explore the relationship between preterm birth, cord adiponectin and ASD risk. All statistical analyses were performed using STATA version 13.0 (StataCorp, College Station, TX). Differences were considered statistically significant if the p value was <0.05.

6.4 Results

The demographic and clinical characteristics of mothers and children in this study are presented in the Supplemental Table 6-1, and have also been documented in earlier studies from this cohort (36, 38-40, 42). We found that risk factors such as advanced maternal age, maternal diabetes, male sex and preterm birth were more common among children with ASD. In addition, ASD children were more likely to have lower birth weight and lower cord adiponectin levels (Supplemental Table 6-1). Mean cord adiponectin levels were significantly different across groups defined by gestational age at birth, with early preterm children (8.29 µg/mL) having the lowest levels, followed by late preterm (11.51 µg/mL) and term infants (16.90 µg/mL) (p value <0.001). Consistent with other studies (14, 32, 43-46), we observed that cord adiponectin was associated with the duration of gestation (Supplemental Table 6-2). Figure 6-1 shows the distribution of cord adiponectin by preterm status. As observed in panel A, the cord adiponectin distribution is shifted to the left for early preterm (<34 weeks) and late preterm (≥ 34 to <37 weeks) babies, when compared to term infants. However, there were no differences in the distribution of early childhood adiponectin levels based on gestational age at birth (Figure 6-2, panel B). Cord adiponectin levels were also correlated with birth weight. Children with lower cord adiponectin levels were likely to have rapid or extremely rapid weight gain (Supplemental Table 6-3), but the association attenuated after adjusting for gestational

age at birth (data not shown). Correlation between cord and early childhood adiponectin was weak (r=0.18), although children whose cord adiponectin were in low, medium or high tertiles likely continued to stay in their respective categories at early childhood (Supplemental Figure 6-2).

6.4.1 Adiponectin and ASD risk

A total of 674 children, including 634 neurotypical and 40 ASD children, were part of this analysis. Figure 6-2 shows the distribution of cord and early childhood adiponectin levels by ASD status. The mean cord adiponectin level is reduced among children later diagnosed with ASD (Supplemental Table 6-1) and this is evident by the distinct lower cord adiponectin distribution observed in Figure 1, panel A. On the other hand, there was no difference in the mean early childhood adiponectin levels between neurotypical and ASD children (Figure 6-1, panel B).

Table 6-1 presents the association between cord adiponectin and subsequent ASD risk. Higher cord adiponectin levels were associated with a lower risk of ASD (OR: 0.51; 95% CI: 0.35, 0.75). When cord adiponectin levels were categorized into quartiles, an inverse dose-response relationship was observed. Compared to children with the lowest cord adiponectin levels (quartile 1), the highest cord adiponectin ($OR_{quartile\ 4}$: 0.23, 95% CI: 0.08, 0.62) was associated with reduced risk of ASD in unadjusted analyses. This association persisted after adjustment for potential confounders (including child's sex, race, maternal education, maternal age, parity, smoking status, pre-pregnancy BMI, maternal diabetes status and follow-up time), ($aOR_{quartile\ 4}$: 0.14, 95% CI: 0.04, 0.46; model 1). Sequentially adjusting for additional covariates including gestational age (model 2), cord insulin (model 3), cord leptin (model 4) and weight gain during

first year of life (model 5) did not alter the association between cord adiponectin and ASD (Table 6-1). This finding was also robust to how adiponectin was categorized (tertiles, quintiles, extreme levels) (Supplemental Table 6-4). Finally, further adjusting for early childhood adiponectin did not alter the association.

A total of 638 children had data on early childhood adiponectin, of which 36 were subsequently diagnosed with ASD. As seen in Supplemental Figure 6-3, there was no difference in the timing of adiponectin measurement between neurotypical children and those that had ASD. In an unadjusted model, early childhood adiponectin was not associated with ASD risk (OR: 0.71; 95% CI: 0.45, 1.13) (Table 6-2). After adjusting for potential confounders (including maternal age, diabetes status, pre-pregnancy BMI, sex, race, preterm birth, age of measurement, follow-up time and breastfeeding status; model 1), higher early childhood adiponectin levels were inversely associated with the risk of ASD (aOR: 0.54; 95% CI: 0.33, 0.90). Similar to cord adiponectin, when early childhood adiponectin was categorized as quartiles, those with the highest levels (quartile 4) were associated with the lowest ASD risk ($aOR_{quartile\ 4}$: 0.29; 95% CI: 0.09, 0.94). The association persisted when adjusting for early childhood insulin (model 3), early childhood leptin (model 4) and weight gain during first year of life (model 5) and approached significance when adjusted for gestational age at birth (model 2). However, after adjusting for cord adiponectin levels (model 6), the association between early childhood adiponectin and ASD was no longer significant. We repeated the analysis by categorizing early childhood adiponectin as tertiles, quintiles and extreme values, and observed similar findings (Supplemental Table 6-5).

Sensitivity analyses using stringent ASD case and neurotypical definitions yielded consistent and stronger associations for both cord and early childhood adiponectin (Supplemental Tables 6-9). Repeating the analysis using ASD subjects that were diagnosed >2 years did not alter the findings.

Since leptin is linked to ASD in several studies (20, 21, 47-49), including ours, and adiponectin-leptin ratio is considered as a marker of metabolic syndrome (50), we assessed the association between log-transformed adiponectin-leptin ratio and subsequent ASD risk. We conducted this analysis for both cord and early childhood ratios (Supplemental tables 6-10 and 6-11). While the associations were statistically significant, the ratio did not predict ASD risk beyond the independent effects of leptin and/or adiponectin.

Next, the joint effects of preterm birth and adiponectin levels on the risk of ASD were assessed. Children that had term birth (≥37 completed weeks of gestation) and high cord adiponectin levels (defined as ≥50th percentile) were the reference group (Table 6-3). After adjusting for confounders, the risk of ASD was not different between preterm (<37 completed weeks of gestation) and term children, as long as they had high cord adiponectin. ASD risk was elevated among term children with low adiponectin (defined as < 50th percentile) in both unadjusted and adjusted models (aOR: 3.06; 95% CI: 1.27, 7.40). Among preterm children with low adiponectin, the ASD risk was significantly higher than the reference group in the unadjusted model and approached statistical significance, after adjusting for confounders (aOR: 2.62; 95% CI: 0.99, 6.99).

6.4.2 Mediation analysis: An exploration

Since the temporal ordering of preterm birth and altered adiponectin levels is unclear (12), mediation analysis was explored in two ways: 1) Cord adiponectin as a mediator in the relationship between preterm birth (independent variable) and ASD risk (dependent variable); and 2) Preterm birth as a mediator in the relationship between cord adiponectin (independent variable) and ASD risk (dependent variable). As seen in Supplemental Table 6-10, early preterm (independent variable) was associated with ASD risk (dependent variable) (step 1), both early and late preterm birth was associated with adiponectin (mediator) (step 2), and cord adiponectin was associated with ASD risk (step 3). In the last regression model (step 4), the association attenuated suggesting that cord adiponectin mediated the relationship between early preterm birth and ASD risk (Supplemental Table 6-12). In the second mediation analysis, preterm birth was considered as mediator in the association between cord adiponectin levels and ASD. Here, cord adiponectin was associated with ASD risk (step 1), preterm birth was associated with cord adiponectin (step 2) and early preterm birth was associated with ASD risk (step 3). In the regression model, adjusting for preterm birth did not alter the association between cord adiponectin and ASD risk, suggesting that there was no mediation (Supplemental Table 6-13). The role of early childhood adiponectin mediating the association between preterm birth and ASD risk is presented in Supplemental Table 6-14.

6.5 Discussion

To our knowledge, this is the first study to prospectively assess adiponectin levels in cord blood (a proxy of fetal adiponectin) and in early childhood venous blood in relation to ASD risk. We find cord blood adiponectin levels to be inversely associated with the risk of ASD. In

comparison, the association between early childhood adiponectin and ASD was less robust and was further weakened after adjustment of cord adiponectin. These associations remained consistent after additional analyses, including sequential adjustment for potential confounders and other metabolic biomarkers (such as leptin, insulin) and after more stringent ASD and neurotypical classifications of outcome.

Our findings are in line with the observations of Fujita-Shimizu et al. who noted that adiponectin levels are lower in subjects with ASD (15). Specifically, they reported a negative correlation between adiponectin and social development in children, characterized by the Autism Diagnostic Interview Revised (15). Consistent with these findings, Rodrigues et al., noted that adiponectin levels were inversely correlated with the severity of autism symptoms (21). Lisik et al. showed that adiponectin levels were lower in those with Fragile X Syndrome (16). These findings suggest that adiponectin may have a larger role to play in neurodevelopmental processes, rather than merely regulate energy expenditure or serve as a biomarker for the onset of metabolic syndrome (51, 52).

Adiponectin is detected in cord serum as early as 24 weeks of gestation, after which the concentration rises 20-fold until term (30). This increase during third trimester mirrors the increase in adiposity (14) and is in stark contrast with children and adults in whom adiponectin concentration is inversely associated with body fat percentage (30, 35). Cord adiponectin does not correlate with maternal adiponectin suggesting the fetal origins of cord adiponectin (14, 28, 53-55). In the fetus, adiponectin is secreted by muscle and vascular cells, in addition to adipocytes (56).

While cord adiponectin is associated with ASD, it is intriguing that early childhood adiponectin demonstrates a less robust association. Adiponectin expression and its relationship to growth parameters and fat distribution seem to evolve temporally, from birth to childhood (51, 57, 58). At birth, adiponectin is positively correlated with growth parameters and fat mass (51, 58-61). Subsequent to this initial time window, there is possibly a shift in this relationship noting an inverse association between adiponectin and fat mass (43, 51, 58, 62). Taken together, these findings suggest that the role of adiponectin *in utero* may be fundamentally different than early childhood adiponectin (43, 57). The discrepancy between adiponectin at different time points has been hypothesized to the differential origination of adiponectin, variation in fat storage, secretion and modulation of adiponectin (43, 56). While the evidence discussed here is in the context of obesity, adiponectin's effect may not be different for neurodevelopmental outcomes such as ASD.

Adiponectin has been previously shown to be altered in preterm babies, however, for the first time we jointly assessed adiponectin and preterm birth in the context of ASD and showed that lower adiponectin levels could increase ASD risk, irrespective of the gestational age. As a next step, we conducted a preliminary mediation analysis. Given the uncertainty in the biological temporal relationship between preterm birth and cord adiponectin (meaning whether preterm birth alters adiponectin levels, or changes in adiponectin triggers preterm birth) (12), our analysis assessed both as mediators. In this exploratory analysis, cord adiponectin mediated the relationship between preterm birth and ASD; whereas, preterm birth did not seem to mediate the association between cord adiponectin and ASD. These findings are preliminary and suggestive of a potential mediating role of adiponectin in relationship between

preterm birth and ASD. More research in animal models and human studies is warranted to further clarify this finding.

6.5.1 Biological plausibility

Several lines of evidence support the biological plausibility of adiponectin's role in ASD. Adiponectin suppresses the macrophage production of pro-inflammatory cytokines such as TNF- α, IL-6 (53), Interferon (IFN)-γ (12), which are noted to be elevated in children with ASD (4, 63, 64). Consistent findings suggest that IL-6 was significantly increased in the anterior cingulated gyrus, frontal cortices and cerebellum(64) and TNF was increased almost 50 times in the cerebrospinal fluid (26). Similarly, IFN-γ is elevated in the brain of ASD subjects, when compared to controls (64). Adiponectin signaling inhibits NF-κB, an essential transcription factor that mediates and regulates inflammatory and stress-related protein expression (13), which is aberrantly expressed in subjects with ASD (65).

Beyond its role in influencing other cytokines, emerging evidence suggests that adiponectin may be implicated in brain functions (66). Adiponectin enters the brain from circulation and directly targets neurons (66-70). Its receptors are widely expressed in the dentate gyrus of hippocampus (68), hypothalamus, cortex and pituitary glands (53, 66). Given this broad distribution of adiponectin receptors in different brain regions, adiponectin's role may be broader than the regulation of metabolic homeostasis and could plan an important role in the 'adipocyte-brain cross-talk' (66). Adiponectin is involved in neurogenesis, dendritic spine remodeling, hippocampal neural stem cell proliferation and dentate gyrus neuronal excitability (67-70). It also promotes adaptive neuroplasticity and may possess cerebra-protective role, likely mediated through the eNOS signal pathway (53, 66, 71). Abnormal development of

dentate gyrus of hippocampus, an important center for learning and memory, is likely implicated in the pathophysiology of ASD (3). Adiponectin may also possess higher brain functions. For example, diminished adiponectin levels are associated with clinically significant affective episodes and subjects with major depressive symptoms are known to have lower adiponectin levels (71). Adiponectin knockout mice have exhibited depressive-like behavior (67). Further, hypoadiponectinemia is thought to increase sympathetic nervous system activity – which is observed in depression (72).

6.5.2 Limitations and strengths

There are limitations to highlight. First, only total adiponectin and not the distinct forms such as low-molecular weight trimmers, medium-molecular-weight hexamers and high-molecular-weight oligomers (HMW) was examined. It is possible that these individual components could have different roles and should be further explored in the context of ASD (12). However, research in pediatric populations has found no difference in the role of total vs. HMW adiponectin (57). Second, ASD assessment relied on EMR data, which can introduce outcome misclassification in unpredictable ways. However, the results of our sensitivity analysis showed consistent associations when using more stringent outcome classification for both cases and neurotypical children. Third, because of the relatively small sample of ASD children in the cohort, our estimates may be subject to random variation. Fourth, although well-known ASD risk factors were adjusted for, there is a possibility of residual confounding. Finally, this study consisted primarily of urban, low-income minority population with high risk for preterm births. Thus, the findings may not be generalizable to other U.S. populations with different characteristics, although, few ASD studies have focused on this important group.

Despite these limitations, this study has a number of strengths. This study is based on a well-designed prospective birth cohort in an urban under-represented minority population. This is one of the first longitudinal studies to measure adiponectin at two-time points (birth and early childhood) prior to ASD diagnosis. Among studies that have looked at adipocytokines in the context of ASD, most of them were cross-sectional, and very few have used cord and/or newborn samples (73). Additionally, using a preterm enriched cohort, this study was uniquely poised to explore a novel question on the joint effect and mediating role of adiponectin in explaining the relationship between preterm birth and ASD risk and highlighted research gaps that can be explored by future studies.

6.6 Conclusions

In summary, our study found an inverse association between cord adiponectin levels and subsequent ASD risk in childhood. The effects of lower adiponectin on increased ASD risk were demonstrated independent of gestational age, early childhood adiponectin, and other important covariables. We would like to emphasize that our findings be regarded as hypothesis generating. Further research is required to elucidate whether cord and/or early childhood adiponectin can be used as a biomarker for identifying children that are at high risk for ASD and serve as potential molecular target for developing novel interventions.

6.7 References

1. Lyall K, Croen L, Daniels J, Fallin MD, Ladd-Acosta C, Lee BK, et al. The Changing Epidemiology of Autism Spectrum Disorders. Annu Rev Public Health. 2017;38:81-102.
2. Akintunde ME, Rose M, Krakowiak P, Heuer L, Ashwood P, Hansen R, et al. Increased production of IL-17 in children with autism spectrum disorders and co-morbid asthma. J Neuroimmunol. 2015;286:33-41.
3. Ito H, Morishita R, Nagata KI. Autism spectrum disorder-associated genes and the development of dentate granule cells. Med Mol Morphol. 2017;50(3):123-9.
4. Ashwood P, Krakowiak P, Hertz-Picciotto I, Hansen R, Pessah I, Van de Water J. Elevated plasma cytokines in autism spectrum disorders provide evidence of immune dysfunction and are associated with impaired behavioral outcome. Brain Behav Immun. 2011;25(1):40-5.
5. Fiorentino M, Sapone A, Senger S, Camhi SS, Kadzielski SM, Buie TM, et al. Blood-brain barrier and intestinal epithelial barrier alterations in autism spectrum disorders. Mol Autism. 2016;7:49.
6. Wozniak RH, Leezenbaum NB, Northrup JB, West KL, Iverson JM. The development of autism spectrum disorders: variability and causal complexity. Wiley Interdiscip Rev Cogn Sci. 2017;8(1-2).
7. Molloy CA, Morrow AL, Meinzen-Derr J, Schleifer K, Dienger K, Manning-Courtney P, et al. Elevated cytokine levels in children with autism spectrum disorder. J Neuroimmunol. 2006;172(1-2):198-205.
8. Ghaffari MA, Mousavinejad E, Riahi F, Mousavinejad M, Afsharmanesh MR. Increased Serum Levels of Tumor Necrosis Factor-Alpha, Resistin, and Visfatin in the Children with Autism Spectrum Disorders: A Case-Control Study. Neurol Res Int. 2016;2016:9060751.
9. Ashwood P, Wills S, Van de Water J. The immune response in autism: a new frontier for autism research. J Leukoc Biol. 2006;80(1):1-15.
10. Erdei C, Dammann O. The Perfect Storm: Preterm Birth, Neurodevelopmental Mechanisms, and Autism Causation. Perspect Biol Med. 2014;57(4):470-81.
11. Hansen-Pupp I, Hellgren G, Hard AL, Smith L, Hellstrom A, Lofqvist C. Early Surge in Circulatory Adiponectin Is Associated With Improved Growth at Near Term in Very Preterm Infants. J Clin Endocrinol Metab. 2015;100(6):2380-7.
12. Mazaki-Tovi S, Romero R, Vaisbuch E, Erez O, Mittal P, Chaiworapongsa T, et al. Dysregulation of maternal serum adiponectin in preterm labor. J Matern Fetal Neonatal Med. 2009;22(10):887-904.
13. Villarreal-Molina MT, Antuna-Puente B. Adiponectin: anti-inflammatory and cardioprotective effects. Biochimie. 2012;94(10):2143-9.
14. Lenz AM, Diamond F. The Importance of the Adiponectin and Leptin Relationship in In Utero and Infant Growth. V. P, editor. New York, NY: Springer; 2012.
15. Fujita-Shimizu A, Suzuki K, Nakamura K, Miyachi T, Matsuzaki H, Kajizuka M, et al. Decreased serum levels of adiponectin in subjects with autism. Prog Neuropsychopharmacol Biol Psychiatry. 2010;34(3):455-8.
16. Lisik MZ, Gutmajster E, Sieron AL. Plasma Levels of Leptin and Adiponectin in Fragile X Syndrome. Neuroimmunomodulation. 2016;23(4):239-43.

17. Mansur RB, Rizzo LB, Santos CM, Asevedo E, Cunha GR, Noto MN, et al. Adipokines, metabolic dysfunction and illness course in bipolar disorder. J Psychiatr Res. 2016;74:63-9.
18. Hu Y, Dong X, Chen J. Adiponectin and depression: A meta-analysis. Biomed Rep. 2015;3(1):38-42.
19. Burd I, Balakrishnan B, Kannan S. Models of fetal brain injury, intrauterine inflammation, and preterm birth. Am J Reprod Immunol. 2012;67(4):287-94.
20. Blardi P, de Lalla A, Ceccatelli L, Vanessa G, Auteri A, Hayek J. Variations of plasma leptin and adiponectin levels in autistic patients. Neurosci Lett. 2010;479(1):54-7.
21. Rodrigues DH, Rocha NP, Sousa LF, Barbosa IG, Kummer A, Teixeira AL. Changes in adipokine levels in autism spectrum disorders. Neuropsychobiology. 2014;69(1):6-10.
22. Schendel D, Bhasin TK. Birth weight and gestational age characteristics of children with autism, including a comparison with other developmental disabilities. Pediatrics. 2008;121(6):1155-64.
23. Kuzniewicz MW, Wi S, Qian Y, Walsh EM, Armstrong MA, Croen LA. Prevalence and neonatal factors associated with autism spectrum disorders in preterm infants. J Pediatr. 2014;164(1):20-5.
24. Movsas TZ, Paneth N. The effect of gestational age on symptom severity in children with autism spectrum disorder. J Autism Dev Disord. 2012;42(11):2431-9.
25. Fezer GF, Matos MB, Nau AL, Zeigelboim BS, Marques JM, Liberalesso PBN. Perinatal Features of Children with Autism Spectrum Disorder. Rev Paul Pediatr. 2017;35(2):130-5.
26. Angelidou A, Asadi S, Alysandratos KD, Karagkouni A, Kourembanas S, Theoharides TC. Perinatal stress, brain inflammation and risk of autism-review and proposal. BMC Pediatr. 2012;12:89.
27. Darcy-Mahoney A, Minter B, Higgins M, Guo Y, Williams B, Head Zauche LM, et al. Probability of an Autism Diagnosis by Gestational Age. Newborn Infant Nurs Rev. 2016;16(4):322-6.
28. Saito M, Nishimura K, Nozue H, Miyazono Y, Kamoda T. Changes in serum adiponectin levels from birth to term-equivalent age are associated with postnatal weight gain in preterm infants. Neonatology. 2011;100(1):93-8.
29. Oberthuer A, Donmez F, Oberhauser F, Hahn M, Hoppenz M, Hoehn T, et al. Hypoadiponectinemia in extremely low gestational age newborns with severe hyperglycemia--a matched-paired analysis. PloS one. 2012;7(6):e38481.
30. Kajantie E, Hytinantti T, Hovi P, Andersson S. Cord plasma adiponectin: a 20-fold rise between 24 weeks gestation and term. J Clin Endocrinol Metab. 2004;89(8):4031-6.
31. Lindsay RS, Walker JD, Havel PJ, Hamilton BA, Calder AA, Johnstone FD, et al. Adiponectin is present in cord blood but is unrelated to birth weight. Diabetes Care. 2003;26(8):2244-9.
32. Nakano Y, Itabashi K, Sakurai M, Aizawa M, Dobashi K, Mizuno K. Preterm infants have altered adiponectin levels at term-equivalent age even if they do not present with extrauterine growth restriction. Horm Res Paediatr. 2013;80(3):147-53.
33. Terrazzan AC, Procianoy RS, Silveira RC. Neonatal cord blood adiponectin and insulin levels in very low birth weight preterm and healthy full-term infants. J Matern Fetal Neonatal Med. 2014;27(6):616-20.

34. Yoshida T, Nagasaki H, Asato Y, Ohta T. The ratio of high-molecular weight adiponectin and total adiponectin differs in preterm and term infants. Pediatr Res. 2009;65(5):580-3.
35. Siahanidou T, Mandyla H, Papassotiriou GP, Papassotiriou I, Chrousos G. Circulating levels of adiponectin in preterm infants. Arch Dis Child Fetal Neonatal Ed. 2007;92(4):F286-90.
36. Wang G, Hu FB, Mistry KB, Zhang C, Ren F, Huo Y, et al. Association Between Maternal Prepregnancy Body Mass Index and Plasma Folate Concentrations With Child Metabolic Health. JAMA Pediatr. 2016;170(8):e160845.
37. Wang X, Zuckerman B, Pearson C, Kaufman G, Chen C, Wang G, et al. Maternal cigarette smoking, metabolic gene polymorphism, and infant birth weight. Jama. 2002;287(2):195-202.
38. Wang G, Divall S, Radovick S, Paige D, Ning Y, Chen Z, et al. Preterm birth and random plasma insulin levels at birth and in early childhood. Jama. 2014;311(6):587-96.
39. Raghavan R, Riley AW, Volk H, Caruso D, Hironaka L, Sices L, et al. Maternal Multivitamin Intake, Plasma Folate and Vitamin B12 Levels and Autism Spectrum Disorder Risk in Offspring. Paediatric and perinatal epidemiology. 2017.
40. Li M, Fallin MD, Riley A, Landa R, Walker SO, Silverstein M, et al. The Association of Maternal Obesity and Diabetes With Autism and Other Developmental Disabilities. Pediatrics. 2016;137(2):e20152206.
41. Wang G, Johnson S, Gong Y, Polk S, Divall S, Radovick S, et al. Weight Gain in Infancy and Overweight or Obesity in Childhood across the Gestational Spectrum: a Prospective Birth Cohort Study. Sci Rep. 2016;6:29867.
42. Brucato M, Ladd-Acosta C, Li M, Caruso D, Hong X, Kaczaniuk J, et al. Prenatal exposure to fever is associated with autism spectrum disorder in the boston birth cohort. Autism Res. 2017;10(11):1878-90.
43. Mantzoros CS, Rifas-Shiman SL, Williams CJ, Fargnoli JL, Kelesidis T, Gillman MW. Cord blood leptin and adiponectin as predictors of adiposity in children at 3 years of age: a prospective cohort study. Pediatrics. 2009;123(2):682-9.
44. Yeung EH, McLain AC, Anderson N, Lawrence D, Boghossian NS, Druschel C, et al. Newborn Adipokines and Birth Outcomes. Paediatric and perinatal epidemiology. 2015;29(4):317-25.
45. Martos-Moreno GA, Barrios V, Saenz de Pipaon M, Pozo J, Dorronsoro I, Martinez-Biarge M, et al. Influence of prematurity and growth restriction on the adipokine profile, IGF1, and ghrelin levels in cord blood: relationship with glucose metabolism. Eur J Endocrinol. 2009;161(3):381-9.
46. Hellgren G, Engstrom E, Smith LE, Lofqvist C, Hellstrom A. Effect of Preterm Birth on Postnatal Apolipoprotein and Adipocytokine Profiles. Neonatology. 2015;108(1):16-22.
47. Ashwood P, Kwong C, Hansen R, Hertz-Picciotto I, Croen L, Krakowiak P, et al. Brief report: plasma leptin levels are elevated in autism: association with early onset phenotype? J Autism Dev Disord. 2008;38(1):169-75.
48. Al-Zaid FS, Alhader AA, Al-Ayadhi LY. Altered ghrelin levels in boys with autism: a novel finding associated with hormonal dysregulation. Sci Rep. 2014;4:6478.
49. Essa M.M., Braidy N., Al-Sharbati M.M., Al-Farsi YM, Ali A, Waly M.I., et al. Elevated plasma leptin levels in autisic children of Sultanate of Oman. International Journal of Biological & Medical Research. 2011;2(3):803-5.

50. Inoue M, Maehata E, Yano M, Taniyama M, Suzuki S. Correlation between the adiponectin-leptin ratio and parameters of insulin resistance in patients with type 2 diabetes. Metabolism. 2005;54(3):281-6.
51. Inami I, Okada T, Fujita H, Makimoto M, Hosono S, Minato M, et al. Impact of serum adiponectin concentration on birth size and early postnatal growth. Pediatr Res. 2007;61(5 Pt 1):604-6.
52. Blardi P, de Lalla A, D'Ambrogio T, Vonella G, Ceccatelli L, Auteri A, et al. Long-term plasma levels of leptin and adiponectin in Rett syndrome. Clin Endocrinol (Oxf). 2009;70(5):706-9.
53. Brochu-Gaudreau K, Rehfeldt C, Blouin R, Bordignon V, Murphy BD, Palin MF. Adiponectin action from head to toe. Endocrine. 2010;37(1):11-32.
54. Dawczynski K, de Vries H, Beck JF, Schleussner E, Wittig S, Proquitte H. Adiponectin serum concentrations in newborn at delivery appear to be of fetal origin. J Pediatr Endocrinol Metab. 2014;27(3-4):273-8.
55. Mazaki-Tovi S, Kanety H, Pariente C, Hemi R, Efraty Y, Schiff E, et al. Determining the source of fetal adiponectin. J Reprod Med. 2007;52(9):774-8.
56. Zhang ZQ, Lu QG, Huang J, Jiao CY, Huang SM, Mao LM. Maternal and cord blood adiponectin levels in relation to post-natal body size in infants in the first year of life: a prospective study. BMC Pregnancy Childbirth. 2016;16(1):189.
57. Meyer DM, Brei C, Stecher L, Much D, Brunner S, Hauner H. Cord blood and child plasma adiponectin levels in relation to childhood obesity risk and fat distribution up to 5 y. Pediatr Res. 2017;81(5):745-51.
58. Kotani Y, Yokota I, Kitamura S, Matsuda J, Naito E, Kuroda Y. Plasma adiponectin levels in newborns are higher than those in adults and positively correlated with birth weight. Clinical endocrinology. 2004;61(4):418-23.
59. Tsai PJ, Yu CH, Hsu SP, Lee YH, Chiou CH, Hsu YW, et al. Cord plasma concentrations of adiponectin and leptin in healthy term neonates: positive correlation with birthweight and neonatal adiposity. Clinical endocrinology. 2004;61(1):88-93.
60. Pardo IM, Geloneze B, Tambascia MA, Barros-Filho AA. Hyperadiponectinemia in newborns: relationship with leptin levels and birth weight. Obes Res. 2004;12(3):521-4.
61. Weyermann M, Beermann C, Brenner H, Rothenbacher D. Adiponectin and leptin in maternal serum, cord blood, and breast milk. Clin Chem. 2006;52(11):2095-102.
62. Stefan N, Bunt JC, Salbe AD, Funahashi T, Matsuzawa Y, Tataranni PA. Plasma adiponectin concentrations in children: relationships with obesity and insulinemia. J Clin Endocrinol Metab. 2002;87(10):4652-6.
63. Chez MG, Dowling T, Patel PB, Khanna P, Kominsky M. Elevation of tumor necrosis factor-alpha in cerebrospinal fluid of autistic children. Pediatr Neurol. 2007;36(6):361-5.
64. Wei H, Alberts I, Li X. Brain IL-6 and autism. Neuroscience. 2013;252:320-5.
65. Young AM, Campbell E, Lynch S, Suckling J, Powis SJ. Aberrant NF-kappaB expression in autism spectrum condition: a mechanism for neuroinflammation. Front Psychiatry. 2011;2:27.
66. Thundyil J, Pavlovski D, Sobey CG, Arumugam TV. Adiponectin receptor signalling in the brain. Br J Pharmacol. 2012;165(2):313-27.
67. Ng RC, Chan KH. Potential Neuroprotective Effects of Adiponectin in Alzheimer's Disease. Int J Mol Sci. 2017;18(3).

68. Zhang D, Wang X, Lu XY. Adiponectin Exerts Neurotrophic Effects on Dendritic Arborization, Spinogenesis, and Neurogenesis of the Dentate Gyrus of Male Mice. Endocrinology. 2016;157(7):2853-69.
69. Zhang D, Wang X, Wang B, Garza JC, Fang X, Wang J, et al. Adiponectin regulates contextual fear extinction and intrinsic excitability of dentate gyrus granule neurons through AdipoR2 receptors. Mol Psychiatry. 2017;22(7):1044-55.
70. Song J, Kang SM, Kim E, Kim CH, Song HT, Lee JE. Adiponectin receptor-mediated signaling ameliorates cerebral cell damage and regulates the neurogenesis of neural stem cells at high glucose concentrations: an in vivo and in vitro study. Cell Death Dis. 2015;6:e1844.
71. Machado-Vieira R, Gold PW, Luckenbaugh DA, Ballard ED, Richards EM, Henter ID, et al. The role of adipokines in the rapid antidepressant effects of ketamine. Mol Psychiatry. 2017;22(1):127-33.
72. Lehto SM, Huotari A, Niskanen L, Tolmunen T, Koivumaa-Honkanen H, Honkalampi K, et al. Serum adiponectin and resistin levels in major depressive disorder. Acta Psychiatr Scand. 2010;121(3):209-15.
73. Zerbo O, Yoshida C, Grether JK, Van de Water J, Ashwood P, Delorenze GN, et al. Neonatal cytokines and chemokines and risk of Autism Spectrum Disorder: the Early Markers for Autism (EMA) study: a case-control study. J Neuroinflammation. 2014;11:113.

Appendix

Table 6-1 Association between cord plasma adiponectin levels and ASD risk in children

	Total n	ASD n	OR	95% CI	p value
Unadjusted					
Continuous	674	40	0.51	0.35, 0.75	0.001
Quartile					
Q1	674	40	Ref		
Q2			0.36	0.16, 0.85	0.02
Q3			0.32	0.13, 0.77	0.01
Q4			0.23	0.08, 0.62	0.004
Model 1[b]					
Continuous	674	40	0.50	0.33, 0.77	0.002
Quartile					
Q1	674	40	Ref		
Q2			0.39	0.15, 1.01	0.05
Q3			0.40	0.15, 1.10	0.08
Q4			0.14	0.04, 0.46	0.001
Model 2[c]					
Continuous	674	40	0.51	0.32, 0.82	0.006
Quartile					
Q1	674	40	Ref		
Q2			0.38	0.14, 1.03	0.06
Q3			0.38	0.13, 1.12	0.08
Q4			0.13	0.04, 0.45	0.001
Model 3[d]					
Continuous	674	40	0.47	0.30, 0.74	0.001
Quartile					
Q1	674	40	Ref		
Q2			0.41	0.16, 1.08	0.07
Q3			0.32	0.11, 0.95	0.04
Q4			0.14	0.04, 0.45	0.001
Model 4[e]					
Continuous	674	40	0.46	0.29, 0.73	0.001
Quartile					
Q1	674	40	Ref		
Q2			0.38	0.14, 1.01	0.05
Q3			0.30	0.10, 0.89	0.03

Q4			0.13	0.04, 0.43	0.001
Model 5[f]					
Continuous	674	40	0.51	0.32, 0.83	0.006
Quartile					
Q1	674	40	Ref		
Q2			0.46	0.16, 1.34	0.16
Q3			0.44	0.15, 1.33	0.15
Q4			0.12	0.03, 0.48	0.003
Model 6[g]					
Continuous	674	40	0.36	0.21, 0.62	<0.001
Quartile					
Q1	674	40	Ref		
Q2			0.32	0.11, 0.91	0.03
Q3			0.23	0.07, 0.77	0.02
Q4			0.08	0.02, 0.34	0.001

[a]Adiponectin was entered as a continuous variable in one model and categorical variable in another model

[b]Model 1: Adjusted for child's sex, race, maternal education, maternal age, parity, smoking status, pre-pregnancy BMI, diabetes status, follow-up time

[c]Model 2: Model 1 + gestational age

[d]Model 3: Model 1 + cord insulin

[e]Model 4: Model 1 + cord leptin

[f]Model 5: Model 1 + weight gain during first year of life

[g]Model 6: Model 1 + early childhood adiponectin

Table 6-2 Association between early childhood plasma adiponectin levels and ASD risk in children

	Total n	ASD n	OR	95% CI	p value
Unadjusted					
Continuous	638	36	0.71	0.45, 1.13	0.15
Quartile					
Q1	638	36	Ref		
Q2			0.70	0.27, 1.79	0.45
Q3			0.88	0.36, 2.13	0.78
Q4			0.61	0.23, 1.61	0.32
Model 1[b]					
Continuous	638	36	0.54	0.33, 0.90	0.02
Quartile					
Q1	638	36	Ref		
Q2			0.65	0.24, 1.77	0.40
Q3			0.68	0.25, 1.82	0.44
Q4			0.29	0.09, 0.94	0.04
Model 2[c]					
Continuous	638	36	0.59	0.35, 0.99	0.05
Quartile					
Q1	638	36	Ref		
Q2			0.71	0.25, 1.97	0.51
Q3			0.80	0.29, 2.19	0.66
Q4			0.31	0.09, 1.04	0.06
Model 3[d]					
Continuous	638	36	0.53	0.32, 0.88	0.01
Quartile					
Q1	638	36	Ref		
Q2			0.64	0.23, 1.76	0.39
Q3			0.68	0.25, 1.86	0.45
Q4			0.26	0.08, 0.87	0.03
Model 4[e]					
Continuous	638	36	0.49	0.29, 0.83	0.008
Quartile					
Q1	638	36	Ref		
Q2			0.64	0.23, 1.81	0.41
Q3			0.68	0.24, 1.89	0.46
Q4			0.23	0.06, 0.79	0.02
Model 5[f]					
Continuous	638	36	0.51	0.30, 0.86	0.01

Quartile					
Q1	638	36	Ref		
Q2			0.63	0.20, 1.98	0.43
Q3			0.59	0.20, 1.72	0.34
Q4			0.27	0.08, 0.93	0.04
Model 6[g]					
Continuous	638	36	0.67	0.34, 1.33	0.25
Quartile					
Q1	638	36	Ref		
Q2			0.72	0.17, 2.95	0.65
Q3			0.81	0.22, 3.01	0.75
Q4			0.64	0.14, 2.99	0.58

[a]Adiponectin was entered as a continuous variable in one model and categorical variable in another model

[b]Model 1: Adjusted for maternal age, diabetes status, maternal BMI, sex, race, age of measurement, follow-up time and breastfeeding status

[c]Model 2: Model 1 + gestational age at birth

[d]Model 3: Model 1 + early childhood insulin

[e]Model 4: Model 1 + early childhood leptin

[f]Model 5: Model 1 + weight gain during first year of life

[g]Model 6: Model 1 + cord adiponectin

Table 6-3 Joint effects of preterm birth and cord adiponectin in predicting ASD risk

Joint effects of preterm birth[a] and adiponectin[b]	n	Unadjusted	P value	Adjusted[c]	P value
Full term + High adiponectin	309	Ref		Ref	
Preterm + High adiponectin	28	1.0 (0.12, 8.07)	1.0	1.22 (0.14, 10.87)	0.86
Full term + Low adiponectin	221	2.26 (1.04, 4.92)	0.04	3.06 (1.27, 7.40)	0.01
Preterm + Low adiponectin	116	2.84 (1.20, 6.74)	0.02	2.62 (0.99, 6.99)	0.05

[a] – Full term defined as ≥ 37 completed weeks of gestation; preterm defined as <37 completed weeks of gestation

[b] - High cord adiponectin – defined as ≥50th percentile; Low cord adiponectin – defined as <50th percentile

[c] – Adjusted for child's sex, maternal education, maternal age, parity, maternal BMI, maternal smoking status, diabetes, race ethnicity and follow-up time

Figure 6-1 Distribution of plasma adiponectin levels in cord blood and in early childhood venous blood, stratified by preterm status

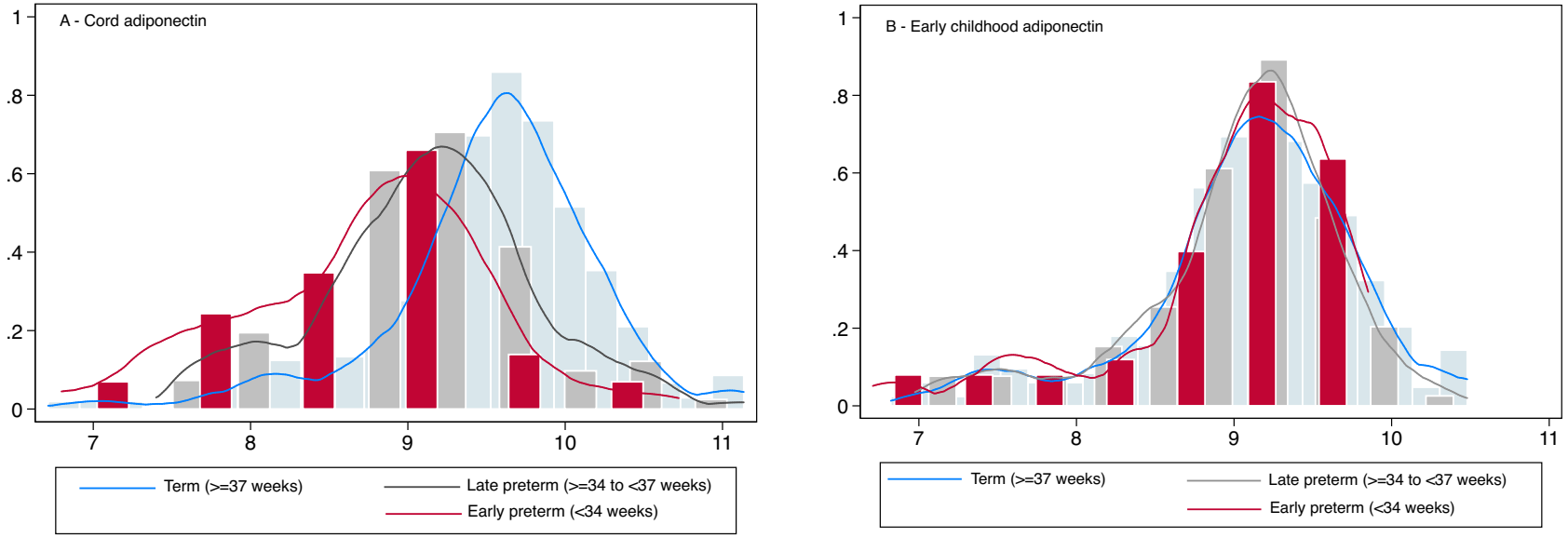


Figure 6-1 describes the distribution of cord adiponectin in panel A and early childhood plasma adiponectin in panel B. The distributions in both these panels are categorized by gestational age: 1) Term (≥ 37 completed weeks of gestation); 2) Late preterm (≥ 34 to <37 completed weeks of gestation); and 3) Early preterm (<34 completed weeks of gestation)

Figure 6-2 Distribution of plasma adiponectin levels in cord blood and in early childhood venous blood, stratified by neurotypical children vs. those with ASD

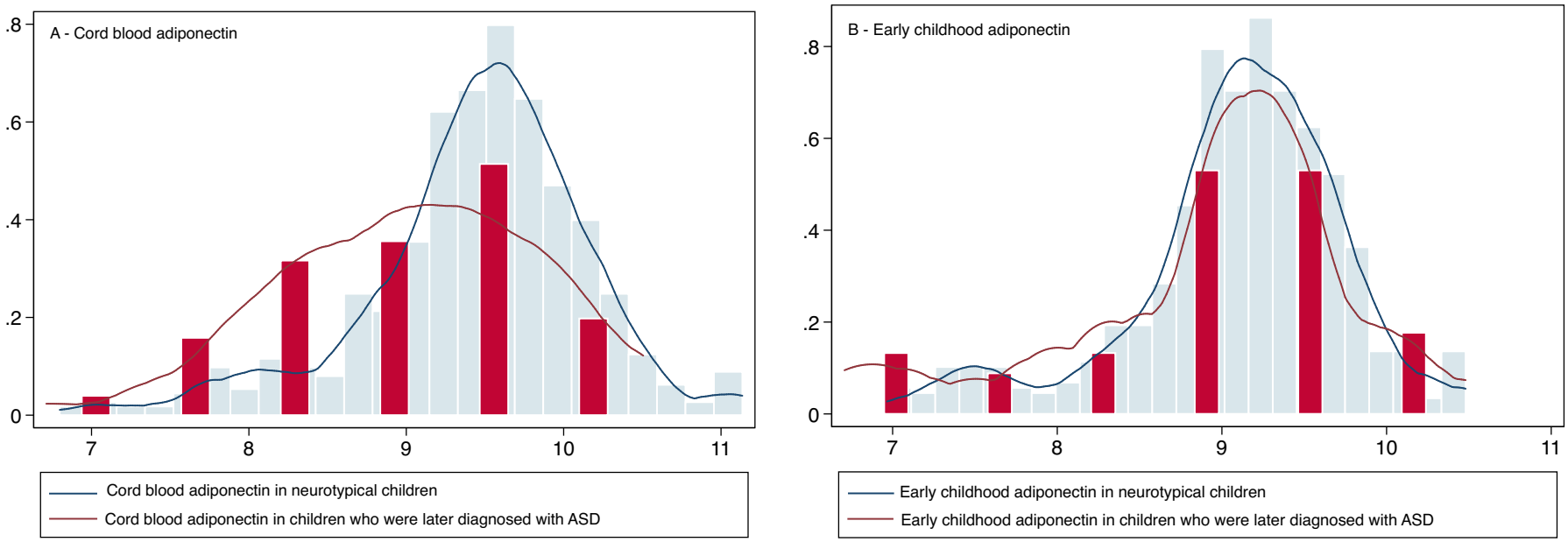


Figure 6-2 describes the distribution of cord adiponectin in panel A and early childhood plasma adiponectin in panel B. The distributions in both these panels are categorized by ASD status: 1) Neurotypical children; and 2) ASD children

Supplement Table 6-1: Maternal and child characteristics by child's case status (neurotypical vs. ASD) in the Boston Birth Cohort

	Neurotypical (n=792)	ASD (n=55)	p value
Characteristics			
Mothers			
Age at birth (yrs), mean (SD)	28.19 (6.56)	30.35 (6.16)	0.02
Parity (%)			0.89
0	333 (42.05)	21 (38.18)	
1 or more	458 (57.83)	34 (61.82)	
Missing	1 (0.16)	0 (0.00)	
Mother's education (%)			0.85
High School or less	510 (64.39)	33 (60.00)	
Some college or more	275 (34.72)	21 (38.18)	
Missing	7 (0.88)	1 (1.82)	
Maternal BMI (%)			0.21
Underweight (<18.5) + Normal Weight (≥18.5-<25)	385 (48.61)	20 (36.36)	
Overweight (25-29.9)	245 (30.93)	19 (34.55)	
Obesity (>=30)	162 (20.45)	16 (29.09)	
Diabetes (%)			0.04
No	718 (90.66)	47 (85.45)	
Gestational	50 (6.31)	2 (3.64)	
Diabetes mellitus	24 (3.03)	6 (10.91)	
Smoking during & 3 months prior to pregnancy (%)			0.45
No	677 (85.48)	42 (76.36)	
Yes	109 (13.76)	12 (21.82)	
Missing	6 (0.76)	1 (1.82)	
Child			
Gender (%)			<0.001
Male	337 (42.55)	40 (72.73)	
Female	455 (57.45)	15 (27.27)	
Race-ethnicity (%)			0.83
Black	453 (57.20)	34 (61.82)	
White	48 (6.06)	4 (7.27)	
Hispanic	186 (23.48)	11 (20.00)	
Other	100 (12.63)	6 (10.91)	
Missing	5 (0.63)	0 (0.00)	
Gestational age (%)			0.04
Term	601 (75.88)	34 (61.82)	

Late preterm (≥34 - <37 weeks)	128 (16.16)	10 (18.18)	
Early preterm (<34 weeks)	63 (7.95)	11 (20.00)	
Birth weight (g)	3017.85 (713.20)	2803.73 (886.24)	0.03
Year of birth (%)			0.66
1998-2006	429 (54.17)	30 (54.55)	
2007-2013	363 (45.83)	25 (45.45)	
Cord blood adiponectin (SD)[a] (µg/mL)	15.80 (10.84)	11.37 (88.27)	0.01
Early childhood adiponectin (SD)[b] (µg/mL)	10.67 (62.15)	10.11 (73.59)	0.61
First year weight gain pattern (%)[c]			0.05
On target	182 (33.96)	9 (18.75)	
Slow	65 (12.13)	6 (12.50)	
Rapid weight gain	92 (17.16)	6 (12.50)	
Extremely rapid weight gain	197 (36.75)	27 (56.25)	
Mode of feeding (%)			0.86
Formula	183 (23.11)	11 (20.00)	
Both	549 (69.32)	37 (67.27)	
Breastfeeding	54 (6.82)	6 (10.91)	
Missing	6 (0.76)	1 (1.82)	
[a]n=674 (Neurotypical n=634; ASD n=40)			
[b]n=638 (Neurotypical n=602; ASD n=36)			
[c]n=584 (Neurotypical n=536; ASD n=48)			

Supplemental Table 6-2: Prenatal and perinatal predictors of cord blood and early childhood adiponectin in the Boston Birth Cohort

	Mean cord adiponectin (µg/mL)	P value	Mean early childhood adiponectin (µg/mL)	P value
Maternal factors				
BMI		0.67		0.28
<25	15.53		10.72	
≥25 to <30	15.14		10.05	
≥30	16.21		11.12	
Diabetes		0.75		
No	15.58		10.60	0.93
Yes	15.15		10.67	
Smoking		0.06		0.15
No	15.86		10.78	
Yes	13.65		9.78	
Race		0.13		0.03
Black	15.06		10.05	
White	14.50		12.17	
Hispanic	17.35		11.62	
Other	15.06		10.67	
Child Factors				
IUGR		0.13		0.07
AGA	16.02		10.35	
SGA	12.99		11.34	
LGA	15.19		12.40	
Weight gain during 1st year[a]				0.68
On target			10.84	
Slow			10.94	
Rapid			11.06	
Extremely Rapid			10.21	
Gestational age		<0.001		0.14
<34 weeks	8.29		9.51	
≥34 to <37 weeks	11.51		9.96	
≥37 weeks	16.90		10.89	
Sex		0.57		0.99
Boys	15.28		10.60	
Girls	15.75		10.61	
Breast feeding[a]				0.57
Bottle only			10.76	
Both bottle and breast			10.48	

feeding				
Only breast feeding			11.45	

[a] Cord blood leptin levels were not calculated for postnatal variables (weight gain during 1st year of life and breastfeeding)

Supplemental Table 6-3: Predictors of cord adiponectin (categorized into quartiles) in the Boston Birth Cohort

	Cord adiponectin				
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P value
Gestational age (%)					<0.001
≥ 37 weeks	94 (17.74)	127 (23.96)	155 (29.25)	154 (29.06)	
≥ 34 to <37 weeks	45 (45.00)	32 (32.00)	11 (11.11)	12 (12.00)	
< 34 weeks	28 (63.64)	11 (25.00)	3 (6.82)	2 (4.55)	
In utero growth (%)					0.23
AGA	86 (77.68)	93 (76.23)	99 (79.84)	104 (81.89)	
SGA	17 (15.32)	10 (8.20)	12 (9.68)	10 (7.87)	
LGA	8 (7.21)	19 (15.57)	13 (10.48)	13 (10.24)	
Birth weight (%)					<0.001
Normal (≥ 2500 g)	106 (63.47)	135 (79.88)	157 (92.90)	161 (95.83)	
Low (<2500 g)	61 (36.53)	34 (20.12)	12 (7.10)	7 (4.17)	
Weight gain (%)					0.001
On track	31 (28.70)	36 (32.73)	54 (45.76)	45 (36.59)	
Slow	6 (5.56)	17 (15.45)	17 (14.41)	17 (13.82)	
Rapid	19 (17.59)	12 (10.91)	22 (18.64)	27 (21.95)	
Extremely rapid	52 (48.15)	45 (40.91)	25 (21.19)	34 (27.64)	
Maternal pre-pregnancy BMI (%)					0.92
<25	85 (50.90)	80 (47.06)	78 (46.15)	82 (48.81)	
≥ 25 to <30	52 (31.14)	57 (33.53)	58 (34.32)	49 (28.17)	
≥ 30	30 (17.96)	33 (19.41)	33 (19.53)	37 (22.02)	
Diabetes (%)					0.55
No	151 (90.42)	148 (87.06)	152 (89.94)	154 (91.67)	
Yes	16 (9.58)	22 (12.94)	17 (10.06)	14 (8.33)	
Maternal obesity & diabetes					0.99
No obesity & diabetes	80 (68.38)	76 (67.86)	73 (68.22)	79 (66.95)	
Obesity or diabetes	34 (29.06)	34 (30.36)	32 (29.91)	35 (29.66)	
Obesity & diabetes	3 (2.56)	2 (1.79)	2 (1.87)	4 (3.39)	
Early child adiponectin					<0.001
Quartile 1	34 (32.69)	26 (21.31)	22 (19.30)	21 (15.56)	
Quartile 2	33 (31.73)	37 (30.33)	21 (18.42)	23 (17.04)	
Quartile 3	23 (22.12)	36 (29.51)	41 (35.96)	26 (19.26)	
Quartile 4	14 (13.46)	23 (18.85)	30 (26.32)	65 (48.15)	
Mean maternal age (yrs)	29.24	28.71	28.41	27.52	0.12

Supplemental Table 6-4: Association between cord adiponectin levels (tertiles, quintiles, extreme values) and ASD risk in children in the Boston Birth Cohort (n=674 including ASD (n=40))

	Unadjusted			Model 1			Model 2			Model 3			Model 4			Model 5		
	OR	95% CI	p	OR	95% CI	p	OR	95% CI	p	OR	95% CI	p	OR	95% CI	p	OR	95% CI	p
Tertile																		
Q1	Ref			Ref			Ref			Ref			Ref			Ref		
Q2	0.43	0.20, 0.93	0.03	0.50	0.21, 1.19	0.12	0.51	0.20, 1.27	0.15	0.45	0.18, 1.11	0.08	0.43	0.17, 1.08	0.07	0.49	0.18, 1.31	0.15
Q3	0.33	0.14, 0.75	0.009	0.26	0.10, 0.68	0.006	0.26	0.09, 0.72	0.009	0.25	0.09, 0.64	0.004	0.24	0.09, 0.64	0.004	0.25	0.09, 0.70	0.009
Quintile																		
Q1	Ref			Ref			Ref			Ref			Ref			Ref		
Q2	0.30	0.11, 0.77	0.01	0.36	0.13, 1.02	0.05	0.36	0.12, 1.05	0.06	0.36	0.12, 1.06	0.06	0.34	0.12, 0.99	0.05	0.43	0.14, 1.30	0.13
Q3	0.41	0.17, 0.98	0.04	0.49	0.18, 1.30	0.15	0.47	0.16, 1.35	0.16	0.41	0.14, 1.16	0.09	0.38	0.13, 1.10	0.07	0.52	0.17, 1.63	0.26
Q4	0.15	0.04, 0.51	0.002	0.20	0.05, 0.74	0.02	0.19	0.05, 0.76	0.02	0.19	0.05, 0.72	0.01	0.17	0.04, 0.68	0.01	0.26	0.06, 1.06	0.06
Q5	0.25	0.09, 0.69	0.008	0.16	0.04, 0.53	0.003	0.15	0.04, 0.53	0.003	0.15	0.05, 0.52	0.002	0.15	0.04, 0.50	0.002	0.13	0.03, 0.54	0.005
Extreme values																		
<20th	Ref			Ref			Ref			Ref			Ref			Ref		
>=20th to <80th	0.28	0.14, 0.56	<0.001	0.35	0.16, 0.76	0.008	0.34	0.14, 0.80	0.01	0.32	0.14, 0.72	0.006	0.3	0.13, 0.69	0.004	0.40	0.17, 0.98	0.05
>=80th	0.25	0.09, 0.69	0.008	0.16	0.05, 0.53	0.003	0.15	0.04, 0.53	0.003	0.15	0.05, 0.51	0.002	0.15	0.04, 0.50	0.002	0.13	0.03, 0.54	0.005

Model 1: Adjusted for child's sex, race, maternal education, maternal age, parity, smoking status, pre-pregnancy BMI, diabetes status, follow-up time
Model 2: Model 1 + gestational age
Model 3: Model 1 + cord insulin
Model 4: Model 1 + cord leptin
Model 5: Model 1 + weight gain during first year of life

Supplemental Table 6-5: Association between early childhood adiponectin levels (tertiles, quintiles, extreme values) and ASD risk in children in the Boston Birth Cohort

			Unadjusted			Model 1			Model 2			Model 3		
	Total n	ASD n	OR	95% CI	p value	OR	95% CI	p value	OR	95% CI	p value	OR	95% CI	p value
Tertile														
Q1	638	36	Ref			Ref			Ref			Ref		
Q2			1.14	0.53, 2.46	0.74	0.88	0.38, 2.04	0.76	1.03	0.43, 2.45	0.95	1.19	0.38, 3.76	0.77
Q3			0.57	0.23, 1.41	0.22	0.26	0.09, 0.75	0.01	0.28	0.09, 0.85	0.03	0.39	0.10, 1.59	0.19
Quintile														
Q1	638	36	Ref			Ref			Ref			Ref		
Q2			0.17	0.04, 0.76	0.02	0.19	0.04, 0.92	0.04	0.19	0.04, 0.95	0.04	0.17	0.02, 1.62	0.12
Q3			1.06	0.45, 2.51	0.89	0.88	0.34, 2.27	0.79	1.14	0.42, 3.09	0.80	1.58	0.43, 5.82	0.49
Q4			0.41	0.14, 1.23	0.11	0.22	0.06, 0.75	0.02	0.24	0.07, 0.86	0.03	0.11	0.01, 0.91	0.04
Q5			0.51	0.18, 1.44	0.20	0.29	0.09, 0.96	0.04	0.33	0.09, 1.12	0.08	0.68	0.14, 3.39	0.64
Extreme values (percentile)														
<20th	638	36	Ref			Ref			Ref			Ref		
>=20th to <80th			0.54	0.25, 1.16	0.11	0.42	0.18, 1.00	0.05	0.48	0.20, 1.16	0.10	0.49	0.15, 1.58	0.23
>=80th			0.51	0.18, 1.44	0.20	0.30	0.09, 1.00	0.05	0.33	0.10, 1.13	0.08	0.69	0.15, 3.23	0.63

Model 1: Adjusted for maternal age, diabetes status, maternal BMI, sex, race, age of measurement, follow-up time and breastfeeding status
Model 2: Model 1 + gestational age
Model 3: Model 1 + cord adiponectin

Supplemental Table 6-6: Sensitivity Analysis on the association between cord adiponectin and subsequent ASD risk in the Boston Birth Cohort using cases that were diagnosed at least twice and by a specialist[a]

	Total n	ASD n	OR	95% CI	p value
Unadjusted					
Continuous	674	30	0.41	0.27, 0.62	<0.001
Quartile					
Q1	674	30	Ref		
Q2			0.38	0.15, 0.94	0.04
Q3			0.16	0.05, 0.55	0.004
Q4			0.16	0.05, 0.56	0.004
Model 1					
Continuous	674	30	0.40	0.25, 0.66	<0.001
Quartile					
Q1	674	30	Ref		
Q2			0.40	0.15, 1.10	0.08
Q3			0.18	0.05, 0.70	0.01
Q4			0.13	0.04, 0.50	0.003
Model 2					
Continuous	674	30	0.44	0.26, 0.74	0.002
Quartile					
Q1	674	30	Ref		
Q2			0.41	0.14, 1.18	0.10
Q3			0.19	0.04, 0.79	0.02
Q4			0.14	0.03, 0.54	0.005
Model 3					
Continuous	674	30	0.39	0.23, 0.64	<0.001
Quartile					
Q1	674	30	Ref		
Q2			0.42	0.15, 1.17	0.1
Q3			0.18	0.04, 0.70	0.01
Q4			0.13	0.03, 0.49	0.002
Model 4					
Continuous	674	30	0.38	0.23, 0.64	<0.001
Quartile					
Q1	674	30	Ref		
Q2			0.40	0.14, 1.11	0.08
Q3			0.16	0.04, 0.67	0.01
Q4			0.12	0.03, 0.47	0.002
Model 5					

Continuous	674	30	0.38	0.22, 0.66	0.001
Quartile					
Q1	674	30	Ref		
Q2			0.43	0.14, 1.34	0.15
Q3			0.18	0.04, 0.76	0.02
Q4			0.09	0.02, 0.44	0.003

[a]Adiponectin was entered as a continuous variable in one model and categorical variable in another model

Model 1: Adjusted for child's sex, race, maternal education, maternal age, parity, smoking status, pre-pregnancy BMI, diabetes status, follow-up time

Model 2: Model 1 + gestational age

Model 3: Model 1 + cord insulin

Model 4: Model 1 + cord leptin

Model 5: Model 1 + weight gain during first year of life

Supplemental Table 6-7: Sensitivity Analysis on the association between early childhood adiponectin and subsequent ASD risk in the Boston Birth Cohort using cases that were diagnosed at least twice and by a specialist[a]

	Total n	ASD n	OR	95% CI	p value
Unadjusted					
Continuous	638	28	0.61	0.37, 1.00	0.05
Quartile					
Q1	638	28	Ref		
Q2			0.46	0.15, 1.40	0.17
Q3			0.87	0.34, 2.26	0.78
Q4			0.47	0.15, 1.43	0.18
Model 1					
Continuous	638	28	0.46	0.27, 0.79	0.005
Quartile					
Q1	638	28	Ref		
Q2			0.40	0.12, 1.29	0.13
Q3			0.64	0.22, 1.86	0.41
Q4			0.20	0.05, 0.78	0.02
Model 2					
Continuous	638	28	0.49	0.28, 0.87	0.02
Quartile					
Q1	638	28	Ref		
Q2			0.43	0.13, 1.45	0.17
Q3			0.75	0.25, 2.27	0.61
Q4			0.21	0.05, 0.88	0.03
Model 3					
Continuous	638	28	0.45	0.26, 0.78	0.004
Quartile					
Q1	638	28	Ref		
Q2			0.40	0.12, 1.30	0.13
Q3			0.65	0.22, 1.91	0.43
Q4			0.19	0.05, 0.76	0.02
Model 4					
Continuous	638	28	0.42	0.24, 0.74	0.003
Quartile					
Q1	638	28	Ref		
Q2			0.40	0.12, 1.33	0.14
Q3			0.62	0.21, 1.85	0.39
Q4			0.16	0.04, 0.68	0.01
Model 5					

Continuous	638	28	0.42	0.24, 0.75	0.003
Quartile					
Q1	638	28	Ref		
Q2			0.47	0.13, 1.76	0.26
Q3			0.61	0.19, 1.95	0.41
Q4			0.20	0.05, 0.86	0.03
Model 6					
Continuous	638	28	0.46	0.22, 0.96	0.04
Quartile					
Q1	638	28	Ref		
Q2			0.31	0.07, 1.50	0.15
Q3			0.50	0.12, 2.03	0.33
Q4			0.22	0.03, 1.44	0.12

[a]Adiponectin was entered as a continuous variable in one model and categorical variable in another model

Model 1: Adjusted for maternal age, diabetes status, maternal BMI, sex, race, age of measurement, follow-up time and breastfeeding status

Model 2: Model 1 + gestational age

Model 3: Model 1 + early childhood insulin

Model 4: Model 1 + early childhood leptin

Model 5: Model 1 + weight gain during first year of life

Model 6: Model 1 + cord adiponectin

Supplemental Table 6-8: Sensitivity Analysis on the association between cord adiponectin and ASD risk in the Boston Birth Cohort using stringent neurotypical subjects[a]

	Total n	ASD n	OR	95% CI	p value
Unadjusted					
Continuous	389	40	0.48	0.31, 0.72	<0.001
Quartile					
Q1	389	40	Ref		
Q2			0.32	0.13, 0.76	0.01
Q3			0.31	0.12, 0.78	0.01
Q4			0.19	0.07, 0.52	0.001
Model 1					
Continuous	389	40	0.48	0.29, 0.79	0.004
Quartile					
Q1	389	40	Ref		
Q2			0.34	0.12, 0.98	0.05
Q3			0.51	0.17, 1.51	0.22
Q4			0.11	0.03, 0.39	0.001
Model 2					
Continuous	389	40	0.48	0.28, 0.81	0.006
Quartile					
Q1	389	40	Ref		
Q2			0.32	0.11, 0.95	0.04
Q3			0.49	0.16, 1.52	0.22
Q4			0.10	0.02, 0.37	0.001
Model 3					
Continuous	389	40	0.42	0.25, 0.72	0.001
Quartile					
Q1	389	40	Ref		
Q2			0.37	0.12, 1.12	0.08
Q3			0.37	0.12, 1.20	0.10
Q4			0.10	0.03, 0.36	0.001
Model 4					
Continuous	389	40	0.39	0.22, 0.68	0.001
Quartile					
Q1	389	40	Ref		
Q2			0.30	0.10, 0.92	0.04
Q3			0.33	0.10, 1.06	0.06
Q4			0.08	0.02, 0.32	<0.001
Model 5					
Continuous	389	40	0.50	0.29, 0.86	0.010

Quartile					
Q1	389	40	Ref		
Q2			0.39	0.12, 1.30	0.13
Q3			0.63	0.19, 2.09	0.45
Q4			0.09	0.02, 0.38	0.001

[a]Adiponectin was entered as a continuous variable in one model and categorical variable in another model

Model 1: Adjusted for child's sex, race, maternal education, maternal age, parity, smoking status, pre-pregnancy BMI, diabetes status, follow-up time

Model 2: Model 1 + gestational age

Model 3: Model 1 + cord insulin

Model 4: Model 1 + cord leptin

Model 5: Model 1 + weight gain during first year of life

Supplemental Table 6-9: Sensitivity Analysis on the association between early childhood adiponectin and ASD risk in the Boston Birth Cohort using stringent neurotypical subjects[a]

	Total n	ASD n	OR	95% CI	p value
Unadjusted					
Continuous	378	36	0.71	0.44, 1.13	0.15
Quartile					
Q1	378	36	Ref		
Q2			0.58	0.22, 1.52	0.27
Q3			0.78	0.32, 1.95	0.60
Q4			0.55	0.20, 1.50	0.25
Model 1					
Continuous	378	36	0.43	0.24, 0.78	0.005
Quartile					
Q1	378	36	Ref		
Q2			0.51	0.17, 1.55	0.23
Q3			0.43	0.14, 1.34	0.15
Q4			0.14	0.04, 0.55	0.005
Model 2					
Continuous	378	36	0.47	0.25, 0.87	0.02
Quartile					
Q1	378	36	Ref		
Q2			0.55	0.17, 1.75	0.31
Q3			0.50	0.15, 1.64	0.26
Q4			0.17	0.04, 0.68	0.01
Model 3					
Continuous	378	36	0.45	0.25, 0.81	0.01
Quartile					
Q1	378	36	Ref		
Q2			0.51	0.16, 1.56	0.24
Q3			0.48	0.15, 1.49	0.20
Q4			0.15	0.04, 0.60	0.007
Model 4					
Continuous	378	36	0.40	0.21, 0.75	0.004
Quartile					
Q1	378	36	Ref		
Q2			0.50	0.16, 1.58	0.24
Q3			0.47	0.15, 1.53	0.21
Q4			0.13	0.03, 0.55	0.005
Model 5					
Continuous	378	36	0.41	0.22, 0.77	0.005

Quartile					
Q1	378	36	Ref		
Q2			0.80	0.22, 2.93	0.75
Q3			0.41	0.12, 1.43	0.16
Q4			0.15	0.04, 0.63	0.01
Model 6					
Continuous	378	36	0.44	0.19, 1.01	0.05
Quartile					
Q1	378	36	Ref		
Q2			0.61	0.12, 3.04	0.55
Q3			0.41	0.09, 1.91	0.26
Q4			0.22	0.04, 1.32	0.10

[a]Adiponectin was entered as a continuous variable in one model and categorical variable in another model

Model 1: Adjusted for maternal age, diabetes status, maternal BMI, sex, race, age of measurement, follow-up time and breastfeeding status

Model 2: Model 1 + gestational age

Model 3: Model 1 + early childhood insulin

Model 4: Model 1 + early childhood leptin

Model 5: Model 1 + weight gain during first year of life

Model 6: Model 1 + cord adiponectin

Supplemental Table 10: Association between cord adiponectin leptin ratio and ASD risk in the Boston Birth Cohort[a]

			Unadjusted			Model 1			Model 2			Model 3		
	Total n	ASD n	OR	95% CI	P value	OR	95% CI	p value	OR	95% CI	p value	OR	95% CI	p value
Log AL ratio	642	38	0.88	0.66, 1.18	0.40	0.75	0.55, 1.03	0.08	0.71	0.51, 0.97	0.03	0.65	0.45, 0.95	0.03
Tertile														
Q1	642	38	Ref			Ref			Ref			Ref		
Q2			1.00	0.45, 2.21	1.00	0.97	0.40, 2.37	0.95	0.94	0.38, 2.30	0.89	0.70	0.25, 1.95	0.50
Q3			0.89	0.40, 2.00	0.78	0.61	0.24, 1.54	0.29	0.47	0.17, 1.25	0.13	0.38	0.12, 1.18	0.09
Quartile														
Q1	642	38	Ref			Ref			Ref			Ref		
Q2			0.99	0.42, 2.36	0.99	1.00	0.39, 2.62	0.99	0.98	0.37, 2.55	0.96	0.69	0.23, 2.07	0.51
Q3			0.71	0.28, 1.82	0.48	0.46	0.16, 1.33	0.15	0.43	0.14, 1.28	0.13	0.21	0.06, 0.81	0.02
Q4			0.71	0.28, 1.81	0.47	0.43	0.15, 1.28	0.13	0.34	0.11, 1.04	0.06	0.26	0.07, 0.93	0.04
Quintile														
Q1	642	38	Ref			Ref			Ref			Ref		
Q2			0.88	0.35, 2.26	0.80	0.75	0.26, 2.15	0.59	0.74	0.26, 2.12	0.57	0.53	0.16, 1.81	0.32
Q3			0.58	0.20, 1.65	0.31	0.45	0.14, 1.47	0.19	0.45	0.14, 1.49	0.19	0.29	0.08, 1.11	0.07
Q4			0.68	0.25, 1.84	0.44	0.39	0.12, 1.24	0.11	0.35	0.11, 1.14	0.08	0.23	0.06, 0.89	0.03
Q5			0.58	0.20, 1.65	0.31	0.31	0.09, 1.03	0.06	0.24	0.07, 0.83	0.02	0.17	0.04, 0.68	0.01
Extreme values														
<20th ptile	642	38	Ref			Ref			Ref			Ref		
>=20th to <80th ptile			0.71	0.33, 1.55	0.39	0.52	0.21, 1.27	0.15	0.50	0.20, 1.24	0.13	0.34	0.12, 0.95	0.04
>=80th ptile			0.58	0.20, 1.65	0.31	0.32	0.10, 1.05	0.06	0.25	0.07, 0.85	0.03	0.18	0.04, 0.71	0.02

Model 1: Adjusted for child's sex, race, maternal education, maternal age, parity, smoking status, pre-pregnancy BMI, diabetes status, follow-up time
Model 2: Model 1 + gestational age
Model 3: Model 1 + Weight gain during first year of life

Supplemental Table 6-11: Association between early childhood adiponectin/leptin ratio and risk of ASD in children in the Boston Birth Cohort

			Unadjusted			Model 1			Model 2			Model 3		
	Total n	ASD n	OR	95% CI	p	OR	95% CI	p	OR	95% CI	p	OR	95% CI	p
Log AL ratio	636	36	0.66	0.51, 0.85	0.001	0.51	0.36, 0.71	<0.001	0.50	0.36, 0.71	<0.001	0.53	0.35, 0.80	0.003
Tertile														
Q1	636	36	Ref			Ref			Ref			Ref		
Q2			0.39	0.18, 0.86	0.02	0.28	0.11, 0.67	0.004	0.23	0.10, 0.59	0.002	0.20	0.06, 0.66	0.008
Q3			0.20	0.07, 0.55	0.002	0.12	0.04, 0.36	<0.001	0.11	0.04, 0.35	<0.001	0.14	0.04, 0.51	0.003
Quartile														
Q1	636	36	Ref			Ref			Ref			Ref		
Q2			0.67	0.30, 1.53	0.35	0.48	0.19, 1.20	0.12	0.45	0.17, 1.18	0.11	0.54	0.16, 1.76	0.30
Q3			0.47	0.18, 1.19	0.11	0.32	0.11, 0.93	0.04	0.29	0.10, 0.88	0.03	0.40	0.11, 1.49	0.17
Q4			0.26	0.08, 0.80	0.02	0.15	0.04, 0.53	0.003	0.15	0.04, 0.51	0.003	0.20	0.05, 0.89	0.04
Quintile														
Q1	636	36	Ref			Ref			Ref			Ref		
Q2			1.10	0.47, 2.56	0.82	0.70	0.27, 1.83	0.47	0.60	0.22, 1.62	0.31	1.23	0.35, 4.35	0.74
Q3			0.46	0.16, 1.28	0.14	0.28	0.09, 0.89	0.03	0.24	0.07, 0.81	0.02	0.30	0.06, 1.54	0.15
Q4			0.25	0.07, 0.91	0.04	0.13	0.03, 0.53	0.004	0.12	0.03, 0.52	0.004	0.31	0.06, 1.55	0.15
Q5			0.24	0.07, 0.88	0.03	0.13	0.03, 0.53	0.004	0.11	0.03, 0.48	0.003	0.19	0.03, 1.16	0.07
Extreme values														
<20th ptile	636	36	Ref			Ref			Ref			Ref		
≥ 20th to <80th ptile			0.59	0.28, 1.26	0.18	0.36	0.15, 0.86	0.02	0.32	0.32, 0.78	0.01	0.60	0.19, 1.94	0.40
≥80th ptile			0.24	0.07, 0.88	0.030	0.14	0.03, 0.56	0.006	0.12	0.03, 0.50	0.004	0.21	0.03, 1.23	0.08

Model 1: Adjusted for child's sex, race, age of measurement, breast feeding status, follow-up time
Model 2: Model 1+ gestational age
Model 3: Model 1 + cord AL ratio

Supplemental Table 6-12: Exploratory mediation analysis using Baron and Kenny - Cord adiponectin mediating the association between preterm birth and ASD (n=674) in the Boston Birth Cohort

	Unadjusted (OR)	Adjusted[a] (OR)
Step 1: Association between preterm birth and ASD		
Full term	Ref	Ref
Late preterm	1.14 (0.46, 2.84); p=0.77	0.83 (0.31, 2.24); p=0.72
Early preterm	2.83 (1.10, 7.26); p=0.03	3.71 (1.19, 11.56); p=0.02
Step 2: Association between cord adiponectin and preterm birth[b]		
Adiponectin in full term	Ref	Ref
Adiponectin in late preterm	-0.83 (-1.12, -0.53); p<0.001	-0.82 (-1.13, -0.50); p<0.001
Adiponectin in early preterm	-1.37 (-1.76, -0.97); p<0.001	-1.49 (-1.91, -1.06); p<0.001
Step 3: Association between cord adiponectin and ASD		
Cord adiponectin (continuous)	0.51 (0.35, 0.75); p=0.001	0.50 (0.33, 0.77); p=0.002
Step 4: Association between preterm and ASD, after adjusting for cord adiponectin[c]		
Full term	Ref	Ref
Late preterm	0.89 (0.35, 2.25); p=0.81	0.57 (0.20, 1.65); p=0.30
Early preterm	1.73 (0.63, 4.74); p=0.28	1.91 (0.54, 6.70); p=0.31

[a] – Adjusted for child's sex, maternal education, maternal age, parity, maternal BMI, maternal smoking status, diabetes, race ethnicity and follow-up time

[b] – Coefficients in step 2 are not log transformed

[c] – In step 4 unadjusted model, cord adiponectin was included. In the adjusted model, all covariates listed above + cord adiponectin was adjusted

Supplemental Table 6-13: Exploratory mediation analysis using Baron and Kenny – Preterm birth mediating the association between cord adiponectin and ASD (n=674) in the Boston Birth Cohort

	Unadjusted (OR)	Adjusted[a] (OR)
Step 1: Association between cord adiponectin and ASD		
Cord adiponectin (continuous)	0.51 (0.35, 0.75); p=0.001	0.50 (0.33, 0.77); p=0.002
Step 2: Association between preterm and cord adiponectin[b]		
Full term	Ref	Ref
Late preterm	-0.42 (-0.56, -0.27); p<0.001	-0.41 (-0.56, -0.26); p<0.001
Early preterm	-0.80 (-1.01, -0.59); p<0.001	-0.82 (-1.03, -0.61); p<0.001
Step 3: Association between preterm and ASD		
Full term	Ref	Ref
Late preterm	1.14 (0.46, 2.84); p=0.77	0.83 (0.31, 2.24); p=0.72
Early preterm	2.83 (1.10, 7.26); p=0.03	3.71 (1.19, 11.56); p=0.02
Step 4: Association between cord adiponectin and ASD, after adjusting for preterm[c]		
Cord adiponectin (continuous)	0.54 (0.36, 0.81); p=0.003	0.51 (0.32, 0.82); p=0.006

[a] – Adjusted for child's sex, maternal education, maternal age, parity, maternal BMI, maternal smoking status, diabetes, race ethnicity and follow-up time

[b] – Coefficients in step 2 are not log transformed

[c] – In the unadjusted model, preterm was included. In the adjusted model, all covariates listed above + preterm was adjusted

Supplemental Table 6-14: Exploratory mediation analysis using Baron and Kenny – Early childhood adiponectin mediating the association between preterm birth and ASD (n=638) in the Boston Birth Cohort

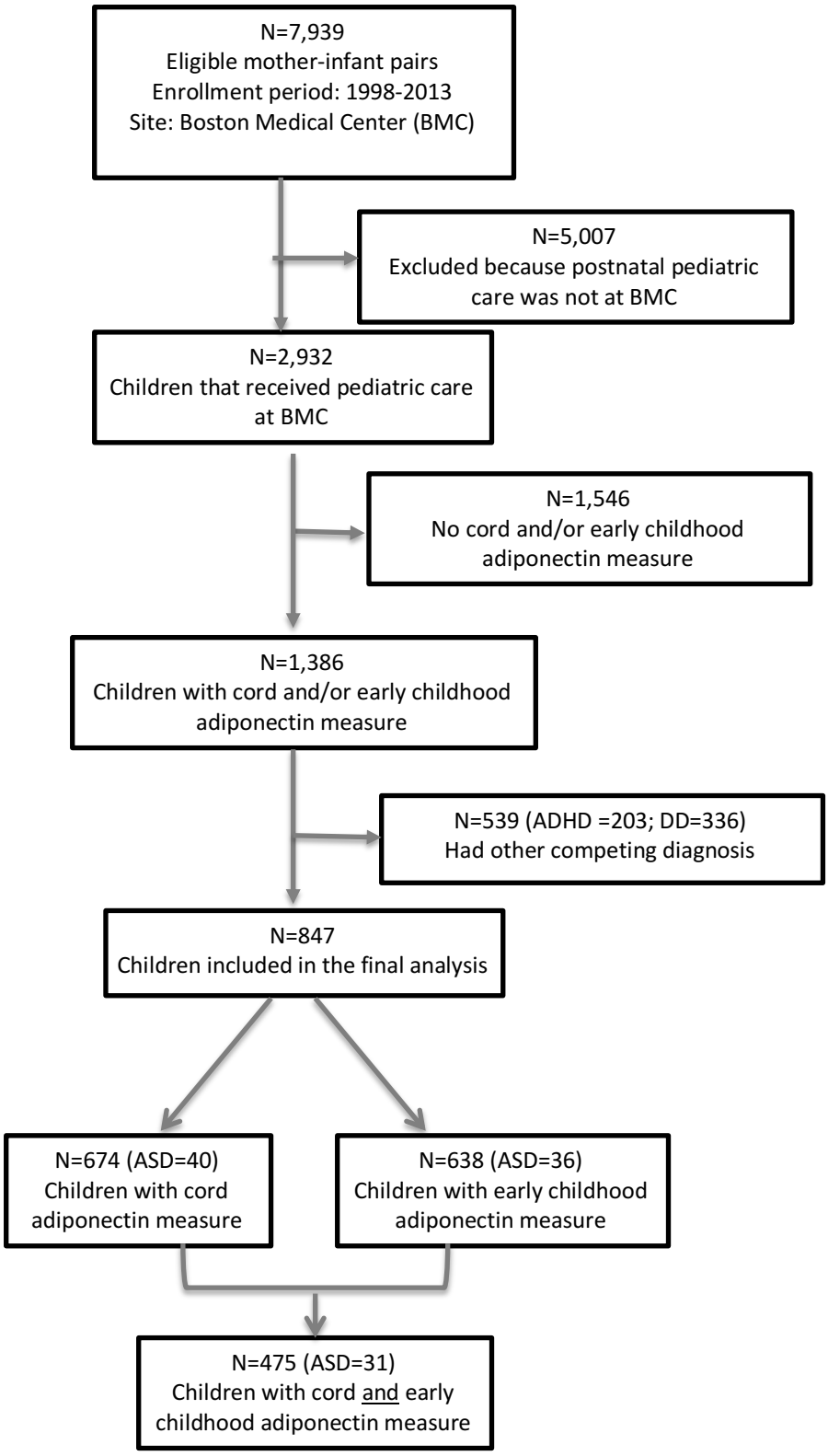
	Unadjusted (OR)	Adjusted[a] (OR)
Step 1: Association between preterm birth and ASD		
Full term	Ref	Ref
Late preterm	0.71 (0.24, 2.10); p=0.54	0.59 (0.19, 1.86); p=0.37
Early preterm	3.19 (1.36, 7.50); p=0.008	3.94 (1.47, 10.52); p=0.006
Step 2: Association between early childhood adiponectin and preterm birth[b]		
Adiponectin in full term	Ref	Ref
Adiponectin in late preterm	-0.15 (-0.45, 0.16); p=0.34	-0.20 (-0.52, 0.13); p=0.23
Adiponectin in early preterm	-0.26 (-0.65, 0.14); p=0.20	-0.32 (-0.73, 0.09); p=0.12
Step 3: Association between early childhood adiponectin and ASD		
Early childhood adiponectin (continuous)	0.71 (0.45, 1.13); p=0.15	0.54 (0.33, 0.90); p=0.02
Step 4: Association between preterm and ASD, after adjusting for early childhood adiponectin[c]		
Full term	Ref	Ref
Late preterm	0.70 (0.24, 2.06); p=0.51	0.58 (0.19, 1.83); p=0.36
Early preterm	3.08 (1.31, 7.27); p=0.01	3.42 (1.23, 9.46); p=0.02

[a] - Adjusted for child's sex, race, follow-up time, age of biomarker measurement, breastfeeding status, maternal age, pre-pregnancy BMI and maternal diabetes status

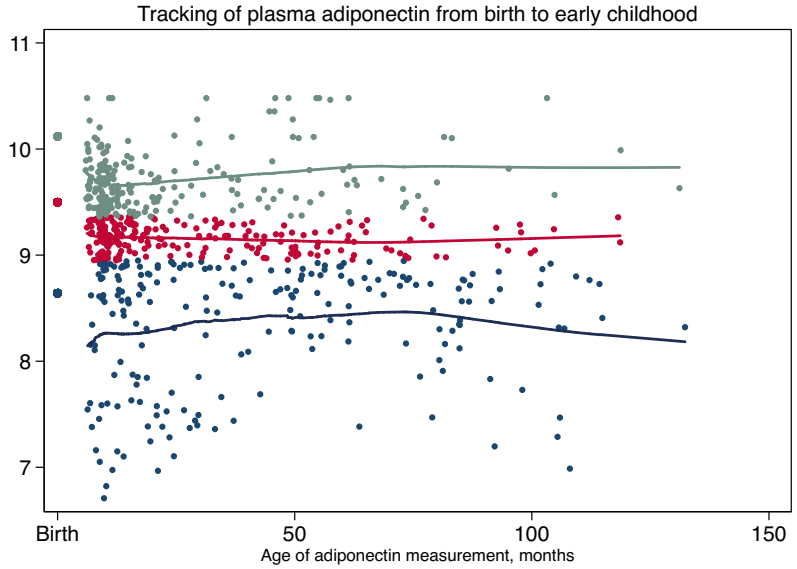
[b] – Coefficients in step 2 are not log transformed

[c] – In step 4 unadjusted model, early childhood adiponectin was included. In the adjusted model, all covariates listed above cord adiponectin was adjusted

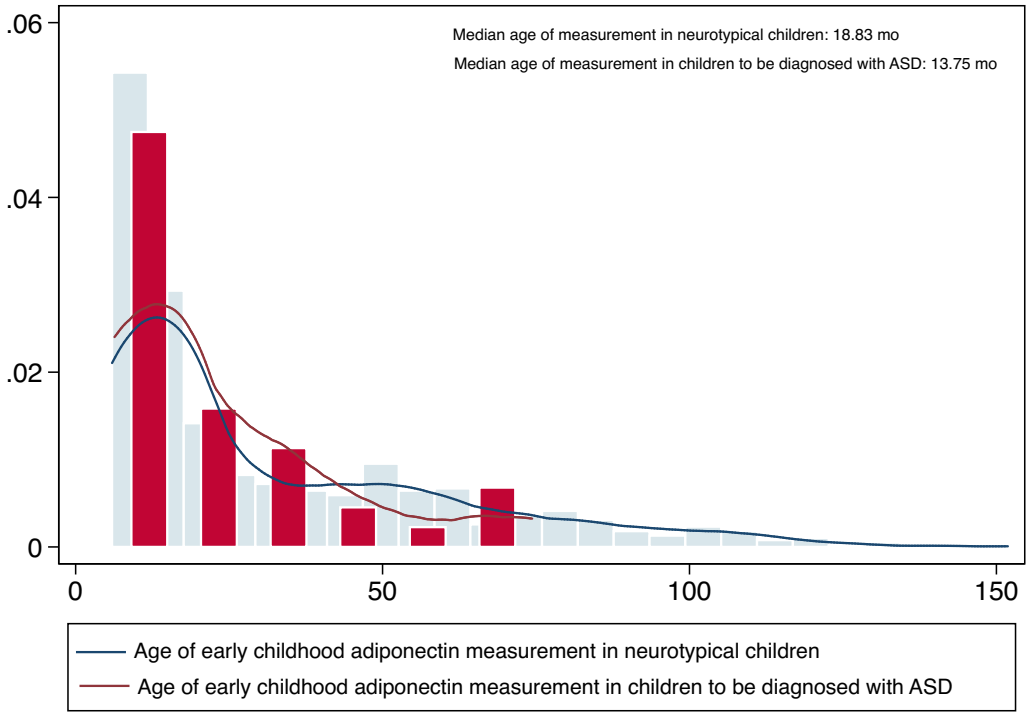
Supplemental Figure 6-1: Flowchart of initial enrollment and postnatal follow-up of the BBC and the Sample Included in the analysis



Supplemental Figure 6-2: Tracking of plasma adiponectin from birth to early childhood



Supplemental Figure 6-3: Distribution of age of early childhood adiponectin measurement



CHAPTER 7

Public Health, Clinical and Research Implications

This chapter begins with discussing the public health and clinical implications on the role of nutritional and metabolic factors in ASD, followed by a section on research implications.

7.1 Public health and clinical implications: Nutrition perspective

7.1.1 Folate, the double-edged sword

Mandatory folic acid fortification of grain products has been hailed as possibly "the most important science-driven intervention in nutrition and public health in decades" (1). In the U.S., folic acid fortification has led to 26-47% reduction in NTD rates (2-5) with the current incidence of 3.0-6.3 per 10,000 live births (6). Despite the success of the fortification efforts, there has been lingering concerns that folic acid intake may be insufficient among some sub-groups of U.S. women (7). In response to this concern, FDA recently approved corn masa flour fortification to improve folate status among Hispanic population (6, 8).

At the other end of the spectrum, there have been some apprehensions about exposing population to too much folic acid and the long-term health consequences associated with it (9, 10). These concerns include masking of vitamin B12, tumor progression, aberrant DNA methylation, diminished natural killer cell cytotoxicity (11), aberrant embryonic development, insulin resistance (12), adverse neurocognitive development (13, 14), increased risk of asthma and respiratory infections (4, 15, 16). Trends overtime suggests that serum folate levels in the U.S. have increased 2.5 times, since fortification and the prevalence of low folate levels has plummeted to ≤1%, across all demographic subgroups (5). Several studies have shown that unmetabolized folic acid (UMFA) is detected in the entire U.S. population, including maternal and fetal circulation and this has been attributed to high levels of synthetic folic acid from supplements and/or fortified foods (4, 9, 17, 18). There is growing interest about the potential

long-term effects of folic acid consumption in the population (19, 20), which had prompted the NIH's Office of Dietary Supplements and National Institute of Environmental Health Sciences to convene an expert panel to identify the research needs around safe use of high intakes of folic acid (21).

7.1.1.1 Sources of folic acid intake in pregnant population

The US Preventative Task Force (USPSTF) recommends women to consume 400-800 μg of folic acid per day. Despite this range, most women in the U.S. generally consume higher doses of folic acid from supplements (≥800 μg/day), rather than a lower standard multivitamin dose of 400 μg/day (22, 23). Recent estimates from a nationally representative sample suggest that U.S. women consume 817 μg/day of folic acid from dietary supplements (22). This is equivalent to 1,389 μg of dietary folate equivalents (DFE), after accounting for 1.7 times greater bioavailability of folic acid when compared to dietary folate (21). Diet continues to be another important source with women receiving ~575 μg DFE from the dietary sources, including natural folate and fortified and enriched foods (24). Taken together, some sub-groups of pregnant women may be consuming folic acid, in excess of 1000 μg/day, which is the FDA's safe upper level (UL) of daily intake (25). While ensuring that women receive adequate folic acid from at least from one of the sources (supplement or fortified foods), it is possible that some sub-groups of women may unintentionally be exposed to high levels of folic acid. This is especially relevant for those who may be consuming adequate quantities from multiple sources.

7.1.1.2 Future policy considerations

First, there is an urgent need to review and update the UL based on the current state of science, which was originally meant to be updated within a decade (26). When establishing the safe UL, it may be important to develop population sub-group specific UL based on their ability to metabolize and absorb folic acid (27). The UL of 1000 μg/day (or 1 mg/day) for folate was proposed primarily for those individuals with vitamin B12 deficiency in whom excess folate may exacerbate neurological damage (26, 27). Hence, it is unclear if it is appropriate to use the same UL as a universal cut-off for all conditions across all populations (27, 28). Further, the DRI/IOM set the UL for folate before studies questioning the safety of folic acid were published (4, 11, 15, 26, 27, 29).

Second, there is no consensus on upper plasma folate levels (10, 30) and time is ripe for authoritative agencies to determine the upper level threshold taking into consideration the pregnancy state, and other individual and assay specific factors (17, 31). Although the WHO has proposed a cut-off of 45.3 nmol/L for elevated levels, it was based on assay's upper limit capabilities and not on biological implications for health (32). It is notable that in our study we did not find adverse effects when considering the WHO cut-off, but we observed effects in the extreme tail (≥ 60.3 nmol/L). Studies like ours underscore the need to have cut-offs that captures not only deficiency and normal range, but also excess and most importantly extreme levels (33).

Third, maternal folate exposure has been beneficially and detrimentally linked to a number of conditions in the offspring, including neural tube defects (NTD), congenital anomalies, insulin resistance, neurodevelopmental condition (12-14, 34). In the U.S., folate levels are not clinically monitored, although there is a desire to do so, at least prior to and

during pregnancy (33). The rationale for monitoring folate levels is to ensure that women are not folate deficient, at the same time are not exposed to exceptionally high levels, thus minimizing the harm across the range of perinatal outcomes (33).

Finally, there is a need to investigate whether it is essential to continue prenatal supplements beyond first trimester. This is especially relevant given that repeated consumption of small amount of folic acid, over the course of pregnancy, can result in accumulation in serum as well as in cerebrospinal fluid (9, 28). Although, the USPSTF recommends folic acid supplementation for preconception to first trimester only (35), a majority of women however continue to use multivitamin supplements until the last trimester (22). It is paradoxical that women in their third trimester are more likely to use supplements than those in their first trimester (22). Thus, it is not only the dosage, but the duration of exposure of folic acid may also matter (28).

7.1.1.3 Considering the context while comparing study findings

In the past decade, studies have been published on prenatal folic acid supplementation and ASD (36-40). While these findings may seem disparate at the face value, understanding the context may provide a better perspective on some of these differences. This section lays out important contextual factors that one shall consider when comparing findings across studies.

- **Folate-replete vs. folate-deplete population**: Over 80 countries including the U.S., and Canada have mandatory folic acid fortification (41). While liberal voluntary fortification policies are in place in countries like Ireland, others limit (e.g. Netherlands) or even prohibit (e.g. Denmark) folic acid fortification (41, 42). Folate status is found to be lowest in populations without access to fortified foods and highest in countries with

mandatory folic acid fortification (41). It is interesting to note that many studies that reported beneficial effects of folic acid supplementation in the context of ASD were conducted in countries that did not have mandatory fortification. For example, in the Norwegian study, the authors reported that none of the foods that the participants consumed were folic acid fortified and the only source of synthetic folic acid were from supplements with a dosage of ≤400 μg/day (39). Another recent study that showed a protective effect of folic acid supplementation was conducted in Israel, a country without mandatory folic acid fortification. In fact, the study reported a prevalence of folate deficiency in pregnant women (36). Thus, the population's underlying folate status and fortification policies in the country in which the study was conducted, should be taken into account when interpreting and comparing study findings.

- **Genetic differences**: One of the important factors in elucidating the relationship between folic acid and ASD risk is the underlying prevalence of methylenetetrahydrofolate reductase (*MTHFR*) gene polymorphism. As mentioned in chapter 2, every population has *MTHFR* polymorphism, but the prevalence may vary based on the race/ethnicity. In the California based CHARGE study, the authors noted an inverse association between folic acid exposure during preconception and early pregnancy and ASD risk. Interestingly, they reported that the association between folic acid and ASD risk was observed only when the mother and/or child had *MTHFR* variant genotype (38). However, we did not observe a similar protective effect in this study. One of the reasons might be that majority of the subjects in our cohort were blacks, who are reported to have the lowest frequency of 677T (<2%) (43). Also, our hypothesis

is in line with the conclusion of a meta-analysis that stated that relationship between folic acid and ASD risk is contingent on the *MTHFR* genotype and differences in the genotype could explain some of the variation in the study findings (44).

- **Race/ethnic differences in folate metabolism**: Blacks in the U.S. have traditionally lower serum and RBC folate concentrations; paradoxically, they also have the lowest NTD rates, when compared to other racial ethnic groups (17). A recent NHANES study noted that non-Hispanic blacks (NHB) are also likely to have higher levels of UMFA, probably indicating a mismatch between folic acid supply and demand (17). This may be suggestive of lower folic acid requirements or genetic differences in the ability to metabolize folic acid in NHB when compared to other sub-groups (17). Similarly, NHANES data noted that NHB have higher levels of vitamin B12, when compared to whites and Hispanics (45). Although we did not find differences in ASD risk by race/ethnicity, it is nevertheless important to consider these different aspects while interpreting study findings.

7.1.2 Vitamin B12

Another nutrient considered in this dissertation is vitamin B12. Although vitamin B12 is intricately involved in one carbon metabolism along with folate, the latter has received a lot more attention from a national and international policy perspective. Recently, there has been a proposal to implement flour fortification of vitamin B12 in the U.S. (28, 46, 47). The driver for such a proposal has been the following – 1) emerging evidence that there is an association between vitamin B12 and NTD, 2) exacerbation of the symptoms of vitamin B12 deficiency with folic acid fortification (48), and 3) high prevalence of vitamin B12 deficiency in elderly (47).

Considering that vitamin B12 fortification may somewhat be similar to the past fortification efforts, much can be learnt from those experiences. Below are some of the contextual issues that are worth considering before embarking on another fortification program.

1. Vitamin B12 deficiency in the U.S. is limited to a sub-set of the population (e.g. elderly) (48). This raises the question whether entire population should be exposed to meet the needs of a small section of the population (48).
2. High prevalence of vitamin B12 deficiency is often due to poor absorption of vitamin B12, rather than deficiency per se. Further, much of the deficiency is sub-clinical and rarely manifests as a notable morbidity (46).
3. Stability of cyanocobalamin, most commonly used vitamin B12 form in supplements, is rather questionable. They can form cobalamin analogues that can interfere with normal vitamin B12 metabolism and transport (48). Even though the synthetic form of vitamin B12 (cyanocobalamin) is readily converted to the active form in the body, substantial accumulation of non-physiologic cobalamin has been noted under certain circumstances (49).
4. Vitamin B12 is tightly regulated in the body. This may be because of the diminished nutrient supply or exposing to large quantities might potentially be harmful (46). Emerging research suggests that elevated levels of vitamin B12 is associated with systemic inflammation, venous thromboembolism, increased cancer risk and higher mortality risk, mainly in elderly (50-52). With studies like ours (and others) are showing

adverse effects, gaining a thorough understanding on vitamin B12 metabolism may be important before initiating fortification efforts.

5. Optimum dosage for vitamin B12 that is effective, yet not toxic is unknown (48). The IOM/DRI has not established a UL for vitamin B12 because of its low potential for toxicity (53). With the emergence of new knowledge, it may be time to revisit the UL for vitamin B12.
6. Similar to plasma folate, there is no consensus on the upper bound cut-off for plasma vitamin B12. The cut-off for excess plasma vitamin B12 has ranged widely in the literature (anywhere from 500 pg/mL to 1084 pg/mL) (50, 54-56).
7. There have been no clinical trials that have shown that vitamin B12 administration prevents NTD or improves outcome in elderly population. Without well-established clinical trials to prove one way or the other, it may be too premature to embark on a population wide effort.

7.2 Public health and clinical implications: Metabolic perspective

7.2.1 Potential for ASD biomarkers

Traditionally, ASD diagnosis has been based on symptom clusters (57); however, precise diagnosis in young children before 3 years is challenging considering the etiological heterogeneity and substantial clinical variations. Unlike other conditions, there are no reliable biomarkers for ASD diagnosis (58, 59). However, recently numerous biomarkers including biochemical, morphological, immunological, hormonal, neurophysiological, neuroanatomic and neuropsychological markers have been proposed (58). Among these, adipokines have received increased attention, mainly because of its potential to identify at least sub-groups of ASD

individuals who may have some form of immune system dysregulation (58). This study, for the first time, has suggested that cord adiponectin and early childhood leptin can potentially serve as a predictive biomarker of ASD. In addition, our study was able to hint at some of the metabolic pathways in which these adipokines may be involved. This may expand the possibility of considering adiponectin as a stand-alone biomarker or leptin, in combination with early life growth indicators (such as rapid weight gain).

While this early work is promising, several steps should be taken before possibly considering these biomarkers at a clinical or population level. Briefly, it is important to understand the role of leptin and adiponectin in different ASD sub-types (58). Our study findings require additional confirmation by other independent studies. If the evidence is consistent, it will be useful for intervention studies to assess these adipokines in order to understand if the interventions are altering or targeting these metabolic biomarkers (57). Ultimately, clinical trials should be conducted to evaluate if an active intervention can result in a favorable clinical response (58).

7.2.2 Managing catch-up growth in ASD

Encouraging postnatal catch-up growth has been controversial (60). Traditionally, clinicians in the NICU have done all they could to promote postnatal growth so that the IUGR or preterm babies become comparable to that of an uncompromised fetus of an equal postmenstrual age (61). However, promoting accelerated postnatal growth has been questioned and has lately been a subject of debate (62, 63). The Latin America SGA consensus guidelines recommended that SGA infants should not be allowed weight gain that is rapid or excessive (64).

The conundrum is that lack of postnatal catch-up growth is a significant risk factor for cognitive impairments and can exacerbate motor, language and developmental delays (65, 66). However, our study (including many others) has shown that accelerated growth during first year of life may be associated with an increased ASD risk. In addition, infants with postnatal rapid catch-up growth, have other adverse outcomes such as more central fat distribution by 5 years of age (67), greater propensity to later cardiovascular disease, insulin resistance, leptin resistance, obesity and high blood pressure as adults (63, 68). Somatic overgrowth in the postnatal period may not be deterministic of ASD; yet, in the context of neurodevelopment, should catch-up growth be promoted or not? And, if acceleration of growth (including head circumference) is an adaptive or compensatory response, can altering the growth trajectory have an impact on ASD etiology? Clearly, more research is needed to answer these questions. If further studies provide evidence for this association, gender-specific clinical guidance on optimum postnatal growth that could maximize health benefits at the same time minimize any potential risk of neurological development can be considered.

7.3. Research Implications

This dissertation has addressed several important questions on the role of nutritional and metabolic factors in ASD. Since this study is one of the first prospective birth cohort studies to show some of the associations, more research in diverse populations is needed to validate our findings. During the course of this dissertation, many more questions have emerged that were not tackled in this study for a variety of reasons including project scope, availability of

time, resources and data. Nevertheless, they are important research gaps worthy of future investigation and have been elaborated below -

7.3.1 Folate, vitamin B12 and ASD

1. Across all three specific aims, outcome was assessed based on physician diagnosis using EMR. However, future studies could consider tools such as Autism diagnostic interview-revised (ADI-R) and Autism diagnostic observation schedule (ADOS), which are considered to be the 'gold standard' for ASD diagnosis (59).
2. Most studies conducted thus far, have solely assessed multivitamin supplement intake as the exposure (36, 38, 39) and have often not considered dietary folate (including fortified, enriched foods and natural folate from the diet). In a country like the U.S., diet contributes a significant amount of folate/folic acid and incomplete exposure ascertainment could preclude studies from observing the associations. Future studies should consider maternal dietary folate intake from all sources as well as biomarkers when assessing ASD risk in children.
3. Unmetabolized folic acid (UMFA), which is ubiquitously detected in the U.S. population (17), is speculated to impact folate metabolism and is thought to be responsible for adverse effects (4, 10). However, none of the studies, thus far, have assessed UMFA in the context of ASD and is an important research question to address.
4. Our study was conducted in a population that is mostly folate replete, with possibly lower folate requirements (based on preliminary knowledge on folate metabolism in blacks (17) and low prevalence of *MTHFR* C677T polymorphism). It will be interesting to

replicate our study in another population without mandatory fortification and/or higher prevalence of *MTHFR* polymorphism.

5. To our knowledge, ours is the only study that assessed third trimester maternal plasma folate and vitamin B12 and its association with ASD in offspring. Studies that measured supplement intake or biomarkers during second trimester reported no association (39) (37, 69). Interestingly, some of these studies reported a protective effect earlier during pregnancy (37, 39). Based on these findings, it can be speculated that there may be unique window of opportunities, during which folate may play a role in ASD (36, 69, 70); however, this has not been systematically examined. Hence, there is a critical research need to understand the relationship between folate and vitamin B12 status at different time points (including preconception, first, second and third trimesters) and subsequent ASD risk.
6. In addition to the ones mentioned already, below are some mechanistic research questions, if explored could provide additional insights on the pathways involving folate/folic acid.
    a. Our study demonstrated the joint effect of elevated folate and vitamin B12 in adversely affecting ASD risk. Although it is well known that folate and vitamin B12 are intricately involved in the one-carbon metabolism, the underlying mechanism that may exacerbate ASD risk is unknown and is worth investigating.
    b. Animal models have suggested that high folic acid supplementation during gestation modulates gene expression and alter brain development and behavior

in offspring (70-72). These findings need to be replicated in humans to understand if similar epigenetic alterations occur.

c. Animal models that assess individual nutrient's effect (folate, vitamin B12) on brain development may be required to unravel the role of each one carbon metabolite during various stages of neurodevelopment (73).

7.3.2 Leptin, Adiponectin and ASD

1. This dissertation presents a new finding that leptin and adiponectin, prior to ASD diagnosis, are independently associated with ASD. However, there is a lack of clarity whether abnormal levels of these adipokines is deterministic of ASD or is it merely associated with the underlying condition (57).
2. There is an urgent need to conduct additional studies to further understand the impact of these adipokine levels on neurological consequences (74), as well as the mechanism behind the role of adipokines in mediating these relationships.
3. Several studies, including ours, have suggested immune system aberrations in ASD. But there is limited understanding on the relationships between immune profiles and ASD symptoms and severity (58, 75). In addition, it may also be worthwhile to explore if abnormalities in adipokines are observed commonly in the presence of certain co-morbidities (e.g. gastrointestinal dysfunction, sleep disorder) (58) or other inflammatory cytokine aberrations.
4. Based on the findings from this study, we have theorized the possibility of considering leptin and adiponectin as early life biomarkers of ASD with full cognizance about the critical steps that are required to validate leptin and/or adiponectin as a useful

biomarker of ASD. If additional studies support our findings, research can be undertaken to validate these biomarkers.

5. Many studies, including ours, have demonstrated that catch-up growth during infancy is associated with subsequent ASD diagnosis. If catch-up growth is important, does timing or magnitude of catch-up growth matter in the context of ASD? Also, can rapid weight gain (or other auxological parameters) individually or in combination with leptin reliably predict ASD?
6. Adiponectin circulates in multiple forms, with high-molecular weight adiponectin considered to be the active form (76). Although this study assessed total adiponectin, it has been suggested that the biological effects may vary by multimeric complex (77). The role of these multimeric adiponectin complexes has not been assessed in the context of ASD and is an important research gap to pursue.

The research gaps or questions stated above are just examples of future research possibilities that are informed and stimulated by this dissertation. At present, one of the biggest challenges in this current research is the inability to temporally attribute neurobehavioral outcomes to early nutritional exposures or metabolic imbalances that may have happened years ago. There are multiple levels between the actual insult (be it genetic, epigenetic or environment alteration) and the behavioral manifestation, with no direct evident link in between each layer. Further, anatomic, spectroscopic or functional neuroimaging techniques are not sensitive enough to detect subtle alterations in brain structure due to sub-optimal nutritional exposures or immunological aberration (78, 79). One option to circumvent these

challenges is for researchers to rely on a layered, multidisciplinary approach (as proposed in chapter 2) using developmentally appropriate pre-clinical models to provide the plausible biological proof for the role of sub-optimal nutrition or adipokine alteration in neurobehavioral manifestation in ASD (78-80). I foresee that the coming decade will be an exciting time to further the understanding of the ASD etiology, by leveraging the rapid advancement in biomedical research and biotechnology especially in large prospective birth cohorts.

7.4 References

1. Johnston RB, Jr. Will increasing folic acid in fortified grain products further reduce neural tube defects without causing harm?: consideration of the evidence. Pediatr Res. 2008;63(1):2-8.
2. Weissenborn A, Ehlers A, Hirsch-Ernst KI, Lampen A, Niemann B. [A two-faced vitamin : Folic acid - prevention or promotion of colon cancer?]. Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz. 2017.
3. Cornel MC, de Smit DJ, de Jong-van den Berg LT. Folic acid--the scientific debate as a base for public health policy. Reprod Toxicol. 2005;20(3):411-5.
4. Plumptre L, Masih SP, Ly A, Aufreiter S, Sohn KJ, Croxford R, et al. High concentrations of folate and unmetabolized folic acid in a cohort of pregnant Canadian women and umbilical cord blood. The American journal of clinical nutrition. 2015;102(4):848-57.
5. Pfeiffer CM, Hughes JP, Lacher DA, Bailey RL, Berry RJ, Zhang M, et al. Estimation of trends in serum and RBC folate in the U.S. population from pre- to postfortification using assay-adjusted data from the NHANES 1988-2010. J Nutr. 2012;142(5):886-93.
6. Au KS, Findley TO, Northrup H. Finding the genetic mechanisms of folate deficiency and neural tube defects-Leaving no stone unturned. Am J Med Genet A. 2017;173(11):3042-57.
7. Mitchell LE. Folic Acid for the Prevention of Neural Tube Defects: The US Preventive Services Task Force Statement on Folic Acid Supplementation in the Era of Mandatory Folic Acid Fortification. JAMA Pediatr. 2017.
8. Administration USFD. FDA approves folic acid fortification of corn masa flour. FDA; 2016.
9. Kalmbach RD, Choumenkovitch SF, Troen AM, D'Agostino R, Jacques PF, Selhub J. Circulating folic acid in plasma: relation to folic acid fortification. The American journal of clinical nutrition. 2008;88(3):763-8.
10. Smith AD, Kim YI, Refsum H. Is folic acid good for everyone? The American journal of clinical nutrition. 2008;87(3):517-33.
11. Paniz C, Bertinato JF, Lucena MR, De Carli E, Amorim P, Gomes GW, et al. A Daily Dose of 5 mg Folic Acid for 90 Days Is Associated with Increased Serum Unmetabolized Folic Acid and Reduced Natural Killer Cell Cytotoxicity in Healthy Brazilian Adults. J Nutr. 2017;147(9):1677-85.
12. Yajnik CS, Deshpande SS, Jackson AA, Refsum H, Rao S, Fisher DJ, et al. Vitamin B12 and folate concentrations during pregnancy and insulin resistance in the offspring: the Pune Maternal Nutrition Study. Diabetologia. 2008;51(1):29-38.
13. Valera-Gran D, Garcia de la Hera M, Navarrete-Munoz EM, Fernandez-Somoano A, Tardon A, Julvez J, et al. Folic acid supplements during pregnancy and child psychomotor development after the first year of life. JAMA Pediatr. 2014;168(11):e142611.
14. Valera-Gran D, Navarrete-Munoz EM, Garcia de la Hera M, Fernandez-Somoano A, Tardon A, Ibarluzea J, et al. Effect of maternal high dosages of folic acid supplements on neurocognitive development in children at 4-5 y of age: the prospective birth cohort Infancia y Medio Ambiente (INMA) study. The American journal of clinical nutrition. 2017;106(3):878-87.
15. Choi JH, Yates Z, Veysey M, Heo YR, Lucock M. Contemporary issues surrounding folic Acid fortification initiatives. Prev Nutr Food Sci. 2014;19(4):247-60.
16. Selhub J, Rosenberg IH. Excessive folic acid intake and relation to adverse health outcome. Biochimie. 2016;126:71-8.

17. Pfeiffer CM, Sternberg MR, Fazili Z, Yetley EA, Lacher DA, Bailey RL, et al. Unmetabolized folic acid is detected in nearly all serum samples from US children, adolescents, and adults. J Nutr. 2015;145(3):520-31.
18. Palchetti CZ, Paniz C, de Carli E, Marchioni DM, Colli C, Steluti J, et al. Association between Serum Unmetabolized Folic Acid Concentrations and Folic Acid from Fortified Foods. J Am Coll Nutr. 2017;36(7):572-8.
19. Crider KS, Bailey LB, Berry RJ. Folic acid food fortification-its history, effect, concerns, and future directions. Nutrients. 2011;3(3):370-84.
20. Wiens D, DeSoto MC. Is High Folic Acid Intake a Risk Factor for Autism?-A Review. Brain Sci. 2017;7(11).
21. Boyles AL, Yetley EA, Thayer KA, Coates PM. Safe use of high intakes of folic acid: research challenges and paths forward. Nutrition reviews. 2016;74(7):469-74.
22. Branum AM, Bailey R, Singer BJ. Dietary supplement use and folate status during pregnancy in the United States. J Nutr. 2013;143(4):486-92.
23. Hermoso M, Vollhardt C, Bergmann K, Koletzko B. Critical micronutrients in pregnancy, lactation, and infancy: considerations on vitamin D, folic acid, and iron, and priorities for future research. Annals of nutrition & metabolism. 2011;59(1):5-9.
24. Cummings D, Dowling KF, Silverstein NJ, Tanner AS, Eryilmaz H, Smoller JW, et al. A Cross-Sectional Study of Dietary and Genetic Predictors of Blood Folate Levels in Healthy Young Adults. Nutrients. 2017;9(9).
25. Quinlivan EP, Gregory JF, 3rd. Effect of food fortification on folic acid intake in the United States. The American journal of clinical nutrition. 2003;77(1):221-5.
26. Rosenberg I. Getting folic acid nutrition right. The American journal of clinical nutrition. 2010;91(1):3-4.
27. Sweeney MR, Staines A, Daly L, Traynor A, Daly S, Bailey SW, et al. Persistent circulating unmetabolised folic acid in a setting of liberal voluntary folic acid fortification. Implications for further mandatory fortification? BMC Public Health. 2009;9:295.
28. Reynolds EH. What is the safe upper intake level of folic acid for the nervous system? Implications for folic acid fortification policies. Eur J Clin Nutr. 2016;70(5):537-40.
29. Rogers EJ. Has enhanced folate status during pregnancy altered natural selection and possibly Autism prevalence? A closer look at a possible link. Med Hypotheses. 2008;71(3):406-10.
30. McStay CL, Prescott SL, Bower C, Palmer DJ. Maternal Folic Acid Supplementation during Pregnancy and Childhood Allergic Disease Outcomes: A Question of Timing? Nutrients. 2017;9(2).
31. Raghavan R, Ashour FS, Bailey R. A Review of Cutoffs for Nutritional Biomarkers. Adv Nutr. 2016;7(1):112-20.
32. WHO. Serum and red blood cell folate concentrations for assessing folate status in populations. Vitamin and Mineral Nutrition Information System [Internet]. 2012. Available from: http://apps.who.int/iris/bitstream/10665/75584/1/WHO_NMH_NHD_EPG_12.1_eng.pdf.
33. Daniels JL. Considerations for Studying Folate Beyond the Typical Range of Exposure. Paediatric and perinatal epidemiology. 2017.
34. Viswanathan M, Treiman KA, Kish-Doto J, Middleton JC, Coker-Schwimmer EJ, Nicholson WK. Folic Acid Supplementation for the Prevention of Neural Tube Defects: An Updated

Evidence Report and Systematic Review for the US Preventive Services Task Force. Jama. 2017;317(2):190-203.
35. Viswanathan M, Treiman KA, Doto JK, Middleton JC, Coker-Schwimmer EJL, Nicholson WK. Folic Acid Supplementation: An Evidence Review for the US Preventive Services Task Force. U.S. Preventive Services Task Force Evidence Syntheses, formerly Systematic Evidence Reviews. Rockville (MD)2017.
36. Levine SZ, Kodesh A, Viktorin A, Smith L, Uher R, Reichenberg A, et al. Association of Maternal Use of Folic Acid and Multivitamin Supplements in the Periods Before and During Pregnancy With the Risk of Autism Spectrum Disorder in Offspring. JAMA Psychiatry. 2018.
37. Schmidt RJ, Hansen RL, Hartiala J, Allayee H, Schmidt LC, Tancredi DJ, et al. Prenatal vitamins, one-carbon metabolism gene variants, and risk for autism. Epidemiology. 2011;22(4):476-85.
38. Schmidt RJ, Tancredi DJ, Ozonoff S, Hansen RL, Hartiala J, Allayee H, et al. Maternal periconceptional folic acid intake and risk of autism spectrum disorders and developmental delay in the CHARGE (CHildhood Autism Risks from Genetics and Environment) case-control study. The American journal of clinical nutrition. 2012;96(1):80-9.
39. Suren P, Roth C, Bresnahan M, Haugen M, Hornig M, Hirtz D, et al. Association between maternal use of folic acid supplements and risk of autism spectrum disorders in children. Jama. 2013;309(6):570-7.
40. Steenweg-de Graaff J, Ghassabian A, Jaddoe VW, Tiemeier H, Roza SJ. Folate concentrations during pregnancy and autistic traits in the offspring. The Generation R Study. European journal of public health. 2015;25(3):431-3.
41. Biesalski HK, Drewnowski A, Dwyer J, Strain JJ, Weber P, Eggersdorfer M, editors. Sustainable Nutrition in a Changing World. Cambridge, UK: Library of Congress; 2017.
42. Olsen SF, Knudsen VK. Folic acid for the prevention of neural tube defects: the Danish experience. Food and nutrition bulletin. 2008;29(2 Suppl):S205-9.
43. Lizer MH, Bogdan RL, Kidd RS. Comparison of the frequency of the methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism in depressed versus nondepressed patients. J Psychiatr Pract. 2011;17(6):404-9.
44. Pu D, Shen Y, Wu J. Association between MTHFR gene polymorphisms and the risk of autism spectrum disorders: a meta-analysis. Autism Res. 2013;6(5):384-92.
45. Pfeiffer CM, Sternberg MR, Schleicher RL, Haynes BM, Rybak ME, Pirkle JL. The CDC's Second National Report on Biochemical Indicators of Diet and Nutrition in the U.S. Population is a valuable tool for researchers and policy makers. J Nutr. 2013;143(6):938S-47S.
46. Green R. Is it time for vitamin B-12 fortification? What are the questions? The American journal of clinical nutrition. 2009;89(2):712S-6S.
47. Allen LH, Rosenberg IH, Oakley GP, Omenn GS. Considering the case for vitamin B12 fortification of flour. Food and nutrition bulletin. 2010;31(1 Suppl):S36-46.
48. Refsum H, Smith AD. Are we ready for mandatory fortification with vitamin B-12? The American journal of clinical nutrition. 2008;88(2):253-4.
49. Carmel R. Biomarkers of cobalamin (vitamin B-12) status in the epidemiologic setting: a critical overview of context, applications, and performance characteristics of cobalamin, methylmalonic acid, and holotranscobalamin II. The American journal of clinical nutrition. 2011;94(1):348S-58S.

50. Grossfeld A, Dekel S, Lerman Y, Sherman S, Atzmony L, Salai M, et al. Symptomatic venous thromboembolism in elderly patients following major orthopedic surgery of the lower limb is associated with elevated vitamin B12 serum levels. Clin Biochem. 2013;46(1-2):54-8.
51. Ermens AA, Vlasveld LT, Lindemans J. Significance of elevated cobalamin (vitamin B12) levels in blood. Clin Biochem. 2003;36(8):585-90.
52. Arendt JFH, Farkas DK, Pedersen L, Sorensen HT. Elevated plasma vitamin B12 levels and risk of venous thromboembolism among cancer patients: A population-based cohort study. Thromb Res. 2017;156:177-83.
53. Supplements OoD. Vitamin B12: Office of Dietary Supplements, National Institues of Health; 2016 [Available from: https://ods.od.nih.gov/factsheets/VitaminB12-HealthProfessional/ - en5.
54. Cosar A, Ozcan O. Serum vitamin B12 concentrations in elderly patients with symptomatic thromboembolism after orthopedic surgery. Clin Biochem. 2013;46(9):838.
55. Valente E, Scott JM, Ueland PM, Cunningham C, Casey M, Molloy AM. Diagnostic accuracy of holotranscobalamin, methylmalonic acid, serum cobalamin, and other indicators of tissue vitamin B(1)(2) status in the elderly. Clin Chem. 2011;57(6):856-63.
56. Cappello S, Cereda E, Rondanelli M, Klersy C, Cameletti B, Albertini R, et al. Elevated Plasma Vitamin B12 Concentrations Are Independent Predictors of In-Hospital Mortality in Adult Patients at Nutritional Risk. Nutrients. 2016;9(1).
57. Goldani AA, Downs SR, Widjaja F, Lawton B, Hendren RL. Biomarkers in autism. Front Psychiatry. 2014;5:100.
58. Masi A, Glozier N, Dale R, Guastella AJ. The Immune System, Cytokines, and Biomarkers in Autism Spectrum Disorder. Neurosci Bull. 2017;33(2):194-204.
59. Falkmer T, Anderson K, Falkmer M, Horlin C. Diagnostic procedures in autism spectrum disorders: a systematic literature review. Eur Child Adolesc Psychiatry. 2013;22(6):329-40.
60. Lau C, Rogers JM, Desai M, Ross MG. Fetal programming of adult disease: implications for prenatal care. Obstet Gynecol. 2011;117(4):978-85.
61. Young L, Embleton ND, McGuire W. Nutrient-enriched formula versus standard formula for preterm infants following hospital discharge. Cochrane Database Syst Rev. 2016;12:CD004696.
62. Cockerill J, Uthaya S, Dore CJ, Modi N. Accelerated postnatal head growth follows preterm birth. Arch Dis Child Fetal Neonatal Ed. 2006;91(3):F184-7.
63. Singhal A, Cole TJ, Fewtrell M, Deanfield J, Lucas A. Is slower early growth beneficial for long-term cardiovascular health? Circulation. 2004;109(9):1108-13.
64. Nunes M, da Silva CH, Bosa VL, Bernardi JR, Werlang ICR, Goldani MZ, et al. Could a remarkable decrease in leptin and insulin levels from colostrum to mature milk contribute to early growth catch-up of SGA infants? BMC Pregnancy Childbirth. 2017;17(1):410.
65. Houk CP, Lee PA. Early diagnosis and treatment referral of children born small for gestational age without catch-up growth are critical for optimal growth outcomes. Int J Pediatr Endocrinol. 2012;2012(1):11.
66. Takeuchi A, Yorifuji T, Nakamura K, Tamai K, Mori S, Nakamura M, et al. Catch-Up Growth and Neurobehavioral Development among Full-Term, Small-for-Gestational-Age Children: A Nationwide Japanese Population-Based Study. J Pediatr. 2018;192:41-6 e2.

67. Cho WK, Suh BK. Catch-up growth and catch-up fat in children born small for gestational age. Korean J Pediatr. 2016;59(1):1-7.
68. Vickers MH. Developmental programming and adult obesity: the role of leptin. Curr Opin Endocrinol Diabetes Obes. 2007;14(1):17-22.
69. Braun JM, Froehlich T, Kalkbrenner A, Pfeiffer CM, Fazili Z, Yolton K, et al. Brief report: are autistic-behaviors in children related to prenatal vitamin use and maternal whole blood folate concentrations? J Autism Dev Disord. 2014;44(10):2602-7.
70. Barua S, Chadman KK, Kuizon S, Buenaventura D, Stapley NW, Ruocco F, et al. Increasing maternal or post-weaning folic acid alters gene expression and moderately changes behavior in the offspring. PloS one. 2014;9(7):e101674.
71. Barua S, Kuizon S, Brown WT, Junaid MA. High Gestational Folic Acid Supplementation Alters Expression of Imprinted and Candidate Autism Susceptibility Genes in a sex-Specific Manner in Mouse Offspring. J Mol Neurosci. 2016;58(2):277-86.
72. Barua S, Kuizon S, Brown WT, Junaid MA. DNA Methylation Profiling at Single-Base Resolution Reveals Gestational Folic Acid Supplementation Influences the Epigenome of Mouse Offspring Cerebellum. Front Neurosci. 2016;10:168.
73. Fuglestad AJ, Rao R, Georgieff MK. The role of nutrition in cognitive development. In: Handbook in developmental cognitive neuroscience. Cambridge, MA: MIT Press; 2008. Available from: http://hirezz.com/satvenandmer.com/pdf/dha/Nutrition and Cognitive Development .pdf.
74. Goines PE, Ashwood P. Cytokine dysregulation in autism spectrum disorders (ASD): possible role of the environment. Neurotoxicol Teratol. 2013;36:67-81.
75. Ashwood P, Kwong C, Hansen R, Hertz-Picciotto I, Croen L, Krakowiak P, et al. Brief report: plasma leptin levels are elevated in autism: association with early onset phenotype? J Autism Dev Disord. 2008;38(1):169-75.
76. Aso Y, Yamamoto R, Wakabayashi S, Uchida T, Takayanagi K, Takebayashi K, et al. Comparison of serum high-molecular weight (HMW) adiponectin with total adiponectin concentrations in type 2 diabetic patients with coronary artery disease using a novel enzyme-linked immunosorbent assay to detect HMW adiponectin. Diabetes. 2006;55(7):1954-60.
77. Mazaki-Tovi S, Romero R, Vaisbuch E, Erez O, Mittal P, Chaiworapongsa T, et al. Dysregulation of maternal serum adiponectin in preterm labor. J Matern Fetal Neonatal Med. 2009;22(10):887-904.
78. Cusick SE, Georgieff MK. The Role of Nutrition in Brain Development: The Golden Opportunity of the "First 1000 Days". J Pediatr. 2016;175:16-21.
79. Georgieff MK. Nutrition and the developing brain: nutrient priorities and measurement. The American journal of clinical nutrition. 2007;85(2):614S-20S.
80. Georgieff MK. Iron assessment to protect the developing brain. The American journal of clinical nutrition. 2017;106(Suppl 6):1588S-93S.

CHAPTER 8

Conclusions

This concluding chapter provides a summary of the key findings and reviews the strengths and limitations of the dissertation.

8.1 Summary of Findings

This dissertation prospectively investigated the relationship between nutritional and metabolic factors and risk of ASD, using a life-course framework. The analysis was organized around three aims:

1. To evaluate the associations between maternal multivitamin supplement intake, B-vitamin biomarkers (plasma folate and vitamin B12) and ASD risk in children
2. To evaluate the association between cord blood, early childhood plasma leptin, fetal growth and first year weight gain pattern, and risk of ASD in children
3. To evaluate the association between cord blood, early childhood plasma adiponectin and risk of ASD in children

To address these aims, I used data from the BBC, a large well-established prospective birth cohort drawn from urban, low-income minority population seeking obstetric and pediatric health care at the BMC. The BBC is an ASD enriched-cohort due to oversampling of babies born preterm. The findings from this study have relevance to high risk, low-income population in Boston and elsewhere in the U.S. with similar characteristics.

8.1.1 Key Findings

The analyses for Specific aim 1 explored the association between maternal multivitamin supplement intake, maternal plasma folate and vitamin B12 measured at delivery and risk of ASD in the offspring. There was a “U shaped” relationship between multivitamin supplement intake during pregnancy and ASD risk in children. Mothers with moderate multivitamin

supplement intake (3-5 times/week) during pregnancy were associated with the lowest risk of ASD. Using this as the reference group, the risk of ASD in children was elevated among those mothers that had low (≤2 times/week) or high levels (>5 times/week) of multivitamin supplement intake. Maternal folate levels, categorized using the WHO cut off (<13.5 nmol/L – possible deficiency; 13.5-45.3 nmol/L – normal range; >45.3nmol/L – elevated) was not associated with an altered ASD risk. However, very high maternal plasma folate (≥60.3 nmol/L) at delivery was associated with an increased ASD risk, compared to those that had plasma folate in the middle 80th percentile. Elevated plasma vitamin B12 (≥536.8 pmol/L) was also associated with an increased ASD risk.

The analyses for Specific aim 2 assessed the association between leptin, early life growth and ASD and whether leptin mediated the relationship between early life growth and ASD. When compared to those whose growth was on track, infants with extremely rapid weight gain (weight gain *z* score >1.28) during first year of life was associated with a greater ASD risk. Similarly, early life elevated plasma leptin levels, prior to ASD diagnosis, were associated with an increased ASD risk. However, fetal growth and cord leptin levels did not predict ASD risk in this study. Early childhood plasma leptin levels mediated the association between rapid weight gain in infancy and ASD risk. Our findings are consistent with the previous studies that have reported that children with ASD subjects experience rapid growth in early life. In addition to prospectively reporting the association between early childhood leptin and ASD, this study provided a mechanistic insight on the role of leptin in mediating the relationship between accelerated infant weight gain and ASD.

The analyses for Specific aim 3 assessed the relationship between cord and early childhood adiponectin and subsequent ASD risk in children. In this study, we showed that cord adiponectin, an anti-inflammatory cytokine was associated with ASD risk. Specifically, a dose-response relationship was observed and children with highest cord adiponectin levels at birth had the lowest subsequent ASD risk. Early childhood adiponectin, prior to ASD diagnosis, was associated with ASD risk; but this association disappeared after adjusting for cord adiponectin, possibly suggesting a tracking of adiponectin levels from birth to early childhood. The association between cord adiponectin and ASD risk was independent of preterm birth, early childhood plasma adiponectin and many other known risk factors.

8.1.2 Major Contributions to autism research

This dissertation, for the first time, assessed and demonstrated an association between elevated maternal plasma folate and ASD risk in offspring. From a public health perspective, this is an important topic to address, given that at least a fraction of women of reproductive age in the U.S. may consume surplus amounts of folic acid from multiple sources (e.g. prenatal supplement intake, foods fortified and enriched with folic acid), in addition to the natural folate from the diet. We also addressed a novel question on the association between maternal plasma vitamin B12 and ASD risk in children, which, to our knowledge, has never been reported before. Additionally, we showed that a combination of elevated maternal plasma folate and plasma vitamin B12 further increased ASD risk in children. This dissertation was uniquely poised to study ASD risk by assessing both prenatal supplement intake and maternal biomarkers of B vitamins, which provides complementary measures of maternal folate and B12 nutritional status during preconception and each trimester of pregnancy.

Emerging research suggests that leptin, a pro-inflammatory cytokine, may be altered in children with ASD; however, this was based on findings from cross-sectional studies. In this dissertation, we were able to extend the existing knowledge by prospectively considering leptin at two time points (at birth and early childhood, before ASD diagnosis). We also confirmed the previous findings that accelerated weight gain during infancy is linked to increased ASD risk. Although the role of adipokines in ASD has been speculated, our study for the first time provided evidence that leptin may mediate the relationship between accelerated infant growth and increased ASD risk.

Finally, adiponectin's role in ASD has not been longitudinally assessed. This is the first study to demonstrate that cord adiponectin, an anti-inflammatory cytokine, was associated with lower risk of ASD in a dose-response fashion, which is independent of preterm birth, postnatal plasma adiponectin levels, and other known risk factors of ASD. Our research has also paved way for future studies to explore the possibility of considering leptin and adiponectin as early predictive biomarkers of ASD and as therapeutic targets.

8.2 Limitations and Strengths

8.2.1 Limitations

Generalizability: The study population consists mainly of urban low-income minority women in Boston, MA. Due to the unique nature of demographic, environmental, and health service access to this population, caution has to be exercised when generalizing the findings of this study to other U.S. populations with different characteristics. Further, biological differences associated with race-ethnicity (e.g. difference in allele frequency of *MTHFR* gene

polymorphisms between non-Hispanic whites, Hispanics and non-Hispanic blacks) has to be considered when interpreting the results and generalizing the study findings.

Selection bias and differential follow-up: The initial participation rate in the BBC was high, but only a subset continued to receive pediatric care at the BMC and they were the ones that were followed in this cohort. Differential follow-up might have occurred for some participants due to a variety of reasons including – 1) children missing their regular health checkup because of their optimum health status, 2) parents' ignorance, 3) migration and 4) changing of hospitals. The latter option is unlikely because majority of families (>90%) in this cohort qualify for Medicaid (1) and BMC is the primary health care provider of Medicaid in Boston. To understand the impact of differential follow-up, analyses were conducted in this cohort comparing children included and excluded from current analyses in terms of characteristics at baseline and the results did not show statistically significant differences (2).

Residual confounding: This study examined the prospective association between independent variables (such as maternal plasma folate; vitamin B12; cord and early childhood leptin and adiponectin; fetal growth and first year weight gain) and risk of ASD. Despite the evidence of temporal and dose-response relationships, it is important to regard our findings as associations rather than indication of causality. Although the analyses in this study controlled for potential confounders based on key covariates identified in the literature, there might still have been other unmeasured variables that could have influenced the outcome. One example is that fat mass was not directly assessed at the time when leptin was measured and this could have resulted in some residual confounding.

Measurement of key exposure variables: Maternal plasma folate and vitamin B12 were measured 24-72 hours after delivery, which may be reflective of maternal biomarker levels during the third trimester, at best. Plasma folate is a marker of recent dietary intake exposure and may not reflect the long-term status. Due to the cost of biomarker assay, we only measured folate and B12 level in a subset of the study sample. Not including dietary data (such as folate from natural foods as well as those that are fortified and enriched with folic acid) along with supplement intake and biomarker data for the entire sample could be viewed as a limitation.

Additionally, metabolic biomarkers – leptin and adiponectin levels were random, non-fasting measurements and this could have introduced noise and could have biased the results towards null (3, 4). The models for leptin and adiponectin accounted for breastfeeding vs. formula feeding, but more research is required to examine the influences of perinatal nutrition on these biomarkers. Our study measured total adiponectin and not the distinct forms such as the low-molecular weight trimmers, medium-molecular-weight hexamers and high-molecular-weight oligomers. These individual forms of adiponectin could possess diverse roles, and this needs further exploration in the context of ASD. Finally, maternal BMI was calculated based on self-reported height and weight and may have been subjected to reporting bias (4).

Identification of ASD cases: Case and neurotypical development classification was based on physician diagnosis as documented in EMR, which could have possibly resulted in outcome misclassification in unpredictable ways. However, additional sensitivity analyses yielded consistent results when using more stringent outcome classification for both cases and neurotypical children. The relatively small sample size of ASD subjects could have resulted in

imprecise estimates. In this study, ASD was defined using DSM-IV rather than newer DSM-5, since ICD-9 codes were used to diagnose autism. However, studies have shown that there is continuity between DSM-IV and DSM-5 and that the findings from DSM-IV would still be relevant (5).

8.2.2 Strengths

Study Design: The longitudinal design of the BBC allowed us to address the study hypotheses in a prospective fashion. Most of the studies that assessed metabolic biomarkers and ASD were cross-sectional and were conducted after ASD diagnosis. To our knowledge, this is the first time a study has prospectively assessed the relationship between cord leptin and adiponectin (assessed at two-time points) in the context of ASD. Further, assessing these metabolic biomarkers in cord blood and early childhood, prior to ASD diagnosis, helped understand how these biomarkers may be altered during different sensitive periods of development and whether this might influence its association with ASD.

Multimethod data collection and availability of nutritional and metabolic biomarkers: The BBC combines different types of data collection including self-report, standardized questionnaire and biomarker data in mothers and children from prenatal, perinatal and postnatal periods. Triangulating data from multiple sources helped maintain data quality, tapped into the unique strengths of different assessment methods and possibly shedding light on different mechanisms, that can be further explored in animal models and human studies. For example, in the BBC, maternal nutritional status was assessed using self-reported multivitamin intake during pregnancy as well as using nutritional biomarker data measured at delivery, which complemented well with one another in providing a reasonable assessment of B

vitamin nutritional status. Early life longitudinal growth data along with leptin measurements provided mechanistic insights into metabolic pathways implicated in ASD. Similarly, considering adiponectin and preterm delivery together gave a unique perspective on their independent and joint roles in the etiology of ASD.

Understudied US minorities: This dissertation focused on an understudied population in urban Boston, mainly comprised of blacks and Hispanics, who have high prevalence of maternal metabolic conditions, preterm deliveries and ASD. Given the dearth of research on this minority population, addressing key nutritional and metabolic factors from a life-course perspective has shed new light on ASD risk factors, which may have implications for research and prevention of ASD in this vulnerable population and beyond.

8.3 Conclusion

Since Leo Kanner first described autism 75 years ago, the field has made great strides in elucidating the underpinnings of ASD. Although notable progress has been made in ASD diagnosis and treatment (6), the cause is still elusive. In pursuit of understanding the etiology of ASD, this dissertation focused on three important, yet understudied questions. The findings from our study are not deterministic, but have contributed to the field in many ways. Specifically, some results confirmed previous research (e.g. accelerated growth during infancy and ASD, maternal supplement intake and ASD); others extended existing knowledge (e.g. cord adiponectin and early childhood adiponectin association with ASD), while others were completely new and cutting-edge (e.g. excessive plasma folate, vitamin B12 levels and ASD risk; leptin mediating the association of rapid infant weight gain and ASD; mechanistic role of adiponectin in preterm and ASD). This is an exciting time for autism research and the field of

early life origins of ASD is brimming with new research in this era of rapid advancement in biomedical research and biotechnology. The accumulating evidence and newer insights contributed by studies like ours and others will push the field forward and ultimately help to solve the autism puzzle.

8.4 References

1. Nachman RM, Mao G, Zhang X, Hong X, Chen Z, Soria CS, et al. Intrauterine Inflammation and Maternal Exposure to Ambient PM2.5 during Preconception and Specific Periods of Pregnancy: The Boston Birth Cohort. Environmental health perspectives. 2016;124(10):1608-15.
2. Kumar R, Tsai HJ, Hong X, Liu X, Wang G, Pearson C, et al. Race, ancestry, and development of food-allergen sensitization in early childhood. Pediatrics. 2011;128(4):e821-9.
3. Wang G, Divall S, Radovick S, Paige D, Ning Y, Chen Z, et al. Preterm birth and random plasma insulin levels at birth and in early childhood. Jama. 2014;311(6):587-96.
4. Wang G, Hu FB, Mistry KB, Zhang C, Ren F, Huo Y, et al. Association Between Maternal Prepregnancy Body Mass Index and Plasma Folate Concentrations With Child Metabolic Health. JAMA Pediatr. 2016;170(8):e160845.
5. Mazefsky CA, McPartland JC, Gastgeb HZ, Minshew NJ. Brief report: comparability of DSM-IV and DSM-5 ASD research samples. J Autism Dev Disord. 2013;43(5):1236-42.
6. Schaaf CP, Zoghbi HY. Solving the autism puzzle a few pieces at a time. Neuron. 2011;70(5):806-8.

CURRICULUM VITEA

Ramkripa Raghavan
born on May 9, 1981 in Chennai, India
married Nov 15, 2004 to Srikanth Raghavan in Chennai, India

22948 Green Teal Ct
Ashburn, VA 20148
(301) 385 8768
Ramkripa@gmail.com

EDUCATION

Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
Department of Population, Family and Reproductive Health
DrPH candidate
Entered in Fall 2014

Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
Department of Population, Family and Reproductive Health
MPH, May 2008

University of Madras, Chennai, India
Masters in Food and Nutrition, May 2003

University of Madras, Chennai, India
Bachelors in Nutrition, Food Service Management and Dietetics May 2001

WORK EXPERIENCE

Subject Matter Expert and Lead Analyst, Center for Nutrition Policy and Promotion
(Feb 2016 – current; 40 hours/week)

- Lead systematic reviews to support the foundational work for developing dietary guidelines for pregnant women in the U.S.
- Lead systematic reviews on assessing the impact of school-based nutrition programs in developing countries
- Facilitate development of questionnaire content for NHANES
- Conduct systematic review methodological research
- Analyze NHANES data to measure the reach of MyPlate in the U.S. population
- Assess racial/ethnic similarities in HEI score using NHANES data in U.S. children

Consultant, Global Alliance for Improved Nutrition (GAIN)
(May 2015 – May 2016; 20 hours/week)

- Analyze national survey data to understand the impact of vitamin A fortification of vegetable oil in Bangladesh
- Perform secondary analysis on Senegal food fortification dataset to assess methodological differences between food frequency questionnaire and household estimation for capturing daily oil consumption

Research Assistant, Johns Hopkins Bloomberg School of Public Health
(Nov 2014 – June 2016; 20 hours/week)

- Analyze birth cohort data to understand the micronutrient status of urban low-income minority population in the U.S.
- Explore the relationship between maternal prenatal micronutrient status and offspring neurocognitive outcomes

Project Manager, *Eunice Kennedy Shriver* National Institutes of Child Health & Human Development (NICHD), NIH
(Aug 2010 – Jan 2015; 40 hours/week)

- Managed the Biomarkers of Nutrition for Development (BOND) program, designed to harmonize the processes for making decisions on nutritional biomarkers for research, program development and evaluation and generation of evidence based policy
- Managed the "B-24 Project" designed to evaluate the evidence base to support the inclusion of infants and children from birth to 24 months of age in the Dietary Guidelines for Americans
- Managed the "Pre-B" project designed to assess the current knowledge on the nutrient needs and guidelines for nutritional care of preterm infants
- Collaborated with WHO on rolling out nutrition guidelines for adolescents and adults with HIV/AIDS
- Represent NICHD/NIH on the Micronutrient Forum Communications advisory committee
- Provided mentoring and supervised staff, interns and NIH's presidential management fellows

Science Policy Analyst, Fogarty International Center (FIC), NIH
(Jan 2008 – Sep 2010; 40 hours/week)

- Coordinated program evaluation activities for FIC
- Designed and managed an online database that tracks the careers and accomplishments of more than 5000 FIC/NIH trainees
- Performed quantitative analysis on FIC trainee data, FIC extramural investments and NIH's international research investment
- Provided mentoring to staff and Presidential Management Fellows

Research Associate, WESTAT, Rockville, MD (http://www.westat.com)
(March 2006- August 2007; 40 hours/week)
Worked on **Agency for Healthcare Research and Quality (AHRQ)'s** longitudinal Medical Expenditure Panel Study (MEPS). The study provided annual national estimates of the use of healthcare services, charges, quality of care for specific health conditions and payments for the services and health insurance coverage.

Roles and Responsibilities

- Analyzed data on medical events and chronic health conditions that were obtained from NHIS (National Health Interview Study)
- Interpreted results of the analysis and suggested improvements in data quality
- Conducted necessary updates to the MEPS database to facilitate further data analysis

Scientist, M.S. Swaminathan Research Foundation (http://www.mssrf.org),
Chennai, India (December 2002- September 2004; 40 hours/week)

M.S. Swaminathan Research Foundation is a premier non-profit organization that works on food security, biotechnology, ecotechnology and a variety of health related international research projects.

Roles and Responsibilities

Developed and implemented health and nutrition component of the project funded by Commonwealth of Learning, Canada

- Conducted anthropometric, biochemical and dietary analysis to examine the extent of multiple micronutrient deficiencies in children and women of reproductive age in rural India
- Worked with under privileged pregnant and lactating women to improve their health and nutritional status
- Implemented short- and long-term intervention strategies to combat micronutrient deficiencies
- Conducted outreach activities to enhance health and nutritional knowledge, attitudes and practices in rural communities
- Assessed morbidity and mortality in rural children in South India as part of implementing UN's Millennium Development Goals 4 and 5

PROFESIONNAL PUBLICATIONS

1. **Raghavan R**, Riley AW, Volk H, Caruso D, Hironaka L, Sices L, Hong X, Wang G, Ji Y, Brucato M, Wahl A, Stivers T, Pearson C, Zuckerman B, Stuart EA, Landa R, Fallin MD, Wang X. Maternal Multivitamin Intake, Plasma Folate and Vitamin B12 Levels and Autism Spectrum Disorder Risk in Offspring. Paediatric and Perinatal Epidemiology. 2018;32(1):100-111.
2. **Raghavan R**, Zuckerman B, Hong X, Wang G, Ji Y, Paige D, Zhang C, DiBari J, Fallin MD, Wang X. Fetal and infancy growth patterns, cord and early childhood plasma leptin, and development of Autism Spectrum Disorder in the Boston Birth Cohort. (Submitted to Autism Research and currently under revision).
3. Tagtow A, **Raghavan R**. Assessing the reach of MyPlate using National Health and Nutrition Examination Survey data. J Acad Nutr Diet. 2017 Feb; 117(2):181-183.
4. **Raghavan R**, Ashour F, Bailey R. A review on cut points for nutritional biomarkers. Adv Nutr. 2016; 7:1-9.
5. Bailey L.B, Stover P.J, McNulty H.M, Fenech M.F, Gregory J.F, Mills J.L, Pfeiffer C.M, Fazili Z, Zhang M, Ueland P.M, Molloy A.M, Caudill M.A, Shane B, Berry R.J, Bailey R.L, **Raghavan R**, Raiten D.J., Biomarkers of Nutrition for Development (BOND) – Folate Review. J Nutr. 2015; 145: 1635S
6. Rohner F, Zimmermann M, Jooste P, Pandav C, Caldwell K, **Raghavan R**, Raiten D., Biomarkers of Nutrition for Development – Iodine Review. J Nutr. 2014; 144: 8S.
7. Raiten D, **Raghavan R**, Kraemer K., Biomarkers in Growth. Ann Nutr Metab. 2013; 63:293-297.
8. Raiten D, **Raghavan R**, Porter A, Obbagy JE, Spahn JM., Executive Summary: Evaluating the evidence base to support the inclusion of infants and children from birth to 24 months of age in the Dietary Guidelines for Americans – "The B-24 Project". Am J Clin Nutr. 2014 Mar; 99(3):663S-91S.
9. Raiten D, **Raghavan R**, Kraemer K., Biomarkers in Growth: A brief summary of a session at the International Congress of Nutrition. Sight and Life. 2014 Jan; 27(3):72-74.
10. Raiten D, **Raghavan R**. (eds). Evaluating the Diet-Related Scientific Literature for Children from Birth to 24 Months – The B-24 Project. Am J Clin Nutr. 2014 Mar (suppl)

11. Ajuwon GA, Auston I, **Raghavan R**, Sheldon K and Hofman KJ., Assessment of Scholarly Publications of Nigerian Health Sciences Researchers in MEDLINE/PubMed (1996-2007). Sierra Leone Journal of Biomedical Research. 2011 3(2). 89-96.
12. Raiten D, **Raghavan, R.** (eds). Biomarkers of Nutrition for Development (BOND): Building a consensus. Workshop proceedings. Am J Clin Nutr. 2011 94 (suppl).
13. Raiten, D.J., **Raghavan, R.** (eds). Nutrition in Clinical Management of HIV-Infected Adolescents (>14 y old) and Adults including Pregnant and Lactating Women. Am J Clin Nutr. 2011 94 (suppl).

POSTER PRESENTATIONS

1. **Raghavan R**, Spahn J, Dreibelbis C. Identifying Needles in a Haystack: Use of text-mining and machine learning technology to improve efficiency in conducting nutrition-related systematic reviews. Poster presentation at 4th International Symposium on Systematic Review and Meta-Analysis of Laboratory Animal Studies (August, 2017)
2. Spahn J, **Raghavan R,** Obbagy J, English L. Application of text-mining and machine learning technology in nutrition systematic review screening: A pilot study. Poster presentation at 4th International Symposium on Systematic Review and Meta-Analysis of Laboratory Animal Studies (August, 2017)
3. **Raghavan R**, Schap T, Haven J, Tagtow A. Assessing the reach of MyPlate in the U.S. population: An analysis using NHANES data. Poster presentation at the Experimental Biology meeting held at Chicago, IL (April 2017)
4. **Raghavan R**, Aaron GJ, Neufeld L, Nahar B, Knowles J. Impact of vitamin A fortification of edible oil in Bangladesh. Poster presentation at the Experimental Biology meeting held at San Diego, CA (April 2016)
5. **Raghavan R**, Riley A, Caruso D, Hong X, Wang G, Ajao B, Ji Y, Li M, He H, Chen Z, Wang M, Pearson C, Hironaka K, Sices L, Fallin MD, Wang X. Maternal multivitamin supplement intake, plasma folate and vitamin B12 levels during pregnancy and risk of Autism Spectrum Disorders in offspring. Poster presentation at the Wendy Klag Center symposium held at Baltimore, MD (October, 2015)
6. **Raghavan R**, Wang G, Chen Z, Wang X. Vitamin B12, Folate and Homocysteine Levels in the U.S. Urban Minority Mothers Post Delivery. Poster presentation at the Experimental Biology meeting held at Boston, MA (March 2015)
7. Ahluwalia N, Stenberg MR, **Raghavan R**, Looker A, Pfeiffer CM. Race-Ethnic patterns in iron intake and status in U.S. women: findings from NHANES 2003-06. Oral presentation at the Experimental Biology meeting held at Boston, MS (March 2015)
8. **Raghavan R**, Raiten D. Biomarkers of Nutrition for Development (BOND): The Query Based System – presenting a new interactive resource for the community. Poster presentation at the Experimental Biology meeting, San Diego (April 2014)
9. **Raghavan R**, Raiten D. Biomarkers of Nutrition for Development (BOND): An Overview. Poster Presentation at the Healthy Diet for Healthy Living Conference, Brussels, Belgium (May 2014)
10. Ashour F, **Raghavan R**, Ross AC, Meydani S, Raiten D. Inflammation and Nutritional Science for Programs/Policies and Interpretation of Research Evidence (INSPIRE). Poster presented at the Experimental Biology meeting held at Boston, MA (April 2013)
11. **Raghavan R**, Raiten D. Biomarkers of Nutrition for Development. Poster presented at the Marker Initiative in Nutrition Research: Striving for consensus on criteria for evaluating markers meeting organized by ILSI Europe held at Lisbon, Portugal (June 2012)

12. Ashour F, Zimmermann M, Rohner F, Pandav C, Jooste P, Trumbo P, **Raghavan R**, Raiten DJ, BOND Expert Panel Deliberations. Case Study 1: Iodine Consultation. Poster presented at the Experimental Biology meeting held at San Diego, CA (April 2012)
13. Namaste SN, Ashour F, Porter A, **Raghavan R**, Pilch S, Raiten D. Effect modifiers for the safety and efficacy of iron intervention in the context of malaria: a review of data for an individual patient-based meta-analysis. Poster presented at the Experimental Biology meeting held at San Diego, CA (April 2012)

MANUSCRIPT SUBMITTED

Raghavan R, Fallin MD, Hong X, Wang G, Ji Y, Stuart EA, Paige D, Wang X. Cord and early childhood plasma adiponectin levels and autism risk: A prospective birth cohort study (submitted to the Journal of Autism and Developmental Disorders)

Raghavan R, Nahar B, Knowles J, Aaron GJ, Neufeld LM, Rahman S, Mondal P, Ahmed T. Household coverage of vitamin A fortification of edible oil in Bangladesh (submitted to PLoS One)

MANUSCRIPTS IN PREPARATION

Raghavan R, Dreibelbis C, James BJ, Wong Y, Abrams B, Gernand AD, Rasmussen KM, Siega-Riz AM, Stang J, Casavale KO, Spahn JM, Stoody EE. Dietary patterns before and during pregnancy and maternal outcomes: A systematic review

Raghavan R, Dreibelbis C, James BJ, Wong Y, Abrams B, Gernand AD, Rasmussen KM, Siega-Riz AM, Stang J, Casavale KO, Spahn JM, Stoody EE. Dietary patterns before and during pregnancy and birth outcomes: A systematic review

Raghavan R, Spahn JM**,** Dreibelbis C, et al. Application of literature scoping to assess research feasibility: a pilot test and perspective on methodology

INVITED PRESENTATIONS

1. PRFH Wednesday Noon Seminar (November 2016)
2. International Meeting for Autism Research, Baltimore, USA (May 2016)
3. Experimental Biology, San Diego, USA (April 2016)
4. Micronutrient Forum, Addis Ababa, Ethiopia (June 2014)
5. International Vitamin Conference, Washington DC (May 2014)
6. International Congress on Nutrition, Granada, Spain (September 2013)
7. Canadian Nutrition Society Annual Meeting, Quebec City, Canada (May 2013)
8. Hidden Hunger Conference, Stuttgart, Germany (March 2013)
9. Experimental Biology, San Diego, USA (April 2012)
10. ILSI Annual Meeting, Phoenix, Arizona (January 2012)
11. Atlanta Conference on Science and Innovation Policy (September 2009)

HONORS/AWARDS

- John and Alice Chenoweth-Pate Fellowship awardee, 2017
- Young Minority Investigator Oral Competition Finalist Awardee, 2016
- Bernard and Jane Guyer Scholarship awardee, 2016
- Wendy Klag Center for Autism & Developmental Disabilities awardee, 2015

- John and Alice Chenoweth-Pate Fellowship awardee, 2015
- Distinguished Achievement Award by Kelly Government Solutions, 2014
- NIH Director's Excellence Award Winner, 2013
- Distinguished Achievement Award by Kelly Government Solutions, 2013
- Distinguished Achievement Award by Kelly Government Solutions, 2012
- Fogarty International Center's Director Award, 2009

PROFESSIONAL ACTIVITIES

- American Nutrition Society
    - Co-chair of the Mini-symposium: Maternal Factors Related to Pregnancy, Lactation and Infant Health
    - Member of Nutritional Epidemiology and Maternal, Perinatal and Pediatric Research Interest Sections

PEER REVIEW ACTIVITIES

- Nutrition Reviews
- Advances in Nutrition
- Annals of Nutrition and Metabolism

STATISTICAL PACKAGE & COMPUTER KNOWLEDGE

- Expert in Microsoft Office Applications (MS Word, MS Excel, MS Power Point)
- Proficient in STATA, R
- Adept in using EndNote

LANGUAGES KNOWN

- English, Tamil and Hindi

OTHERS

- Trained in Indian classical music and have performed extensively in the U.S. and India and also in radio and reality TV shows
- Runs an Indian classical music school with the goal of propagating Indian music to children and adults in the DC metro area