IMPACT OF PRENATAL MATERNAL STRESS ON INFANT COGNITION AND MODIFICATIONS OF EFFECTS BY EXPOSURE TO PHTHALATES

BY

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DISSERTATION

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ABSTRACT

Recent literature has shown that maternal exposures to both chemical and non-chemical stressors during gestation are risk factors for adverse neurodevelopment. However, there is little evidence on the impact of these, individually or in combination, on cognitive development during the first year of life. Maternal prenatal stress is highly prevalent in the US, with up to 25% of women experiencing clinically relevant symptoms during pregnancy and/or postpartum. Phthalates are endocrine disruptors that are found in a wide range of consumer products including personal care products, children's toys, and food packaging. In an effort to characterize the effects of these stressors on more specific aspects of cognition and obtain results earlier in life that has been done in most previous studies, this dissertation used innovative measures adapted from developmental psychology to assess basic building blocks of cognition in infancy. These include physical reasoning, working memory, attention, and information processing speed. Chapter 3 presents a general profile of the Illinois Kids Development Study (IKIDS) cohort, within which this study took place. IKIDS is a large prospective cohort study of mothers and their infants that consists of mostly white non-Hispanic (80%), college educated (70%) women with household income greater than $60,000, which is reflective of the Champaign-Urbana area. Chapter 4 examines the association between prenatal maternal perceived stress and a sexually dimorphic physical reasoning task at 4.5 months of age. The results suggest that maternal perceived stress is associated with poorer physical reasoning in girls. Chapter 5 examines the associations of other maternal stress measures (stressful life events, and maternal and cord blood telomere length), and phthalate exposure individually with physical reasoning, and also the potential for cumulative effects of stress and phthalates (MEP, DEHP, DINP, anti-androgenic sum, and sum of all phthalates) on physical reasoning. The results revealed a significant association of maternal telomere length with performance on the physical reasoning task in girls. That is longer telomere length was

associated with girls looking longer at the impossible than the possible event. There were no significant associations of stressful life events or cord blood telomere length with physical reasoning. When evaluating the effects of prenatal exposure to phthalates significant associations were found between DEHP at 16-18 weeks of gestation and difference in looking time in girls, between DINP, both at 16-18 weeks and in a pooled sample representing average exposure across pregnancy, and difference in looking time in boys; between Anti-Androgenic sum in the pooled sample and difference in looking time in boys; and between the sum of all phthalates at 16-18 weeks of gestation and difference in looking time in boys. In all cases, higher prenatal exposure to phthalates was associated with boys looking longer at the possible than the impossible event. There was little indication of interactive effects between maternal stress and prenatal phthalate exposure. However, there was an interaction between prenatal maternal perceived stress and DINP in girls. Girls whose mothers were in the medium stress group and had higher exposure to DINP had a greater difference in looking time (looked longer at the impossible than the possible event). Chapter 6 examined the association between prenatal maternal stress alone and cumulative effects of maternal stress and phthalate exposure with several measures of cognition measured at 7.5 months of age. Results showed significant associations between higher perceived stress and decreased novelty preference (a measure of recognition memory) in boys only; shorter visual fixations, which may indicate faster information processing speed, in girls only; and longer trial durations, which suggests a detriment in visual attention, in boys and girls. Stressful life event models showed significant associations with greater novelty preference, shorter visual fixations times, and longer trial durations, again suggesting a detriment in visual attention. Maternal telomere length models showed significant associations of shorter maternal telomere length with greater novelty preference in girls only, and shorter visual fixation times, and longer trial durations in both boys and girls. Cord blood telomere length did not appear to be a good predictor of cognitive outcomes in infants. In addition, in analyses to explore potential cumulative effects of stress and

phthalate exposure on trial duration, there were significant interactions between stress and prenatal exposure to DINP. These results are important as they not only add to the body of evidence that prenatal maternal stress can have a negative impact on cognitive development, they also illustrate that several different measures of stress including perceived stress, actual experience of stressful life events and a biological measure of long-term chronic stress all predict cognitive outcomes infants. In particular, all of these measures predicted longer trial durations, suggesting that maternal stress is associated with detriments in visual attention. The results also reveal that an impact of maternal stress on cognition can be measured as early as 4 months of age and confirm earlier reports that there are sex differences in response to maternal stress. Furthermore, the results presented here add to the growing body of evidence that environmental factors may interact in complex ways to influence cognitive development. Future studies should consider these potential interactions when assessing its effects on neurodevelopment.

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TABLE OF CONTENTS

CHAPTER 1: A GENERAL INTRODUCTION TO THE IMPACT OF PRENATAL MATERNAL STRESS AND PHTHALATES ON NEURODEVELOPMENT

Recent literature has shown that maternal exposures to both chemical and non-chemical stressors during gestation are risk factors for adverse neurodevelopment. However, there is little evidence on the cumulative effects of these stressors. Maternal psychological stress is highly prevalent in the US, with up to 25% of women experiencing clinically significant stress or anxiety during pregnancy and/or postpartum (Kingston et al. 2015). Phthalates are endocrine disruptors that are found in a wide range of consumer products including personal care products, children's toys, food packaging, and medical tubing (Ejaredar et al. 2015). Prenatal exposure to either maternal stress or phthalates has been individually associated with adverse neurodevelopmental outcomes. These exposures are likely to co-occur, yet the potential for cumulative effects on early neurodevelopment has not been investigated. This research evaluated the impact of prenatal maternal stress on infant cognition at 4.5 and 7.5 months of age. Also, whether there are cumulative effects of exposure to prenatal perceived stress and phthalates.

1.1. IMPACT OF MATERNAL PRENATAL STRESS ON PHYSICAL AND NEURODEVELOPMENT

Maternal prenatal stress is highly prevalent and has been associated with adverse physical and neurodevelopmental outcomes in children including lower birth weight, and impaired motor and cognitive development (Barrett et al. 2014; Barrett et al. 2013; Kingston et al. 2015; Sutherland and Brunwasser, 2018; Vehmeijer et al. 2018). When the body is encountered by stress be it physical, emotional, or biochemical, the hypothalamic-pituitary-adrenal (HPA) axis regulates the body's response. The HPA axis end product is cortisol, which plays a critical role in fetal development including lung maturation, normal brain development, and preparation for delivery (Davis and Sandman, 2010; Buss et al. 2012). However, this also means that unusual and elevated levels of stress and stress response in the mother may affect

the developing fetus. There are several possible mechanisms through which prenatal stress may act to impact the fetus. A possible mechanism is through the placental enzyme 11-β hydroxysteroid dehydrogenase- type 2 (11β-HSD2). Studies suggest that greater production of maternal cortisol may lead to dysregulation of 11β-HSD2 (Beijer et al. 2014; Carpenter et al. 2017; Glover and Hill, 2012). This enzyme limits the amount of cortisol that crosses the placenta, and reaches the developing fetus, by converting cortisol to cortisone. Hence, dysregulation may lead to greater exposure of the fetus to cortisol and this in turn may affect neurodevelopment (Glover and Hill, 2012). Studies have shown that the brain has high-levels of glucocorticoid receptors with the highest being the limbic system, the hypothalamus and the cortex (Moisiadis and Matthews, 2014). These are areas that have been shown to regulate the HPA axis, behavior, and memory acquisition. During development glucocorticoids have been shown to also affect neurogenesis and gliogenesis (Moisiadis and Matthews, 2014). Sex steroid pathways may be another possible mechanism. The hypothalamic-pituitary-gonadal (HPG) axis has been shown to be affected by the chronic activation of the HPA axis. The production of cortisol inhibits the release of gonadotropin releasing hormone that, in consequence, affects the rest of the pathway and the production if sex steroids (estrogen in ovaries and testosterone in testes) (Toufexis et al. 2014). Surges of gonadal and adrenal testosterone are important for the sexual-differentiation of the brain (Cowell and Wright, 2017). Finally, it has been suggested that the upregulation of the HPA axis may lead to greater production of androgens by the adrenal cortex and this may impact the normal sex differentiation of the brain (Barrett and Swan, 2015; Cowell and Wright, 2017). The sex steroid pathway seems to also play an important role in the effects of chemical exposure, which is discussed later in this chapter.

When evaluating the impact of prenatal stress on cognitive development the Bayley Scales of Infant Development (BSID) have been most often used, and a recent systematic review and meta-analysis of these studies found that higher maternal prenatal stress or anxiety

was associated with lower scores on the BSID in early childhood (reviewed by Kingston et al. 2015). The BSID is a standardized test that yields two composite scores, the mental development index (MDI) and the psychomotor development index (PDI). The MDI assesses children's cognitive, language and personal/social development, while the PDI assesses fine and gross motor development. As reviewed by Kingston and colleagues (2015), Bergman et al. (2007) reported a negative association between stressful life events during pregnancy and mental development at 14 to 19 months of age. The study used a Stressful Life Event (SLE) questionnaire where women were able to evaluate how stressful events affected their lives ("affected me a lot" vs. "affected me a little"). Thus, it yielded two scores: the objective number of events and the perceived impact of those events. Results showed a significant negative association between both the total number of stressful life events and the perceived impact of those events, and the child's MDI score. Zhu and colleagues (2014) used a 19-item Prenatal SLE survey to assess prenatal levels of stress. Results showed a negative association between prenatal SLE scores during the first trimester and MDI scores at 16 to 18 months of age. An exception to these negative associations was observed by DiPietro and colleagues (2006), they observed significant positive associations between two measures of prenatal stress and MDI scores at 24 months of age. Laplante and colleagues (2004) assessed how objective and subjective maternal stress caused by a natural disaster (the Québec 1998 ice storm) was associated with intellectual and language development at 2 years of age. The objective stress measure included four categories: threat, loss, scope, and change. The subjective stress measure made use of the Impact of Event Scale (IES-R), the scale describes symptoms related to posttraumatic stress. At 2 years of age the infants completed the BSID and the MacArthur-Bates Communicative Development Inventory (MB-CDI). Results showed that objective stress was associated with lower MDI and MCID scores. Subjective stress was not significantly associated with either, BSID or MCID scores.

Later in childhood, cognition is typically assessed with standardized scales that assess Intelligence Quotients (IQ). The literature looking at the association between prenatal maternal stress and child IQ is more inconsistent. Interestingly, Laplante and colleagues (2008) decided to follow up their 2004 study and aimed to determine whether maternal stress predicts offspring's intellectual and language functioning at 5.5 years of age. At 5.5 years of age, children completed the Wechsler Preschool and Primary Scale of Intelligence-Revised (WPPSI-R) and the Peabody Picture Vocabulary Test-Revised (PPVT-R). Results showed that high levels of objective, but not subjective, stress were associated with lower IQ and language abilities in the offspring. More recently, Lamb and colleagues (2014) studied whether prenatal maternal perceived (subjective) stress was associated with offspring's IQ at 7 and 11 years of age. To assess women's perceived stress, women completed a 10-item Perceived Stress Scale right after giving birth. Children completed the Wechsler Intelligence Scale for Children- 3rd edition (WISC-III) at 7 years of age and the Wechsler Abbreviated Scale of Intelligence (WASI) at 11 years of age. Results showed that higher prenatal maternal perceived stress was associated with lower IQ scores at 7 and 11 years of age. Another recent study was conducted by Cortes Hidalgo (2018) they studied the association between global prenatal maternal stress and the offspring's IQ at 6 years of age. The measure of stress covered four stress domains: life stress, contextual stress, personal stress, and interpersonal stress. Children completed a Dutch non-verbal IQ test (Snijders-Oomen Niet-verbale intelligence, 2.5-7 revised). This sample was very diverse with individuals from Dutch, Caribbean, African, Indonesian, Moroccan, and Turkish ethnicities. Results showed no association between prenatal maternal stress and child IQ in most groups with the exception of the Moroccan and Turkish groups. In these groups, higher prenatal stress was associated with lower IQ scores at 5.5 years of age. These inconsistent results suggest that there are multiple factors to take into consideration when assessing the effects of prenatal stress on cognition. There is a need to not only take into account the type of stress being evaluated, but potentially other cofounders (e.g. ethnicity) that

may affect the association. In addition, it is important to consider the cognitive domain that is being assessed.

Maternal stress has also been associated with sexually dimorphic measures of development. Play behavior has been shown be an important aspect of development that is sexually dimorphic in both humans (Alexander et al. 2009) and animals (Pellis, 2002; Ward and Stehm, 1991; Barrett et al. 2014). This difference is thought to result from the action of androgens on the developing male brain (Collaer and Hines, 1995). In humans, sexual dimorphism in play behavior has been shown in infants as young as 3-8 months of age. Alexander and colleagues (2009) showed that 3-8-month-olds show differences in visual attention to dolls and trucks. Girls had a visual preference for the doll and boys had more fixations on the truck. Barrett and colleagues (2014) used the Preschool Activities Inventory (PSAI) to assess the impact of maternal prenatal stress on play behavior in 4- and 5-year-olds in the Study for Future Families (SFF) cohort. Parents were asked to answer the 24-item PSAI questionnaire that consisted of twelve items that were “feminine” and twelve “masculine”. A SLE scale was used to assess prenatal stress. This scale asked about events that occurred during their pregnancy. The items were job loss, serious family illness or injury, death of a close family member, relationship difficulties, serious legal/financial issues, or any other major life events. Later the women were dichotomized as ‘higher stress’ and ‘lower stress’, the higher stress group having one or more SLEs and the lower stress group having no SLEs. Results showed that girls whose mothers were exposed to more life event stressors during pregnancy showed a more masculinized pattern of play behavior than girls whose mothers had less exposure to life event stressors. Conversely, boys exposed to higher life event stressors showed a non-significant trend toward higher feminine PSAI scores than unexposed boys, with no differences on the masculine or composite scales. These findings suggest that male and female fetuses are

differentially sensitive to maternal stress, and that prenatal stress may masculinize the play behavior of girls.

Another sexually dimorphic measure of development regulated by androgen exposure is anogenital distance (AGD). This is the distance between the anus and the genitals. This measure has been shown to be a good biomarker of prenatal androgen exposure, with boys normally having a longer AGD distance than girls. In both rodents and humans this biomarker has been shown to be affected by exposure to prenatal stress (Shono et al. 1991; Dahlöf et al. 1978; Kinsley & Svare, 1988). Barrett and colleagues (2013) showed that female infants that were exposed to more life event stressors in utero had a longer anogenital distance than female infants that had low in utero stress, while boys exposed to higher prenatal stress showed a non-significant trend towards a shorter anogenital distance.

Most studies in the literature have used measures of maternal perceived stress or scales that measure exposure to actual life event stressors. Few studies have assessed associations between biological measures of chronic stress and neurodevelopmental outcomes. One recent approach to assess exposure to chronic stress is telomere length. Exposure to stress and depression has been associated with shorter telomere length, and this is likely happening as a result of oxidative stress (Feiler et al. 2018; Mathur et al. 2016; Oliveira et al. 2016; Wojcicki et al. 2015). Epel and colleagues (2004) examined whether chronic psychosocial stress led to telomere shortening and lowered telomerase function. The study examined two groups of biological mothers who either had a healthy child or a chronically ill child. The study included both objective (event based) and subjective (perceived) stress measures. Results showed that perceived stress was associated with shorter telomere length (TL) and lowered telomerase activity in the mothers, regardless of the child's health status. These results suggest telomere length may be influenced by perceived stress. Interestingly, there is evidence to suggest that maternal stress may also affect the offspring's telomere length. Entringer and colleagues (2011) examined whether maternal psychological stress during pregnancy is associated with shorter

telomere length in the offspring. Stress was defined as the presence of a major negative life event (e.g. death of a family member) during pregnancy. Blood samples to assess telomere length were taken when the offspring reached young adulthood (mean age: 25). Results showed that exposure to maternal psychological stress was associated with significantly shorter telomere length in young adulthood. In 2013, Entringer and colleagues examined whether maternal stress during pregnancy also impacted newborn leukocyte telomere length. Stress was assessed with a 4-item pregnancy-specific scale. This scale asked about concerns and feelings related to being pregnant, the health of the unborn baby, and about labor and delivery. TL was examined in cord blood after birth. Results showed that higher maternal stress was associated with shorter TL in cord blood. Together these results suggest maternal stress affects both the mother's and the offspring's telomere length, that effects in the offspring may vary by sex, and that this is evident as early as birth.

Few studies have looked at the relationship between telomere length and neurodevelopment. Wojcicki and colleagues (2015) evaluated the effects of exposure to maternal depression and child behavior problems on telomere length in early childhood. Blood samples from the children and their mothers were taken at 4 and 5 years of age to assess telomere length. Mothers' depression was assessed at 3, 4, and 5 years of age using the Center for Epidemiologic Studies Depression Scale and the Edinburgh Depression Scale. The Child Behavior Checklist (CBCL) was used to assess the child's behavior at 3, 4, and 5 years of age. Results showed that maternal clinical depression at 3 years of age was associated with shorter TL in their children at 4 and 5 years of age. In addition, shorter TL at 4 and 5 years of age was associated with increased oppositional defiant behavior or anxiety. More recently, Feiler and colleagues (2018) examined the association between relative telomere length (rTL) and cognitive development. As part of the Seychelles Child Development Study (SCDS), cord blood samples were collected immediately after birth. Cognitive development was evaluated multiple ways: at 9 and 30 months of age the BSID-II was administered and at 5 years of age the

Preschool language scale, the Kaufman Brief Intelligence Test, and the CBCL were administered. Results showed a positive significant association between rTL and PDI scores at 30 months of age. There was also a positive significant association between rTL and the letter-word test at 5 years of age. Relative telomere length was not significantly associated with any of the other outcomes analyzed.

Other biological measures include cortisol concentrations in blood and/or saliva. However, these measures capture snapshots of cortisol levels and hypothalamic-pituitary-adrenal (HPA) activity and are not good indicators or chronic or prolonged stress (D'Anna-Hernandez et al. 2011). Recently, hair cortisol has been identified as a more stable and reliable measure of longer-term stress (D'Anna-Hernandez et al. 2011). Each one-centimeter length of hair approximates one month of cortisol accumulation, with the hair closest to the scalp representing the most recent time period (D'Anna-Hernandez et al. 2011; Bowers et al. 2018). There have been some studies that assessed the correlation between hair cortisol levels and maternal perceived stress (Kalra et al. 2007; Braig et al. 2016; Bowers et al. 2018). Karla and colleagues (2007) had pregnant women complete the 10-item PSS and provide a sample of hair at the end of their first trimester or the beginning of their second trimester. Results showed a moderate (r=0.47) correlation between scores on the PSS and hair cortisol. More recently, Braig and colleagues (2016) assessed this correlation, however, perceived stress was assessed using the Tier Inventory of Chronic Stress (SSCS-TICS). This survey aims to assess chronic perceived stress related to both employment and social relationships during the three previous months. Both the survey and hair collection happened right after delivery. Results did not show a correlation (r=0.02) between the SSCS-TICS scores and hair cortisol levels. Another recent study by Bowers and colleagues (2018) also examined the correlation between maternal stress and hair cortisol levels. Women completed multiple surveys that assessed multiple aspects of distress including the 10-item PSS and provided a 3-cm hair sample. Results showed a strong correlation (r=0.62) between perceived stress and hair cortisol levels when accounting for their

adverse childhood experience (ACE) scores (when ACE scores were greater than two). These differing results may be due to multiple differences in the studies. One important difference between the Karla (2007) and Braig (2016) studies is the scale used to assess prenatal stress (PSS vs. SSCS-TICS). Another important difference is the timing of these assessments (beginning vs. end of gestation). The end of gestation cortisol levels might not be as predictive as there is a normal increase of maternal cortisol during pregnancy, with the last trimester having the highest levels, which is important for development of the lungs and for the delivery of the fetus (reviewed by Davis and Sandman, 2010).

A recent study by Enlow and colleagues (2018) looked at the association between maternal hair cortisol and newborn telomere length. Results were sex-specific, with only females having a positive significant association between maternal hair cortisol during pregnancy and telomere length. The present study took into consideration the literature discussed in that it assessed the correlation between different measures of maternal stress (perceived stress, exposure to stressful life events, telomere length and hair cortisol) and the ability of three of these (perceived, subjective, telomere length) to predict cognitive outcomes. Hair cortisol was only available for a relatively small subset of the pregnant women, so it was not possible to examine its association with cognitive outcomes at this time. However, this is a future goal.

1.2. IMPACT OF PRENATAL PHTHALATE EXPOSURE ON PHYSICAL AND NEURODEVELOPMENT

Prenatal exposure to phthalates has been reported to be associated with poorer neonatal neurological outcomes, behavioral problems, cognitive development, mental development and social impairment during childhood, with some researchers observing sex-specific effects (Miodovnik et al. 2014). Phthalates are found in a wide range of consumer products, and as a result there are multiple routes of exposure: oral, dermal and inhalation

(Sathyanarayana, 2008; Miodovnik et al. 2014). Due to their ubiquitous nature, exposure occurs on a daily basis and detectable levels of phthalate metabolites are present in urine samples from both adults and children (Ejaredar et al. 2015). Some researchers have grouped phthalates by their molecular weight for analysis. Phthalates can be classified by their molecular weight, high molecular weight (>50 Dalton) and low molecular weight (<50 Dalton). High molecular weight phthalates such as di(2-ethylhexyl) phthalate (DEHP) and diisononyl phthalate (DINP), are mostly use as plasticizers (Ejaredar et al. 2015; Zhang et al. 2019). While low molecular weight phthalates such as diethyl phthalate (DEP) and dibutyl phthalate (DBP), are used in cosmetics, lotions and other personal care products (Ejaredar et al. 2015; Zhang et al. 2019).

There are several possible biological mechanisms through which phthalates may act to affect neurodevelopment. A possible mechanism is through thyroid hormone disruption. Thyroid hormone has been shown to be a critical for brain development and cognition (Rovet, 2014). As reviewed by Miodovnik and colleagues (2014) phthalate exposure has been associated with decreases in thyroid hormone levels. There are various mechanisms that have been proposed through which phthalates may affect thyroid hormones. Phthalates may alter transcriptional activity of the sodium/iodine symporter; they may interfere with the active form of the hormone, T3 by binding to the carrier protein, transthyretin; or they may act by inhibiting the binding of T3 to the thyroid receptor (Miodovnik et al. 2014). Another possible way phthalates may be acting is as an anti-androgen leading to the disruption of normal sexual-differentiation of the brain. This mechanism has been studied extensively in animal models and phthalates have been shown to reduce fetal testosterone production, in both humans and animals (Weiss, 2012). This fact along with their potency has provided a basis for summing metabolites of these phthalates. Finally, another possible pathway through which phthalates may be affecting neurodevelopment is through lipid metabolism. Normal brain development requires adequate levels of lipids and fatty acids (i.e. DHA and AA) (Miodovnik et al. 2014).

As reviewed by Miodovnik and colleagues (2014), Engel et al. (2009) reported an association between maternal phthalate metabolites in urine and scores on the Brazelton Neonatal Behavioral Assessment Scale (BNBAS). The results showed that girls, but not boys, who had exposure to high molecular weight phthalates, had lower scores in orientation and quality of alertness five days after delivery. Studies related to behavioral development such as Engel et al. (2010) found an association between phthalate metabolites and childhood behavior. Parents completed the Behavior Assessment System for Children-Parent Rating Scales (BASC-PRS) and the Behavior Rating Inventory of Executive Function (BRIEF) when the children were between 4 and 9 years of age. The results showed a decrease in adaptability scores with increasing concentrations of high molecular weight phthalate metabolites in maternal urine. While higher concentrations of low molecular weight phthalate metabolites were associated with greater aggression, poorer attention, more conduct problems and greater depression. These results remained significant for boys, when the results were stratified by sex. Yolton and colleagues (2011) also observed significant association between prenatal exposures to phthalates and neurobehavior, using the NICU Network Neurobehavioral Scale (NNNS), at 5 weeks of age. Specifically, exposure to DBP at 26 weeks of gestation was associated with improved self-regulation and movement quality. While DEHP exposure was associated with non-optimal reflexes in boys.

When considering the association between prenatal exposure to phthalates and cognitive outcomes in humans, one of the more commonly used assessments is the Bayley Scales of Infant Development (BSID), which have also been used in the studies assessing the effects of prenatal maternal stress. Kim and colleagues (2011) used the Korean BSID-II to assess the effects of prenatal exposure to DEHP and DBP on aspects of neurodevelopment in 6-month-old infants. As part of a prospective birth cohort called Mothers and Children's Environmental Health (MOCEH) pregnant women were recruited during their first trimester of pregnancy. A sample of urine was collected during their third trimester of pregnancy (35.7 to

41.7 weeks of gestation) and measured for secondary metabolites of DEHP [mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP); mono(2-ethyl-5-oxohexyl) phthalate (MEOHP)] and DBP [mono-n-butyl phthalate (MnBP)]. Results showed an inverse association between prenatal exposure to MEHHP and PDI scores and MnBP and MDI of 6-month-old infants. They also observed a strong inverse association between prenatal exposure to MEHHP, MEOHP, and MBP and male's performance on the mental and psychomotor indices. This result is consistent with the results reported by Engle (2010), which also suggested that males were more adversely affected by prenatal phthalate exposure.

Another example is a study conducted by Whyatt and colleagues (2012) in which they evaluated the association between prenatal exposure to phthalates and child mental, psychomotor and behavioral development at 3 years of age. Pregnant women were recruited as part of a prospective birth cohort called Columbia Center for Children's Environmental Health (CCCEH). Metabolites of five parent phthalates were measured in maternal urine samples collected at 33 weeks gestation: DnBP, BBzP, DiBP, DEHP and DEP. At three years of age the children completed the BSID-II to assess their mental and psychomotor development and the mothers completed the Child Behavior Checklist (CBCL) to assess the children's behavior development. The results showed associations between prenatal exposure to MnBP and MiBP and decreases in psychomotor development and an increase in odds of psychomotor delay. They also observed a significant association between exposure to MnBP, MiBP, and MBzP and an increase in number of behavioral problems in the internalizing domain. Interestingly, the results also showed a significant association between prenatal exposure to MnBP and a decrease in girls' mental development at 3 years of age. More recently, Doherty et al (2017) and Kim et al (2018) used the BSID to assess the association between prenatal exposure to phthalates and neurodevelopment in 24-month-old children. As part of the Mount Sinai Children's Environmental Health cohort, pregnant women collected a urine sample between 25 and 40 weeks of gestation (Doherty et al. 2017). Doherty and colleagues (2017) saw sex-

specific associations, where higher exposure to phthalate metabolites was associated with lower MDI and PDI scores in girls, whereas, boys had improved performance. As part of the Children's Health and Environmental Chemicals in Korea (CHECK) cohort, pregnant women provided maternal blood and urine samples when visiting the hospital for full-term deliveries (Kim et al 2018). Kim and colleagues (2018) saw an association between higher levels of MEP and lower MDI and PDI scores.

The studies previously discussed reported an association between prenatal exposure to phthalates and neurodevelopment and some saw sex-specific associations. However, these sex-specific associations don't seem to be consistent across studies. Although a direct comparison between studies would be difficult due to their variability (i.e. age of the children at the time of the assessment, assessment used, metabolites analyzed) there are two studies reviewed by Miodovnik et al (2014) that more clearly show an inconsistency between these sex-specific associations. The study led by Kim and colleagues (2011) saw an inverse association between concentrations of MEHHP, MEOHP and MBP and male's performance on the mental and psychomotor indices at 6 months of age. However, a study led by Tellez-Rojo and colleagues (2013) observed an inverse association between concentrations of MEHP, MEHHP, MEOHP, MECPP, and DEHP and girls' performance on the mental development index at 2 to 3 years of age. Similar results were observed by Doherty et al (2017), phthalate metabolites were associated with lower scores in girls at 24 months. These differences and similarities may due to differences in the age of the children at assessment. Another possible difference may due to the power of the analysis. Kim and colleagues (2011) had a sample of 460 mother-infant pairs (225 were female), whereas, Tellez-Rojo and colleagues (2013) had a much smaller sample of 135 (71 were female).

Another assessment that has been used to look at the association between prenatal phthalate exposure and cognitive function is the Wechsler Intelligence Scale for Children, 4th edition (WISC-IV). The WISC-IV measures four areas: verbal comprehension, perceptual

reasoning, working memory, and processing speed. The indices measure verbal concept formation, nonverbal and fluid reasoning, assess children's ability to memorize new information, holding it in short-term memory, and ability to concentrate, manipulate information and focus. An analysis by Factor-Litvak and colleagues (2014) looked at the association between prenatal exposure to phthalates and scores on the four areas of cognitive function that are associated with overall intelligence quotient (IQ) at 7 years of age. The participants included in this study were a part of the CCCEH prospective birth cohort. Results showed that prenatal exposure to metabolites of DnBP and DiBP were inversely associated with the full-scale IQ. In addition, metabolites of DBP and BBzP were inversely associated with perceptual reasoning. Processing speed, verbal comprehension, and working memory were also shown to be negatively affected by metabolites of DBP. There was not a statistically significant sex difference.

Recently, human epidemiological studies have started looking at more specific aspects of neurodevelopment. A recent study by Ipapo and colleagues (2017) looked at the association between prenatal exposure to phthalates and visual recognition memory at 6 months of age, utilizing the CCCEH prospective birth cohort. Visual recognition memory was assessed using the Fagan Test of Infant Intelligence (FTII). The FTII consists of two phases: a familiarization phase where infants are shown two identical pictures and a test phase where the familiar image is paired with a novel one. Results showed sex-specific effects in which females whose mothers had higher prenatal urinary concentrations of phthalate metabolites (MBzP, MEP, and DEHP) showed lower novelty preference scores. This relationship was not seen in the males. Other recent studies have focused on language development (Kim et al. 2017; Bornehag et al. 2018; Olesen et al. 2018). Kim and colleagues (2017) looked at the association of prenatal and child exposure to phthalates and child IQ at 6 years of age in The Environment and Development of Children cohort. Child IQ was assessed using the Korean's Wechsler Intelligence Scale for Children. Interestingly, results showed a significant effect of child urinary DEHP metabolites and lower verbal IQ and vocabulary scores in children, no association with prenatal exposure. In

contrast, Bornehag et al. (2018) and Olesen et al. (2018) both found significant associations of prenatal phthalate exposure and language development. Bornehag and colleagues (2018) studied the association between first-trimester phthalate exposure and language development at 2.5 years of age using the SELMA and TIDES cohorts. Language was assessed using parental questionnaires in which they assessed the numbers of words the child used. In both cohorts, there was a significant association between prenatal exposure to DBP and BBzP metabolites and language delay. Finally, Olesen and colleagues (2018) analyzed the association between prenatal phthalate exposure in the third trimester and language development in 2- and 3-year-olds using the Odense Child Cohort (OCC). Children's language development was evaluated using the MB-CDI. Higher prenatal exposure to DEP, BBzP, and DEHP metabolites were associated with lower language scores in boys, but not girls. Similar to the literature discussed previously, there were some differences in the results and conclusions reached by the different studies. However, these add to the growing literature that phthalates may be affecting multiple aspects of development and these associations may vary by sex of the offspring.

Just as with prenatal maternal stress Swan and colleagues, utilized the Study for Future Families (SFF) to evaluate effects of prenatal exposure to phthalates on play behavior (2010) and anogenital distance (2005). To assess phthalate exposure a urine sample was collected during mid-pregnancy. Samples were measured for secondary metabolites of DEHP [mono-(2-ethylhexyl) phthalate (MEHP); MEHHP; MEOHP; their sums (RDEHP)] and DBP [MnBP; mono-isobutyl phthalate (MiBP); their sums ($\sum \mathrm{DBP}$)]. The Preschool Activities Inventory (PSAI) was used to assess play behavior in 4- and 5-year-olds. Just as in the study of prenatal stress, the parents were asked to complete the 24-item questionnaire. Interestingly, the results showed that prenatal exposure was associated with more feminized pattern of play in males. While females' pattern of play did not seem to be affected. For the anogenital distance analysis, the same phthalate metabolites were included. Physical examinations were completed when the infants

were around one year of age (mean: 12.6 months). Boys' examinations included: anogenital distance, description of the testes and scrotum, and the width of the penis. The description of the testes and scrotum were used to account for differences (i.e. size, location, and whether testes had descended or not) in anogenital distance, these were correlated with such measurements. Results showed that prenatal phthalate exposure was associated with shorter anogenital distance in males. Females were not included in this analysis. Interestingly, when prenatal maternal stress was assessed in this cohort, they saw non-significant trends in the same direction as the ones discussed for phthalates in males (in both anogenital distance and play behavior). These results leave open the possibility of cumulative effects of prenatal stress and phthalate exposure in boys. In contrast, girls play behavior seemed to be sensitive to prenatal maternal stress but not phthalates.

In conclusion, phthalates are known endocrine disruptors that are associated with multiple outcomes in neurodevelopment. However, there is still much uncertainty regarding their mode of action and their sex-specific effects.

1.3. CUMULATIVE EFFECTS OF STRESS AND CHEMICALS

Very few studies have assessed the cumulative effects of prenatal stress and prenatal chemical exposures. To my knowledge only two studies (Barrett et al. 2016 and Arbuckle et al. 2019) have looked at the interaction between prenatal stress and phthalates and a very limited number of other studies (e.g. Cowell et al. 2015, Tamayo y Ortiz et al. 2017, and Zhou et al. 2017) have assessed interactions of maternal stress with other chemicals. Barrett and colleagues (2016) looked at prenatal stress as a possible modifier of phthalate exposure in infants' reproductive development. Pregnant women were recruited as part of The Infant Development and the Environment Study (TIDES). The study focused on prenatal phthalate exposure and stress exposure during the first trimester of pregnancy. A SLE was used to assess prenatal stress, the same survey was used in the Barrett and colleagues (2014) play

behavior study. As a biomarker of prenatal phthalate exposure maternal urine samples were collected during the first trimester of pregnancy and analyzed for phthalate metabolites. Shortly after birth, the infants underwent anogenital distance examinations. Results suggest that prenatal stress (SLEs) may modify the effects of prenatal phthalate exposure. Prenatal phthalate exposure was associated with shorter anogenital distances in the males that were part of the lower stress group. Whereas, for males in the higher stress group there was not a significant association between prenatal phthalate exposure and anogenital distance. This result is surprising based on the previous results where males seemed to be sensitive to exposures to both prenatal maternal stress and phthalates. However, this study focused on stressful events only during the first trimester, whereas, the other analysis looked at stressful life events across pregnancy, regardless of when the stressful event happened. Prenatal phthalate exposure was not significantly associated with infant females anogenital distance, regardless of the stress group they were in.

Similarly, Arbuckle and colleagues (2019) assessed whether prenatal maternal stress modified the effects of phthalates on anogenital distance. Pregnant women were recruited as part of the Maternal-Infant Research on Environmental Chemicals (MIREC) study. Similar to the TIDES study phthalate exposure was assessed during the first trimester of pregnancy. However, the SLE questionnaire was administered 6 months postpartum and it assessed the subjective impact of each event. Women rated how each event impacted them in a 4-point scale that ranged from “not at all” to “very much”. For analyses, stress was dichotomized as “lower stressor”, which were women who didn’t have SLEs or rated them as not stressful versus “higher stressor” which were women who reported 1 or more SLEs that were moderately or very stressful. Shortly after birth, the infants underwent AGD examinations. Results showed a modifying effect of stress on the association between phthalates and AGD, with females having a significantly longer AGD when accounting for both the subjective impact of SLEs and an androgen-disruptor sum (metabolites: MnBP, MBzP, MEOHP, MEHHP, MEP, and MINP). This

result is surprising because there were effects on the opposite sex, females instead of males, and there was a suggestion of cumulative effects of stress and phthalates in females, which was not observed in the previous study. However, this study had two main differences is the assessment of prenatal stress. It not only assessed stressful events, it assessed women's perception of the event, and this assessment was made 6 months postpartum. It is important to note the possible bias introduced by asking women to rate their perception of an event months after it happened, distance from the event may change their initial perception. Although these studies do not have similar findings, they highlight the fact that there are multiple factors (psychological, social, chemical) that could affect specific aspects infants' development and the need to assess how they interact with each other to impact development.

Cowell and colleagues (2015) examined whether prenatal stress modified the effect of black carbon (BC) on memory domains in 6-year-olds. Pregnant women were recruited as a part of the Asthma Coalition on Community, Environment, and Social Stress project. Women's exposure to BC was estimated based on their residence over the entire pregnancy. Prenatal stress was assessed using the Crisis in Family Systems- Revised (CRISYS-R). This 64-item scale was administered during the third trimester of pregnancy and women were asked about occurrences during the previous 6 months. In addition, women were able to classify whether this experience was positive, negative, or neutral. The Wide Range Assessment of Memory and Learning-Second Edition (WRAML2) was used as the neurodevelopment assessment. The WRAML2 has three core indices (the Attention Concentration Index, the Verbal Memory Index, and the Visual Memory Index) that evaluate memory function. Results showed a significant decrease in the Attention Concentration Index scores in boys, when accounting for both BC and prenatal stress suggesting a cumulative effect of the two exposures. Tamayo y Ortiz and colleagues (2017) examined if prenatal exposure to stress modifies the effects of lead on cognitive, language and motor development in 24-month-old infants. Pregnant women were study participants in the Programming Research in Obesity, Growth, Environment and Social

Stressors (PROGRESS) cohort in Mexico City. The CRISYS-R was used to assess prenatal maternal stress, and it was administered during the third trimester. As a biomarker of prenatal lead exposure, maternal venous blood samples were drawn and analyzed during the second and third trimester. The BSID-III was used as the neurodevelopment assessment. Results showed an additive effect where lower BSID scores were observed as blood lead levels and stress levels increased. A similar study was conducted in Shanghai by Zhou and colleagues (2017), in which the main goal was to examine the effects of prenatal lead exposure on the cognitive abilities at 24 to 36-months, and to identify whether maternal stress would exacerbate the effects. The Symptom Checklist-90-Revised (SCL-90-R) was used to assess emotional stress during pregnancy. Prenatal lead exposure was assessed in maternal blood between 24-36 weeks of gestation. Behavioral development was assessed using the Gesell Development Scale. The scale contains 5 domains: gross motor, fine motor, adaptive behavior, language, and social behavior. Results agreed with those obtained by the Tamayo y Ortiz group; they observed poorer performance by the toddlers who had been exposed to both prenatal maternal stress and lead. Interestingly, although these studies used different measures of stress and assessed different chemical exposures and neurodevelopmental outcomes their results suggest a cumulative effect of stress and chemical exposures. These highlight the need for further assessment of the impact of the cumulative effect of multiple factors, including chemical and non-chemical stressors on neurodevelopment.

1.4. RESEARCH GOALS AND APPROACH

As discussed previously, relatively few studies have assessed the effects of maternal prenatal stress on early neurodevelopment and very few have assessed the impact during the first year of life. Furthermore, there is even less known about the cumulative effects of stress and environmental chemical exposures. In addition, most studies have relied on maternal surveys (perceived stress or stressful life events) to measure maternal stress, as these are easy

to obtain. As expressed previously, there is very little data looking at biological measures of chronic stress (e.g. telomere length) as they relate to neurodevelopment and to our knowledge no research has investigated whether maternal reported measures or biological measures are better predictors when studying similar neurodevelopmental outcomes.

The research presented here takes place in the context of the Illinois Kids Development Study (IKIDS). IKIDS is an ongoing prospective pregnancy and birth cohort study that recruits pregnant women early in pregnancy and follows them closely throughout their pregnancy. Their children are closely followed during their first year of life and early childhood. IKIDS aims to study the impact of prenatal environmental factors, including maternal stress and phthalates, on physical and cognitive development in infancy and early childhood. Thus, extensive participant information is available from chemical exposures to data pertaining to health and pregnancy history, demographics and other lifestyle factors.

This research makes use of multiple measures of prenatal stress and evaluates how these relates to each other and to infant cognitive outcomes. These include a well-validated psychological measure of perceived stress (Cohen's perceived stress scale) (Cohen et al. 1988), an objective measure of stressful life events (scale) and a biological measure of the chronic stress response (telomere length in maternal blood and infant cord blood). In addition, for a small subset of the women hair samples were used to measure cortisol concentrations as a measure of chronic stress throughout pregnancy.

In addition, the research assesses whether phthalate exposure modifies the relationship between prenatal maternal stress and cognitive outcomes. The study focuses on diethyl phthalate (DEP) and di-(2-ethylhexyl) phthalate (DEHP) exposure because these two phthalates account for about 35% and 20%, respectively, of the total phthalate metabolites in maternal urine. In addition, diisononyl phthalate (DINP) was assessed as this is a common replacement

for DEHP and exposure has been increasing (CDC, 2019). Women are never exposed to a single parent phthalate; hence, the following sums of multiple phthalates were considered as exposure measures based on mechanism of action: the sum of anti-androgenic phthalates and the sum of all phthalates.

This research takes advantage of research in developmental psychology showing that infant looking behaviors can be used as reliable and stable measures of basic cognitive processes and that these looking behavior measures are predictive of cognitive abilities later in childhood (Aslin, 2007; Rose et al. 2001). These allow for earlier identification of adverse outcomes related to environmental factors and could allow for earlier interventions to potentially prevent adverse impacts on the child. Looking behaviors are recorded via infrared eye-tracking. Eye-tracking allows for a fine-grained analysis of looking responses, allowing the measurement of infants' looking to a more constrained area than in previous studies that used human coders and evaluated the infant's looking behavior to the entire scene. In addition, it allows for a more automated approach where data collection and processing are completed by the eye-tracker. The eye-tracking camera captures video of the infant's eye and uses the location of the pupil and corneal reflection to determine the precise location of the infant's gaze on the television. This system allows precise recording of the location and duration of each visual fixation as well as tracking of the saccades between fixations. This has several advantages, it allows efficient testing of large numbers of infants, yields high quality quantitative data, and requires little training to administer, making this approach ideal for epidemiological settings.

To do this, we created an automated version of a physical reasoning task designed by Baillargeon (1995) to assess young infants' ability to reason about conditions in which objects should remain in place versus when they should fall (Baillargeon, 1995). Baillargeon's work revealed that females develop the understanding that an object that is not physically supported should fall earlier than males do. Girls' ability to detect the impossible event is believed to result

from their earlier development of binocular vision allowing them to make better (more accurate) observations of events around them and learn about support relationships between objects at an earlier age, whereas binocular vision develops more slowly in males due to the actions of androgens on the visual cortex during fetal development (Held, 1993; Held et al. 1996). As discussed previously, both stress and phthalates seem to affect normal sex differences. A sexually dimorphic task helps explore further not only whether there are cumulative effects of stress and phthalates but also whether the two may act together to alter normal sex differences in neurodevelopment. At 7.5 months of age, an automated (eye-tracking) version of the Visual Recognition Memory (VRM) task was used, this was based on previous work by Susan Rose (1992). In the VRM paradigm, the infant sees a series of photographs of human faces and geometrical shapes. Sex differences on this task have not been reported, however, previous research has shown it can be used to obtain reliable, stable, and valid measures of basic cognitive processes including working memory, information processing speed, and visual attention (Rose et al. 2001), and that these measures are predictive of cognitive functioning later in childhood (Rose et al. 1995, 2009).

In summary, the goals of the research presented in this dissertation were to characterize the association of prenatal stress with specific aspects of cognition measured at an earlier age than the outcomes measured in most previous studies of prenatal stress. Furthermore, this study assessed which prenatal stress assessments (perceived stress, actual stressful life events or biological measures of stress) are associated with the cognitive tasks assessed. The study also aimed to understand whether prenatal phthalate exposure has a cumulative effect, adding to the effect of prenatal perceived maternal stress, on cognitive outcomes.

CHAPTER 2: SPECIFIC AIMS

Both maternal prenatal stress and prenatal exposure to endocrine disrupting chemicals (EDCs) have been shown to have adverse effects on child neurodevelopment (Barrett et al. 2013; Barrett et al. 2014; Kingston et al. 2015; Ejaredar et al. 2015; Miodovnik et al. 2014). Maternal prenatal stress is highly prevalent and has been associated with adverse physical and neurodevelopmental outcomes in children including lower birth weight, and impaired motor and cognitive development. Recent evidence has shown that prenatal stress also can alter sex-specific characteristics, like anogenital distance and play behavior (Barrett et al. 2013 & Barrett et al. 2014). However, few studies have assessed the effects of prenatal stress on cognitive development, particularly sexually dimorphic aspects of cognitive development, during infancy.

Phthalates are synthetic endocrine disruptors that can be found in personal care products, children's toys, food packaging, and medical tubing. Several studies have reported that prenatal exposure to phthalates is associated with poorer neonatal neurological outcomes, and with behavioral problems, poorer cognitive development, and social impairment during childhood (Ejaredar et al. 2015; Miodovnik et al. 2014). Phthalate exposure has also been associated with changes in anogenital distance and play behavior, but in a different manner than maternal prenatal stress (Swan et al. 2005; Swan et al. 2010).

Early identification of negative outcomes related to prenatal stress and chemical exposure could allow for earlier interventions to mitigate risk. In an effort to characterize more specific aspects of cognition and obtain results earlier in life than has been done in most previous studies, our research is using innovative measures adapted from developmental psychology to assess basic building blocks of cognition including physical reasoning, working memory, attention and information processing speed early in infancy. The Illinois Kids Development Study (IKIDS), a prospective pregnancy and birth cohort currently under

recruitment at the University of Illinois. IKIDS has gathered a wealth of information on prenatal exposures, and on maternal prenatal health, diet, demographic and lifestyle variables. The research presented here drew on this rich data set of prenatal exposure variables and covariates, together with an approach that uses infrared eye- tracking to examine associations between prenatal exposures and infant looking behaviors. This study is one of the first to assess multiple self-report and biological measures of maternal prenatal stress in relation to cognitive development and assess which best predicts outcomes. It is also the first to investigate whether maternal stress interacts with phthalate exposure to increase the risk of adverse cognitive outcomes. **Accordingly, the specific aims were to:**

Specific Aim 1 (Prenatal stress): (a) Examine the association between maternal prenatal stress and infant cognitive outcomes at 4.5 and 7.5 months of age. (b) Examine which measures of maternal stress best predict infant cognitive outcomes at 4.5 and 7.5 months of age. Stress was measured three ways: perceived stress, stressful events, and a biological measure of the chronic stress response—telomere length in maternal blood and infant cord blood. Additionally, maternal cortisol levels during pregnancy were measured in hair samples from a small subset of women. Thus, correlations with other stress measures were assessed, but it was not possible examine the relationship of maternal hair cortisol to infant cognitive outcomes at this time. Physical reasoning was measured in infants between four and five months of age using a video that consisted of two physical events, one possible and the other impossible (a box that is unsupported but does not fall). If infants understand that the unsupported box should fall, they will look longer at the impossible event. Total looking time to each event was measured using infrared eye-tracking. The outcome variable was the difference in looking time between the two events. At seven to eight months of age, visual recognition memory (VRM) and other cognitive outcomes were measured in a task in which infants see a series of photographs of human faces. Looking behaviors were measured via infrared eye-

tracking. Novelty preference, the proportion of time an infant spends looking at a novel stimulus, was used to measure recognition memory. Average fixation time, the average time an infant spends looking at a stimulus before looking away, was used to measure information processing speed, and time to reach familiarization, the time an infant took to reach looking time criteria, was used as a measure of attention, as off-task behavior prolonged time to reach criterion. **(a) It was hypothesized that higher maternal stress during pregnancy would be associated with altered performance on the physical reasoning task, and with altered working memory, information processing ability and visual attention on the VRM task and that these associations would show a detriment in the girls. (b) Further, it was hypothesized that maternal perceived stress would be a better predictor of infant neurodevelopmental outcomes at 4.5 and 7.5 months of age than other measures of stress.**

Specific Aim 2 (Interaction): Assess whether exposure to phthalates including di-(2-ethylhexyl) phthalate (DEHP), mono-ethyl phthalate (MEP), a replacement phthalate, diisononyl ester (DINP), and sums of phthalates (anti-androgenic sum and the sum of all phthalates) interact with the effects of prenatal stress on these tasks. Phthalate exposure measures focused on MEP and DEHP, because the metabolites of these parent phthalates account for approximately 35 and 20 percent, respectively, of all phthalate metabolites in maternal urine. I also assessed associations with DINP, which is being used as a replacement for DEHP. Women are never exposed to a single parent phthalate; hence, the following sums of multiple phthalates were also considered as exposure measures based on pathway of exposure or mechanism of action: the sum of anti-androgenic phthalates and the sum of all phthalates. **It was hypothesized that phthalate exposure would the modify effects of maternal prenatal stress on the cognitive outcomes and that these effects would be cumulative.**

The results of this study provide insight into the effects of prenatal stress and its potential interaction with phthalates on fundamental building blocks of infant cognition.

Furthermore, because the assessments were done during infancy, earlier in development than most previous studies assessing the impact of maternal stress, this research could ultimately lead to earlier identification of, and more timely intervention for at-risk infants.

CHAPTER 3: COHORT PROFILE: ILLINOIS KIDS DEVELOPMENT STUDY (IKIDS)

3.1 INTRODUCTION

The potential for prenatal and childhood exposure to chemical and non-chemical environmental factors, including endocrine disrupting chemicals (EDCs) and psychosocial stress, to adversely impact child development is a growing public health concern. Phthalates and phenols, known EDCs, are used in many consumer products. As a result, there are multiple routes of exposure (oral, dermal, and inhalation; Miodovnik et al. 2011) in humans and these chemicals or their metabolites are consistently detected in human urine, blood, amniotic fluid, cord blood, and breastmilk (Centers for Disease Control and Prevention, 2019; Hogberg et al. 2008; Lee et al. 2018; Shekhar et al. 2017). Pregnant women and infants are thus vulnerable to the effects of these EDCs. Prenatal exposure to these EDCs or to maternal prenatal stress has been associated with poorer neurodevelopmental outcomes (Barrett et al. 2014; Ejaredar et al. 2015, 2017; Kingston et al. 2015; Miodovnik et al. 2014) however, few studies have directly evaluated the impact of these factors on infant cognitive development. Another concern is the possible cumulative or interactive impact of EDC exposure and prenatal stress, but there is limited information about their individual effects and even less regarding joint effects (Barrett et al. 2014; Tamayo y Ortiz et al. 2017; Zhou et al. 2017). Furthermore, published studies have largely focused on global measures of cognition, rather than measures of specific cognitive domains, and very few have assessed the effects of these prenatal exposures on neurodevelopment during the first year of life.

The Illinois Kids Development Study (IKIDS), an ongoing prospective pregnancy and birth cohort, was established with the main goal of assessing the impact of prenatal exposure to environmental factors on specific domains of cognition and to obtain these results earlier in life than had been done before.

Upon completion of cohort recruitment, this manuscript will be submitted for publication to BMJ Open with authorship as follows: Dzwilewski, K., Merced-Nieves, F.*, Strakovsky, R., Aguiar, A., & Schantz, S. *denotes equal contribution; Dzwilewski created Tables 3.1, 3.2, and 3.7-3.9 and Figures 3.1 to 3.3.*

Another goal was to follow children prospectively and assess whether associations of exposures with cognitive outcomes observed in infancy predict continued risk later in childhood. IKIDS has gathered a wealth of information on prenatal exposures, health, diet, demographics, and lifestyle variables to obtain a holistic view of the prenatal environment. Prenatal exposure to EDCs was assessed by measuring metabolites of interest in an individual urine sample at 16-18 weeks of gestation and in a pooled sample of five maternal urines collected approximately 4-6 weeks apart from 10-14 to 34-36 weeks of gestation, providing an average measure of exposure across pregnancy. The time point of 16-18 weeks of gestation may be of particular importance for the influence of EDCs as it is the time of peak androgen production in male fetuses. Our research group is using innovative measures adapted from developmental psychology which use looking behaviors to assess basic building blocks of cognition including physical reasoning, recognition memory, attention, and information processing speed early in infancy. These measures of looking behavior have been shown to be reliable and stable measures of basic cognitive processes and are predictive of cognitive abilities later in childhood (Rose et al. 1995). We have adapted these measures using state-of-the-art infrared eye tracking technology that provides much more precise estimation of gaze location than traditional methods and automates data collection and processing for use in large-scale epidemiological studies. In this paper, we describe the study design, data collection, baseline characteristics, and exposure characteristics of the IKIDS cohort.

3.2 COHORT DESCRIPTION

3.2.1 Recruitment and Eligibility

IKIDS is a prospective pregnancy and birth cohort located in east central Illinois, USA. Participants were recruited between December 2013 and January 2019 at two local obstetric clinics (Christie Clinic and Carle Physician Group) and gave birth at two local hospitals (OSF

Heart of Mary Medical Center and Carle Foundation Hospital). The research laboratory is located at the University of Illinois at Urbana-Champaign.

Recruiting clinics gave out informational brochures regarding the study to new pregnant obstetrics patients at their first prenatal visits. Patients were asked to fill out a reply card indicating their interest in being contacted about participation in the study. Those who declined to be contacted were asked to provide basic demographic information for the purpose of comparing participants with non-participants. Those who indicated permission to be contacted were called over the telephone to receive more information about participation and determine eligibility. Women were eligible to participate if they had not reached 15 weeks of gestation, were between the ages of 18 and 40 years, did not have a child already participating in IKIDS, were fluent in English, were not carrying multiples, were not in a high-risk pregnancy, resided within a 30-minute drive of the University of Illinois campus, were able to provide a fasting blood sample and five urine samples throughout pregnancy, and were able to bring their infant to campus for 4 visits during the first year of life. Those who were eligible but chose not to participate were asked to provide demographic information for comparison to participants.

3.2.2 Prenatal Data Collection

A timeline of prenatal study visits and data collection is provided in Figure 3.1. Women who chose to participate were enrolled in the study between 10 and 14 weeks of gestation. Baseline data regarding demographics, pregnancy and health history, pregnancy symptoms, medication use, and lifestyle factors were obtained at a home visit shortly following enrollment. Physical measurements were also obtained, including waist and hip circumference, weight, body mass index (BMI), and bioelectrical impedance (BIA) measurement of lean mass, fat mass, and visceral fat.

Participants were asked to provide first morning urine samples at approximately 10-14, 16-18, 22-24, 28-30, and 34-36 weeks of gestation for analysis of phthalate and phenol

metabolites. Specific gravity was measured and urine samples aliquoted within 48 hours after collection, and aliquots were stored at -80 Celsius for later analysis. A fasted blood sample was collected at 16-18 weeks of gestation and processed 2 hours later to obtain serum, plasma, buffy coat, and whole blood which were stored at -80 Celsius for later analysis. Toenail and hair samples were collected at 34-36 weeks of gestation.

At each of the five urine sample time points, interviews were conducted to update health, pregnancy symptoms, medication use, and lifestyle information. Maternal surveys regarding depression symptoms (Edinburgh Postnatal Depression Survey [EPDS]; Cox et al. 1987), perceived stress (Perceived Stress Scale [PSS]; Cohen et al. 1983), and social support (Interpersonal Support Evaluation List [ISEL]; Cohen et al. 1985) were collected at 10-14, 16-18, and 34-36 weeks of gestation. At these time points, mothers were also asked to fill out 24-hour diaries regarding food and drink sources and containers, personal care product use, and household product use for the purpose of identifying exposure sources. A modified Block food frequency questionnaire (FFQ; Brunst et al. 2016; Snook Parrott et al. 2009), which included additional questions specific to seafood consumption and pregnancy supplement use, was completed at 10-14 and 34-36 gestational weeks, as well as a questionnaire about neighborhood and social stress (Krieger et al. 2016) at 16-18 weeks.

3.2.3 Postnatal Data Collection

A timeline of data collection during infancy is provided in Figure 3.2. A cord blood sample was collected at the time of delivery by obstetrics providers. Samples were processed as soon as possible between 2 and 24 hours after collection to obtain serum, plasma, buffy coat, and whole blood which were stored at -80 Celsius for later analysis. Meconium/stool samples were collected by parents or hospital staff, stored at -4 Celsius, and later processed for microbiome analysis. IKIDS researchers visited each mother-infant pair at the hospital within 48 hours following the birth of the infant. An update interview was conducted regarding the last few

weeks of pregnancy (health, symptoms, medications, and lifestyle) and labor and delivery. Maternal surveys including the EPDS, PSS, and ISEL, as well a survey of stressful life events during pregnancy (Barrett et al. 2014), were completed. Researchers measured newborn head circumference, second and fourth digit length, anogenital distance, and recumbent length.

Mothers were invited to bring their infants to the research laboratory for follow-up assessments at 1-5 weeks, 4-5 months, and 7-8 months of age. Interviews were conducted at each visit regarding infant health, medications, feeding practices, and use of plastics and baby care products. Non-nutritive sucking (NNS) data was obtained at each visit using methods described by Zimmerman and colleagues (Barlow et al. 2012). Assessments of visual recognition memory (VRM; 1-5 weeks and 7-8 months), physical reasoning (4-5 months), and psychological reasoning (7-8 months) were performed using infant looking behavior assessment techniques. Infants who completed the memory and attention assessment at 7-8 months of age were invited to return for an additional visit that used electroencephalography (EEG) to collect visual event-related potential (ERP) data during a similar visual assessment paradigm involving familiarization to photographs of faces followed by short test trials displaying both familiar and novel photographs. Mothers were asked to complete the Ages and Stages Questionnaire (ASQ; Squires et al. 2009), EPDS, PSS, and ISEL at 4-5 and 7-8 months. At 4-5 months, infant weight, recumbent length, head circumference, second and fourth digit length, and anogenital distances were measured. Mothers completed the Peabody Picture Vocabulary Test – Fourth Edition (PPVT-IV; Dunn et al. 2007) at either the 4-5 month or 7-8 month visit to obtain maternal verbal IQ. The Pediatric Review and Observation of Children's Environmental Support and Stimulation (PROCESS) Inventory (Casey et al. 1988) was completed at the 7-8 month visit to assess children's home and caregiving environment. A stool sample for microbiome analysis was obtained at 4-5 months and a toenail sample for exposure analysis was collected at 7-8 months.

Figure 3.3 is a timeline of data collection during early childhood. When children reached 2 and 3 years of age, mothers were mailed a packet of questionnaires to complete which included the EPDS, PSS, ISEL, Parent Relationship Questionnaire (PRQ, part of the Behavior Assessment System for Children, 3rd edition; Kamphaus et al. 2015), age-appropriate ASQ, Child Behavior Checklist – 1.5-5 years (CBCL/1.5-5; Achenbach et al. 2000) – an assessment of emotional and behavioral problems, MacArthur-Bates Communicative Development Inventory – Words and Sentences (CDI; Fenson et al. 2007; age 2 only) – a measure of early language development, Speech and Language Assessment Scale (SLAS, Hadley et al. 1993; age 3 only), and Block Kids FFQ (Cullen et al. 2008; age 2 only). Toenails samples were obtained and an update interview regarding child health and product use was conducted over the phone.

Mothers were again invited to bring their child to the research laboratory for a follow-up visit when the child was 46-48 months of age. Mothers were asked to complete an update interview regarding the child's health and product use was well as the EPDS, PSS, ISEL, PRQ, ASQ, CBCL/1.5-5, SLAS, Block Kids FFQ, and Behavior Rating Inventory of Executive Function – Preschool (BRIEF-P; Gioia et al. 2003). Children underwent a battery of cognitive tasks to assess psychological reasoning, working memory, attention, inhibition, and development of number concepts. They also completed the Sentence Recall portion of the Clinical Evaluation of Language Fundamentals Preschool – 2nd Edition (CELF Preschool 2; Wiig et al. 2006) to assess language development. A urine sample, stool sample, toenail sample, and hair sample were also obtained and stored for future analysis.

Maternal medical records for the prenatal period and labor and delivery were obtained, as well as child medical records from the time of birth to one year of age. All participants provided written informed consent for participation and HIPAA waivers for medical record release. The study was approved by the Institutional Review Board at the University of Illinois at Urbana-Champaign.

3.2.4 Retention and Demographics

A flowchart of prenatal recruitment and retention is shown in Figure 3.4. Between December 2013 and January 2019, 8794 women received informational brochures and returned reply cards at their obstetric clinic. Of those, 1702 were ineligible for participation, 4847 declined to be contacted, and 2245 were eligible and indicated interest in being contacted about participation. Of those 2245, 492 were determined to be ineligible at the screening call, 382 declined participation, and 771 could not be contacted or dropped contact before eligibility screening was completed. By January 2019, 600 pregnant women had been enrolled in the IKIDS cohort and 476 had given birth to infants who were enrolled in the study. Twenty-five mothers became ineligible during pregnancy, 74 withdrew before or at the time of birth, and 25 had not yet given birth in January 2019.

Figure 3.5 contains a flowchart of infancy and early childhood participation and retention. During the first year of life, 7 families became ineligible for participation and 12 withdrew participation. As of January 2019, 375 families participated in the follow-up at 4-5 months of age, 351 at 7-8 months of age, 182 at 2 years of age, 105 at 3 years of age, and 43 at 4 years of age.

Twenty-five mothers withdrew from the study or became ineligible before baseline data collection. Demographic information for the remaining 575 women, as well as those with infants enrolled in the study, is given in Table 3.1. The mean ± SD maternal age at enrollment was 30.16 ± 4.28 years. Eighty percent of mothers and about 78 percent of fathers were white and non-Hispanic. Eighty-six percent of mothers were married and another 9 percent co-habited with a partner. Thirty-four percent of mothers had attained a bachelor's degree and another 43 percent held a master's or doctorate-level degree. Fifty percent of mothers were nulliparous and nearly 99 percent had some form of health insurance. Only about 5 percent of mothers reported smoking during the first trimester of pregnancy, and about 29 percent reported consuming more

than one alcoholic beverage on average over a 4-week period in the first trimester while 60 percent reported no alcohol consumption at all during the first trimester. The demographics for mothers with infants enrolled in the study were largely similar to those of the cohort as a whole, although slightly higher percentages were white, non-Hispanic, married, and had attained at least a bachelor's degree.

Demographic information for children enrolled in the study is presented in Table 3.2. About 72 percent of enrolled children were white and non-Hispanic and 49 percent were male. Seventy percent of enrolled infants were delivered vaginally, 13 percent by planned Cesarean section, and 12 percent by unplanned Cesarean section (delivery type data is not yet collected for about 5 percent of enrolled infants but will be obtained via medical record review). The mean ± SD gestational age at birth was 39.30 ± 1.49 weeks.

3.2.5 Maternal Stress

As previously mentioned, participants completed surveys and provided samples that allow us to assess their stress levels during pregnancy. These measures included a well-validated psychological measure of perceived stress (Cohen's perceived stress scale) (Cohen et al. 1988), an objective measure of stressful events (adverse life events scale) and two biological measures of the chronic stress response (maternal hair cortisol and telomere length in maternal blood and infant cord blood). The study administered the Perceived Stress Scale (PSS) two times during pregnancy, at the beginning and end of pregnancy, the Stressful Life Event Scale addressed stressful events that happened any time during pregnancy. The PSS is a 10-item scale that consists of easy-to-understand items and response options; the scale allows evaluations of the extent to which respondents believe their lives are stressful (Cohen et al. 2012). The scale has been validated in multiple groups of people and different countries (Baik et al. 2017; Cohen et al. 2012; Khalili et al 2017; Lee et al. 2012; Leung et al. 2010). In addition, maternal scores on this scale have been associated with behavior problems in childhood (Betts et al. 2014). The Stressful Life Event Scale (SLE) is a 6-item questionnaire that

asks about possible major stressful life events (i.e. loss of job, death in the family) during pregnancy. Similar surveys have been associated with changes in play behavior (Barrett et al. 2014) and with increased behavior problems at 5 years of age as assessed by the Child Behavior Checklist (CBCL) (Betts et al. 2014).

Typically, biomarkers like salivary or blood cortisol are used as indicators of stress, however, these fluctuate in response to acute stress and may not accurately reflect the maternal stress response during pregnancy due to altered levels of enzymes (i.e. HSD11β2) that interact with this system (Cowell and Wright, 2017). Hair cortisol has been identified as a more stable and reliable measure of long-term cortisol accumulation (D'Anna-Hernandez et al. 2011). IKIDS researchers collected a sample of hair from the posterior vertex of the skull at the end of pregnancy (34-36 weeks of pregnancy). The samples were up to a pencil-width in diameter and the cut was made as close as possible to the scalp. A 1-cm segment represents approximately a month of hair growth. Hence, each 3-cm segment contains cortisol (CORT) that has been deposited over approximately the last 3 months. Samples were analyzed in Dr. Jerrold Meyer's laboratory at the University of Massachusetts Amherst in Amherst, MA, USA. A high-sensitivity enzyme immunoassay (EIA) kit was used to extract CORT from 3 cm hair samples (Meyer et al. 2014) representing each pregnancy trimester.

Telomere length (TL) has also been identified as a potentially more stable biomarker of chronic stress (Cowell and Wright, 2017). Telomeres are non-coding DNA base regions that cap the ends of chromosomes and serve a protective role during replication of the cell (Mathur et al. 2016 & Salihu et al. 2016). Multiple studies suggest that chronic stress is associated with shorter telomere length (Mathur et al. 2016 & Oliveira et al. 2016). TL was assessed in two samples- maternal blood at 16-18 weeks of pregnancy and in cord blood at the time of delivery. Samples were analyzed in Dr. Jue Lin's laboratory at University of California San Francisco in San Francisco, CA, USA. The telomere length measurement assay was adapted from the published original method by Cawthon (2002) and represents a ratio of two qPCR reactions:

Telomere over **S**ingle copy gene, (T/S). DNA purification used the Qiagen QiaAmp DNA mini kit. The T/S ratio for each sample was measured twice, each with triplicate wells. When the duplicate T/S value and the initial value varied by more than 7% for any sample, it was run a third time and the two closest values were reported.

Descriptive statistics for each stress measure are shown in Table 3.3. Only a subset of the samples has been analyzed for hair cortisol. Tables 3.4 and 3.5 show the distribution of perceived stress and stressful life events in the IKIDS cohort, as shown, women have relatively low perceived stress scores and a low number of stressful life events during pregnancy. Table 3.6 presents the correlations among stress measures. There were significant correlations between perceived stress scale scores at the two time points, perceived stress scale scores and number of stressful life events, maternal and cord blood telomere length, and hair cortisol in the three segments. Although the PSS scores and SLE were significantly correlated these correlations were weak (r_s=0.18720 and 0.16243, respectively). In addition to the SLEs reported, other events or situations not covered on the scale may also contribute to women's perceived stress. However, the biomarkers of stress were not correlated with either PSS or SLE and hair CORT was not correlated with telomere length.

Thus far, only a few studies have assessed correlations between these various indices of stress. However, the absence of correlations between perceived stress scores and hair cortisol or telomere length in our cohort is in contrast to the limited number of previous studies, which did find that perceived stress in pregnant women was correlated with hair cortisol levels in the women (Bowers et al. 2018 & Kalra et al. 2017) and with shorter telomere length in their newborns (Entringer et al. 2013 & Salihu et al. 2016). These differences might be partially explained by the timing of hair collection and PSS administration. Kalra and colleagues (2017) administered the PSS and collected hair samples at the end of the first trimester/beginning of the second trimester. Whereas, the IKIDS cohort administered the PSS during the first and third trimesters, but hair collection was during the third trimester. It has been reported that there is a

decline in CORT levels in hair over time due to a “washout effect” which is related to repeated exposure to water and shampoo (Meyer et al. 2014). Thus, our measures of CORT in segments of hair representing the first trimester, but not collected until the third trimester could be less accurate due to this washout effect. This could be compounded by the fact that we only have hair CORT data for a relatively small number of women at this time. Bowers and colleagues (2018) observed a modest correlation between PSS scores and hair CORT, which was stronger when they also accounted for the women’s childhood adversity, data that we do not have. Finally, the correlations presented in Table 3.4 do not take into account other factors that may affect an individual’s cortisol level (e.g. medications that contain glucocorticoids).

The lack of correlation between perceived stress and cord blood TL may be due to the differences in the way perceived stress was measured and the time at which it was measured in this study vs the published literature. Entringer and colleagues (2013) evaluated perceived stress related to pregnancy using a different questionnaire that only contained 4 items. This survey was administered at the beginning of pregnancy and it assessed global feelings about pregnancy from feeling about being pregnant to concerns about the health of the unborn baby. Salihu and colleagues (2016) did use a version of the PSS, however, it was administered around the time of delivery.

There was a lack of correlation between maternal TL and the rest of the stress measures. This may be due to the fact that maternal TL is a measure of longer-term chronic stress, with the shortening of TL likely happening as a result of oxidative stress (Feiler et al. 2018; Mathur et al. 2016; Oliveira et al. 2016; Wojcicki et al. 2015). There is evidence to suggest that perceived stress is correlated to telomere length (Epel et al. 2004), however, this was in a chronically stressed sample (mothers taking care of a chronically ill child).

3.2.6 Exposure Analysis

As previously mentioned, participants provided first morning urine samples at approximately 10-14, 16-18, 22-24, 28-30, and 34-36 weeks of gestation. Samples were

collected from the first morning urine void in cups provided by the study and known to be bisphenol A- and phthalate-free. Specific gravity was measured using a handheld refractometer. Urine was aliquoted within 48 hours for each sample, and aliquots were stored at -80 Celsius until exposure analysis. A pooled sample consisting of 0.9 mL of urine from each of the five samples was created for each participant and stored at -80 Celsius until exposure analysis. Because phenols and phthalates have very short half-lives and exposure varies greatly across time within individuals, a single sample is unreliable for making predictions about exposure levels across pregnancy (Fisher et al. 2015; Ye et al. 2011). The pooling of samples has been shown to be cost effective and to provide a more stable estimate of exposures during gestation (Brookmeyer 1999; Liu et al 2003; Saha-Chaudhuri et al. 2013; Sham et al. 2002; Weinberg et al. 1999).

Exposure biomarker analysis was performed on the pooled samples and on individual samples collected at 16-18 weeks of gestation, a potential critical developmental window for impacts of EDC exposure due to peak androgen production in male fetuses around this time (Auyeung et al. 2013). Samples were analyzed at the Centers for Disease Control and Prevention (CDC) Division of Laboratory Sciences in Atlanta, Georgia, USA. Phthalate and phthalate replacement chemical metabolites as well as phenolic compounds were measured in each urine sample by online solid phase extraction-high performance liquid chromatography-isotope dilution tandem mass spectrometry using a modification of the methods described by Silva and colleagues (2007) and Ye and colleagues (2005).

Number of samples analyzed thus far, median biomarker levels and interquartile ranges (IQRs) and limits of detection (LOD), as well number and percentage of samples with no signal detected are presented in Table 3.7 for phthalates, Table 3.8 for phthalate replacement chemicals, and Table 3.9 for phenolic compounds. Data regarding phenolic compound concentrations for the last batch of analyses is not yet available from the CDC, thus the smaller number of samples reported for those analytes. Biomarker concentrations used to calculate

median and IQR were corrected for dilution using specific gravity, according to the following equation:

$$adjusted\ value = \ biomarker\ concentration * \frac{(sample\ specific\ gravity - 1)}{(IKIDS\ median\ specific\ gravity - 1)}$$

The most recent median (95% confidence interval) measures for females from the National Health and Nutrition Examination Survey (NHANES) for each of the biomarkers are included for comparison to a national biomonitoring sample (CDC 2019). Median biomarker levels were similar between IKIDS and the national NHANES sample for most analytes. The IKIDS pooled sample median concentration of mono-carboxyisooctyl phthalate (MCOP), a metabolite of diisononyl phthalate (DINP), was slightly higher than in NHANES. Pooled sample median concentrations of both measured metabolites of di-2-ethylhexyl terephthalate were significantly higher than in NHANES. In both the 16-18 weeks of gestation samples and the pooled samples, median measures of benzophenone-3 and triclosan were markedly higher than those seen in NHANES. Additionally, median measures for ethyl paraben (16-18 weeks sample), propyl paraben (16-18 weeks sample), and methyl paraben (16-18 week and pooled samples) were relatively lower than observed in NHANES. Although some biomarker measures differed from this national sample, most are representative of biomarker levels in the US.

3.3 STRENGTHS AND LIMITATIONS

The IKIDS study has gathered extensive biospecimen and covariate data spanning from the end of the first trimester of pregnancy through the child's fourth birthday. The sociodemographic aspects of the cohort are relatively homogeneous, but largely reflect the local population. Collected covariate data include key demographic information, extensive health and lifestyle variables, and archived biospecimens (urine, blood, hair, nails, and stool) that can be

used to measure additional exposures as well as health outcomes. Longitudinal assessments of physical growth and cognition across infancy and early childhood provide substantial data to assess exposure effects across a variety of domains and developmental time points.

Although the relatively short half-lives of phthalates and phenolic compounds in the human body makes accurate estimation of exposure difficult, the use of pooled samples for urine biomarker analysis is a cost-effective approach to more accurately estimate exposure across the prenatal period. While this does not allow for time-specific assessment of effects, additional aliquots have been archived for future analyses at specific time points across the three trimesters of pregnancy. Additionally, biomarker data for newly introduced replacement chemicals such as DINP and DINCH are available for analysis of the potential adverse effects of these chemicals about which relatively little is known.

State-of-the-art methods are being used to collect data on specific cognitive domains during the first year of life - much earlier than has been done in previous environmental epidemiology studies. While these measures have only been used in relatively small studies in developmental psychology and thus have not been standardized, the large number of infants in this cohort demonstrates their scalability for studies of this size and allows for characterization of infants' performance on these tasks. These assessments provide measures of specific building blocks of cognition that tend to be stable within individuals across time and follow-up throughout early childhood will demonstrate whether these measures will be useful as early predictors of long-term impacts associated with prenatal and early life exposures.

3.4 TABLES AND FIGURES

Table 3.1. Parental demographics for all IKIDS participants and enrolled infants

	All participants (n=575)	Participants with infant enrolled (n=476)
Parental Demographics	**N (%)**	**N (%)**
Maternal race & ethnicity		
White, Non-Hispanic	460 (80.0)	385 (80.9)
White, Hispanic	18 (3.1)	15 (3.2)
Black, Non-Hispanic	42 (7.3)	25 (5.3)
Asian, Non-Hispanic	32 (5.6)	29 (6.1)
Other	22 (3.8)	21 (4.4)
Unknown/Missing	1 (0.2)	1 (0.2)
Paternal race & ethnicity		
White, Non-Hispanic	449 (78.1)	377 (79.2)
White, Hispanic	24 (4.2)	21 (4.4)
Black, Non-Hispanic	49 (8.5)	33 (6.9)
Asian, Non-Hispanic	26 (4.5)	24 (5.0)
Other	25 (4.3)	20 (4.2)
Unknown/Missing	2 (0.3)	1 (0.2)
Maternal marital status		
Married	494 (85.9)	422 (88.7)
Living as married	50 (8.7)	35 (7.4)
Separated/Divorced/Widowed	0 (0.0)	0 (0.0)
Single	31 (5.4)	19 (4.0)
Maternal education		
High school or less	23 (4.0)	13 (2.7)
Some college or technical school	106 (18.4)	75 (15.8)
Bachelor's degree	197 (34.3)	173 (36.3)
Master's or doctorate degree	249 (43.3)	215 (45.2)
Paternal education		
High school or less	60 (10.4)	41 (8.6)
Some college or technical school	130 (22.6)	95 (20.0)
Bachelor's degree	184 (32.0)	160 (33.6)
Master's or doctorate degree	197 (34.3)	177 (37.2)
Unknown/Missing	4 (0.7)	3 (0.6)
Household income		
$0-$49,999	122 (21.2)	87 (18.3)
$50,000-$99,999	272 (47.3)	228 (47.9)
≥$100,000	176 (30.6)	157 (33.0)
Unknown/Missing	5 (0.9)	4 (0.8)
Maternal parity		
0	291 (50.6)	244 (51.3)
1	179 (31.1)	152 (31.9)
≥2	105 (18.3)	80 (16.8)

Table 3.1. (cont.) Parental demographics for all IKIDS participants and enrolled infants

	All participants (n=575)	Participants with infant enrolled (n=476)
Parental Demographics (continued)	**N (%)**	**N (%)**
Maternal health insurance		
Private or provided by employer	490 (85.2)	417 (87.6)
Medicaid	62 (10.8)	43 (9.0)
Military or VA health insurance	12 (2.1)	9 (1.9)
Provided by employer & Medicaid or All Kids	4 (0.7)	2 (0.4)
Uninsured	5 (0.9)	3 (0.6)
Unknown/Missing	2 (0.3)	2 (0.4)
Maternal tobacco smoking during pregnancy reported at 10-14 weeks gestation		
Yes	31 (5.4)	24 (5.0)
No	505 (87.8)	417 (87.6)
Unknown/Missing	39 (6.8)	35 (7.4)
Maternal alcohol consumption		
Gestational weeks 0-4:		
None	348 (60.5)	281 (59.0)
Less than 4 drinks per week	178 (31.0)	153 (32.1)
4-10 drinks per week	42 (7.3)	36 (7.6)
11-21 drinks per week	6 (1.0)	5 (1.1)
Gestational weeks 5-8:		
None	556 (96.7)	461 (96.8)
Less than 4 drinks per week	15 (2.6)	11 (2.3)
4-10 drinks per week	3 (0.5)	3 (0.6)
11-21 drinks per week	0 (0.0)	0 (0.0)
Gestational weeks 9-14:		
None	564 (98.1)	467 (98.1)
Less than 4 drinks per week	9 (1.6)	7 (1.5)
4-10 drinks per week	1 (0.2)	1 (0.2)
11-21 drinks per week	0 (0.0)	0 (0.0)
Unknown/Missing	1 (0.2)	1 (0.2)
	Mean (SD)	**Mean (SD)**
Maternal age at enrollment (years)	30.16 (4.28)	30.30 (4.05)
Maternal verbal IQ (PPVT-IV standard score)		108.20 (10.98) *

Parental demographics for all enrolled participants with demographic information collected (n=575) and participants with an infant enrolled in the study (n=476). All Kids is an Illinois program to provide free or reduced-cost comprehensive health insurance based on family size and household income. *Maternal verbal IQ: n=341, not yet collected for 135 mothers. PPVT-IV: Peabody Picture Vocabulary Test – Fourth Edition.

Table 3.2. Child demographics for infants enrolled in IKIDS

Child Demographics	**N (%)**
Child sex	
Male	234 (49.2)
Female	242 (50.8)
Child race & ethnicity	
White, Non-Hispanic	342 (71.8)
White, Hispanic	27 (5.7)
Black, Non-Hispanic	19 (4.0)
Asian, Non-Hispanic	18 (3.8)
Other	69 (14.5)
Unknown/Missing	1 (0.2)
Delivery type	
Vaginal	332 (69.7)
Planned Cesarean section	63 (13.2)
Unplanned Cesarean section	59 (12.4)
Unknown/Missing	22 (4.6)
	Mean (SD)
Child gestational age at birth	39.30 (1.49)

Child demographics for 476 infants enrolled in IKIDS as of January 2019.

Table 3.3. Descriptive statistics of stress assessments

Stress Assessment (unit)	N	Mean	St. Dev.	Median	Min	Max
PSS at 10-14 weeks of gestation (score)	562	12.24	6.54	11	0	39
PSS at 34-36 weeks of gestation (score)	482	11.25	6.49	10	0	32
SLE (number of events)	445	0.48	0.8	0	0	5
Cord Blood Telomere Length (T/S)	268	1.35	0.19	1.35	0.87	1.92
Maternal Telomere Length (T/S)	272	0.88	0.14	0.86	0.61	1.37
Hair Cortisol-1st trimester (pg/mg)	61	3.66	10.5	0.8	0.1	74.8
Hair Cortisol-2nd trimester (pg/mg)	62	3.33	8.46	1.2	0.2	63
Hair Cortisol-3rd trimester (pg/mg)	65	3.83	3.83	2.3	0.3	19.6

Table 3.4. Distribution of Perceived Stress Scale scores

	N (%)	Median (Range)
Prenatal Maternal Stress		
Weeks of gestation		
10 to 14		11 (0,39)
34 to 36		10 (0,32)
Categories		
Low	146 (24.3)	
Medium	137 (22.8)	
High	192 (32)	
Missing/Unknown	125 (20.8)	
Postnatal Maternal Stress		
4.5-month visit		9 (0,35)
4.5-month visit (categories)		
Low	180 (30)	
High	201 (33.5)	
Missing/Unknown	219 (36.5)	
7.5-month visit		9 (0,30)
7.5-month visit (categories)		
Low	154 (25.7)	
High	191 (31.8)	
Missing/Unknown	255 (42.5)	

Table 3.5. Distribution of Stressful Life Events during pregnancy

Stressful Life Event	All infants N (%)	Girls N (%)	Boys N (%)
Have you or your partner experienced job loss or unemployment?			
Yes	36 (8.3)	16 (7.3)	20 (9.3)
No	398 (91.7)	204 (92.7)	194 (90.7)
Have you, your partner, or a close family member been seriously injured or ill?			
Yes	50 (11.5)	29 (13.2)	21 (9.8)
No	384 (88.5)	191 (86.8)	193 (90.2)
Has a close family member (partner, parent, child, or sibling) died?			
Yes	30 (6.9)	12 (5.5)	18 (8.4)
No	404 (93.1)	208 (94.5)	196 (91.6)
Have you experienced divorce, a separation or serious relationship difficulties with your partner?			
Yes	9 (2.1)	4 (1.8)	5 (2.3)
No	425 (97.9)	216 (98.2)	209 (97.3)
Have you or your partner experienced serious legal or financial problems?			
Yes	8 (1.8)	5 (2.3)	3 (1.4)
No	426 (98.2)	215 (97.3)	211 (98.6)
Have you experienced any other major life events that you found highly stressful?			
Yes	69 (16)	38 (17.4)	31 (14.6)
No	363 (84) *	181 (82.6)	182 (85.4)

*Two participants skipped this question.

Table 3.6. Correlation matrix for stress measures

Stress Assessment	PSS (10-14 weeks)	PSS (34-36 weeks)	SLE	Cord Blood TL	Maternal TL	Hair Cortisol (3rd trimester)	Hair Cortisol (2nd trimester)	Hair Cortisol (1st trimester)
PSS (10-14 weeks)	1	0.60842 p<.0001 n=475	0.18720 p<.0001 n=441	-0.02207 p=0.7206 n=265	-0.04272 p=0.4862 n=268	0.15956 p=0.2079 n=64	-0.08717 p=0.5041 n=61	-0.14149 p=0.2809 n=60
PSS (34-36 weeks)		1	0.16243 p=0.0007 n=428	0.00505 p=0.9350 n=263	0.03548 p=0.5638 n=267	0.08318 p=0.5134 n=64	-0.17786 p=0.1777 n=61	-0.05453 p=0.6790 n=60
SLE			1	0.01059 p=0.8706 n=239	-0.01750 p=0.7861 n=243	-0.18912 p=0.1377 n=63	0.07098 p=0.5900 n=60	-0.06807 p=0.6084 n=59
Cord Blood TL				1	0.29021 p<.0001 n=235	-0.07755 p=0.5810 n=53	-0.03131 p=0.8273 n=51	-0.11316 p=0.4388 n=49
Maternal TL					1	-0.03899 p=0.7795 n=54	0.02566 p=0.8581 n=51	-0.05161 p=0.7219 n=50
Hair Cortisol (3rd trimester)						1	0.611155 p=<.0001 n=62	0.58767 p=<.0001 n=61
Hair Cortisol (2nd trimester)							1	0.71583 p=<.0001 n=60
Hair Cortisol (1st trimester)								1

Table 3.7. Phthalate metabolites measured and median measures for urine samples at 16-18 weeks of gestation and pooled samples

					16-18 weeks gestation sample n = 480		Pooled sample n = 482		NHANES Females (2015-2016)
Parent compound	**Parent abbr.**	**Metabolite compound**	**Metabolite abbr.**	**LOD (µg/L)**	**Median (IQR) (µg/L)**	**N (%) with no detection**	**Median (IQR) (µg/L)**	**N (%) with no detection**	**Median (95% CI) (µg/L)**
Benzylbutyl phthalate	BBzP	Mono-benzyl phthalate	MBzP	0.3	4.66 (8.74)	1 (0.2)	5.60 (8.55)	0 (0.0)	4.20 (3.40, 5.00)
Diethyl phthalate	DEP	Mono-ethyl phthalate	MEP	1.2	22.34 (31.61)	4 (0.8)	26.30 (33.59)	0 (0.0)	31.6 (25.6, 41.1)
Di-isodecyl phthalate	DDP	Monocarboxy-isononyl phthalate	MCNP	0.2	1.67 (1.68)	0 (0.0)	2.09 (1.78)	0 (0.0)	1.60 (1.40, 1.90)
Di-n-butyl phthalate	DBP	Mono-n-butyl phthalate	MBP	0.4	11.62 (10.21)	0 (0.0)	13.20 (9.27)	0 (0.0)	10.6 (9.60, 11.9)
		Mono-(3-hydroxybutyl) phthalate	MHBP	0.4	1.11 (1.19)	12 (2.5)	1.29 (1.10)	6 (1.2)	0.90 (0.80, 1.00)
Di-(2-ethylhexyl) phthalate	DEHP	Mono-(2-ethylhexyl) phthalate	MEHP	0.8	1.01 (1.38)	31 (6.5)	1.33 (1.29)	16 (3.3)	1.00 (0.90, 1.10)
		Mono-(2-ethyl-5-hydroxyhexyl) phthalate	MEHHP	0.4	4.65 (4.90)	4 (0.8)	5.80 (4.94)	0 (0.0)	5.40 (4.90, 5.70)
		Mono-(2-ethyl-5-oxohexyl) phthalate	MEOHP	0.2	3.90 (3.89)	3 (0.6)	4.61 (3.48)	0 (0.0)	3.50 (3.20, 3.90)
		Mono-(2-ethyl-5-carboxypentyl) phthalate	MECPP	0.4	7.12 (8.12)	1 (0.2)	9.10 (7.84)	0 (0.0)	8.40 (7.70, 9.30)
Di-isononyl phthalate	DINP	Mono-isononyl phthalate	MINP	0.9	0.45 (0.79)	39 (8.1)	0.75 (1.07)	5 (1.0)	< LOD
		Mono-carboxyisooctyl phthalate	MCOP	0.3	7.13 (14.88)	0 (0.0)	11.51 (20.50)	0 (0.0)	6.90 (5.30, 8.50)
		Mono-oxononyl phthalate	MONP	0.4	1.73 (1.99) *	1 (0.3)	2.64 (2.90) †	0 (0.0)	1.70 (1.50, 2.00)
Di-isobutyl phthalate	DIBP	Mono-hydroxyisobutyl phthalate	MHIBP	0.4	2.93 (2.78)	1 (0.2)	3.20 (2.78)	0 (0.0)	2.70 (2.50, 3.10)
		Mono-isobutyl phthalate	MIBP	0.8	7.77 (7.45)	0 (0.0)	8.78 (7.41)	0 (0.0)	8.70 (8.00, 9.30)
(Metabolite of DBP, DOP, and other high-molecular weight phthalates)		Mono-(3-carboxypropyl) phthalate	MCPP	0.4	1.10 (1.25)	4 (0.8)	1.46 (1.69)	0 (0.0)	1.0 (0.80, 1.20)

LOD: limit of detection, CI: confidence interval. *n=308 (analytical method introduced after initial samples analyzed). †n=309 (analytical method introduced after initial samples analyzed).

Table 3.8. Phthalate replacement chemical metabolites measured and median measures for urine samples at 16-18 weeks of gestation and pooled samples

					16-18 weeks gestation sample n = 480		Pooled sample n = 482		NHANES Females (2015-2016)
Parent compound	**Parent abbr.**	**Metabolite compound**	**Metabolite abbr.**	**LOD (µg/L)**	**Median (IQR) (µg/L)**	**N (%) with no detection**	**Median (IQR) (µg/L)**	**N (%) with no detection**	**Median (95% CI) (µg/L)**
Di-2-ethylhexyl terephthalate	DEHTP	Mono-(2-ethyl-5-carboxypentyl) terephthalate	MECPTP	0.2	20.46 (43.73) *	0 (0.0)	59.56 (109.63) †	0 (0.0)	15.7 (12.6, 19.6)
		Mono-(2-ethyl-5-hydrohexyl) terephthalate	MEHHTP	0.4	3.45 (7.96) *	0 (0.0)	8.21 (15.96) †	0 (0.0)	3.80 (3.20. 4.70)
1,2-Cyclohexane dicarboxylic acid diisononyl ester	DINCH	Cyclohexane-1,2-dicarboxylic acid monocarboxy isooctyl ester	MCOCH	0.5	0.38 (0.37)	81 (16.9)	0.53 (0.64)	48 (10.0)	0.50 (0.50, 0.60)
		Cyclohexane-1,2-dicarboxylic acid monohydroxy isononyl ester	MHINCH	0.4	0.50 (0.60)	22 (4.6)	0.84 (1.12)	3 (0.6)	0.50 (0.40, 0.70)

LOD: limit of detection, CI: confidence interval. *n=308 (analytical method introduced after initial samples analyzed). †n=309 (analytical method introduced after initial samples analyzed.

Table 3.9. Phenolic compound biomarkers measured and median measures for urine samples at 16-18 weeks of gestation and pooled samples

			16-18 weeks gestation sample n = 345		Pooled sample n = 347		NHANES Females (2013-2014)
Compound Name	**Compound Abbreviation**	**LOD (µg/L)**	**Median (IQR) (µg/L)**	**N (%) with no detection**	**Median (IQR) (µg/L)**	**N (%) with no detection**	**Median (95% CI) (µg/L)**
Bisphenol A	BPA	0.2	0.81 (0.95)	1 (0.3)	1.07 (1.05)	2 (0.6)	1.20 (1.10, 1.30)
Bisphenol F	BPF	0.2	0.17 (0.60)	115 (33.3)	0.37 (0.95)	77 (22.2)	0.30 (0.30, 0.50)
Bisphenol S	BPS	0.1	0.35 (0.49)	12 (3.5)	0.48 (0.53)	0 (0.0)	0.40 (0.30, 0.50)
2,4-dichlorophenol	2,4-DCP	0.1	0.85 (0.69)	1 (0.3)	0.66 (0.61)	0 (0.0)	0.60 (0.50, 0.70)
2,5-dichlorophenol	2,5-DCP	0.1	1.17 (1.89)	2 (0.6)	1.50 (2.17)	0 (0.0)	1.70 (1.30, 2.50)
Benzophenone-3	BP-3	0.4	60.23 (196.53)	1 (0.3)	107.36 (240.71)	1 (0.3)	26.2 (19.4, 37.2)
Triclosan	TCS	1.7	10.83 (54.23)	2 (0.6)	18.56 (68.99)	0 (0.0)	6.20 (5.60, 7.10)
Triclocarban	TCC	0.1	0.07 (0.15)	165 (48.8)	0.07 (0.19)	172 (49.6)	< LOD
Butyl paraben	B-PB	0.1	0.15 (0.35)	124 (35.9)	0.11 (0.34)	113 (32.6)	< LOD
Methyl paraben	M-PB	1.0	32.81 (98.72)	0 (0.0)	51.87 (117.46)	0 (0.0)	73.9 (61.0, 94.1)
Propyl paraben	P-PB	0.1	3.50 (15.86)	0 (0.0)	9.81 (24.32)	0 (0.0)	13.5 (10.3, 18.2)
Ethyl paraben	E-PB	1.0	0.86 (3.52)	4 (1.2)	1.33 (6.08)	6 (1.7)	1.60 (1.20, 2.30)

LOD: limit of detection, CI: confidence interval.

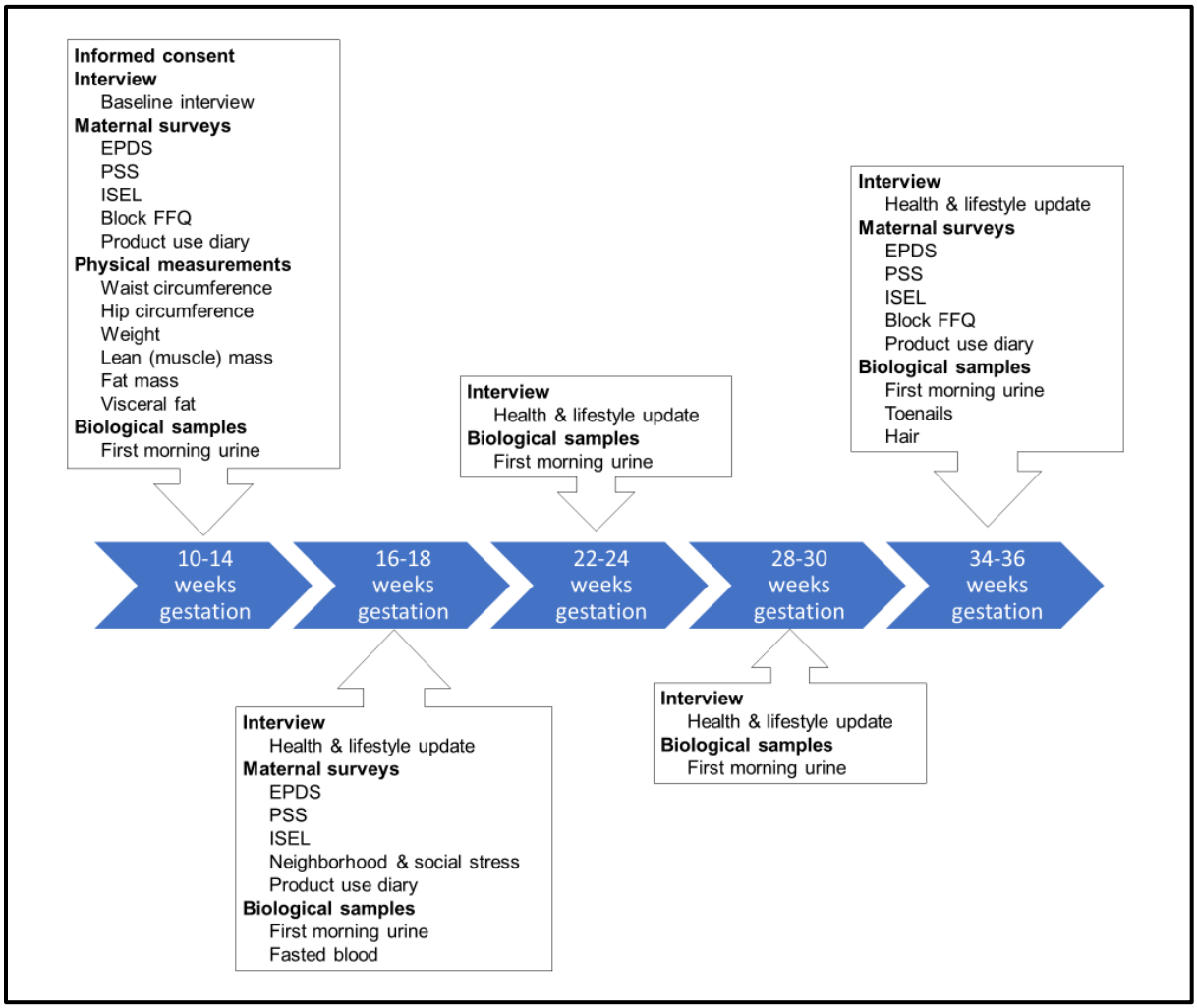

Figure 3.1. Prenatal data collection timeline. EPDS: Edinburgh Postnatal Depression Scale, PSS: Perceived Stress Scale, ISEL: Interpersonal Support Evaluation List, FFQ: Food Frequency Questionnaire.

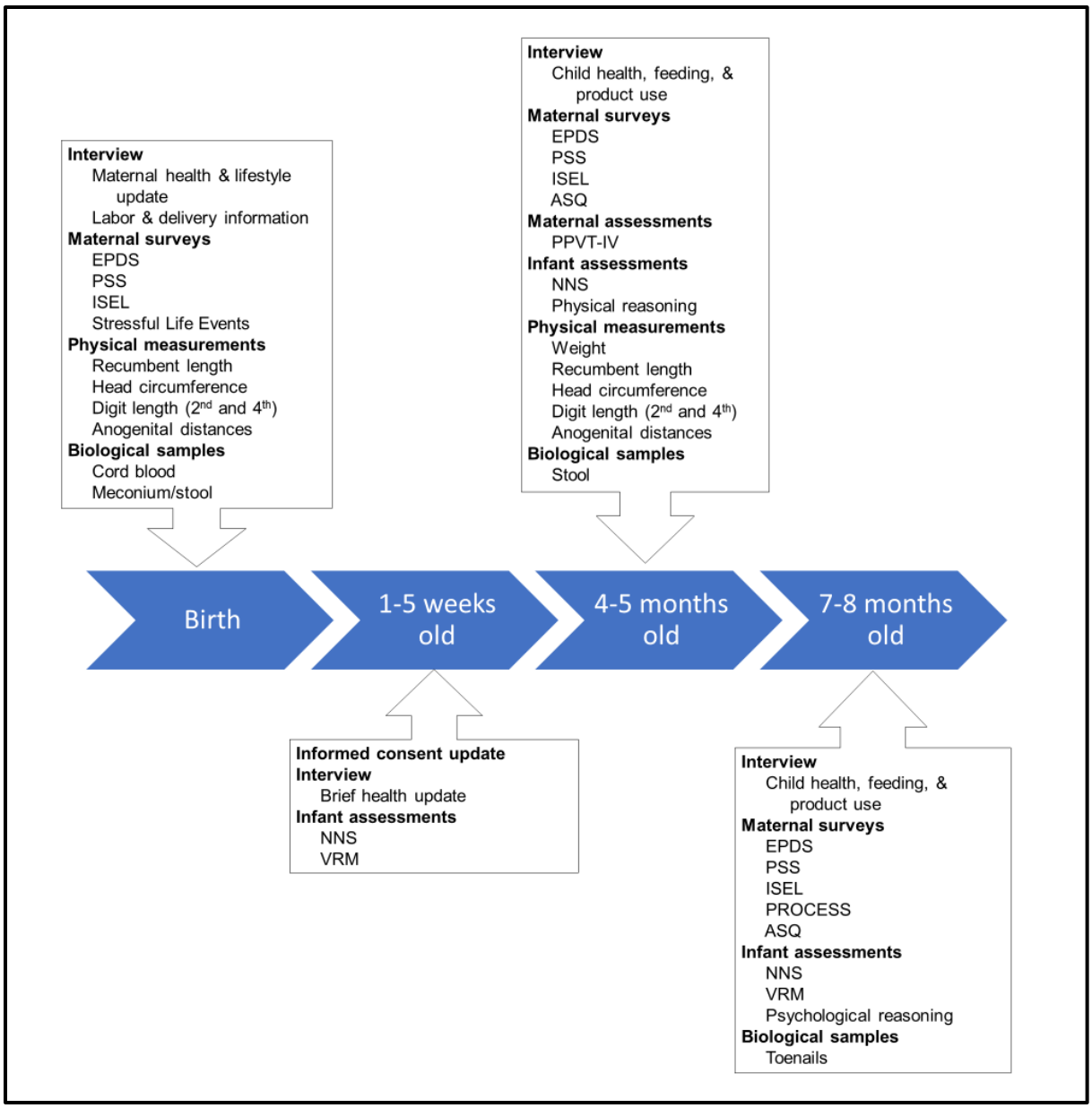

Figure 3.2. Timeline of data collection during infancy. EPDS: Edinburgh Postnatal Depression Scale, PSS: Perceived Stress Scale, ISEL: Interpersonal Support Evaluation List, FFQ: Food Frequency Questionnaire, NNS: non-nutritive sucking, VRM: Visual Recognition Memory, ASQ: Ages & Stages Questionnaire, PPVT-IV: Peabody Picture Vocabulary Test - Fourth Edition, PROCESS: Pediatric Review and Observation of Children's Environmental Support and Stimulation Inventory.

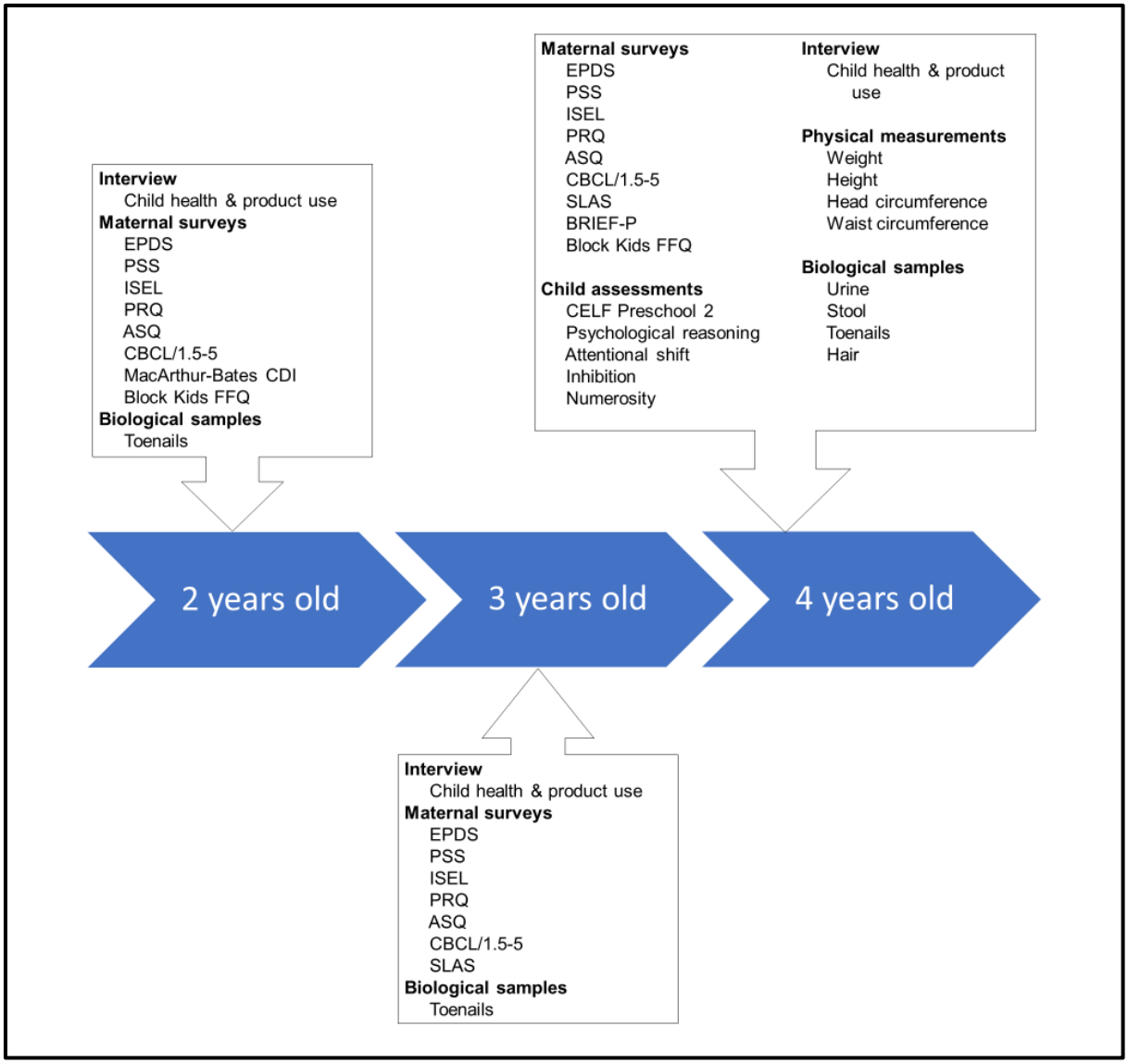

Figure 3.3. Timeline of data collection during early childhood. EPDS: Edinburgh Postnatal Depression Scale, PSS: Perceived Stress Scale, ISEL: Interpersonal Support Evaluation List, PRQ: Parent Relationship Questionnaire, ASQ: Ages & Stages Questionnaire, CBCL/1.5-5: Child Behavior Checklist – 1.5 - 5 years, CDI: Communicative Development Inventory, FFQ: Food Frequency Questionnaire, SLAS: Speech and Language Assessment Scale, BRIEF-P: Behavior Rating Inventory of Executive Function – Preschool, CELF Preschool 2: Clinical Evaluation of Language Fundamentals – Preschool – 2nd Edition (Recalling Sentences section).

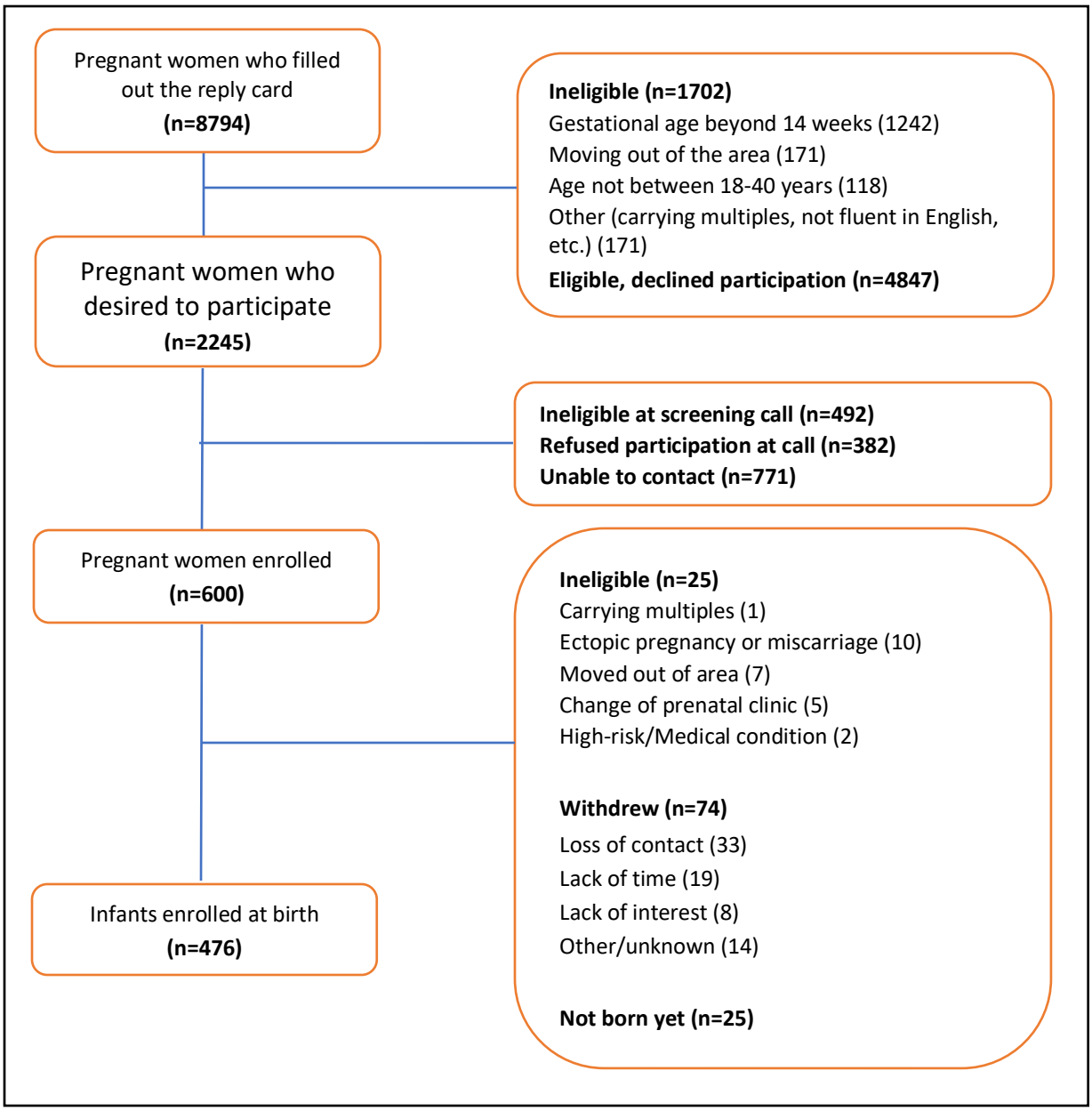

Figure 3.4. Flowchart of recruitment and retention during the prenatal period.

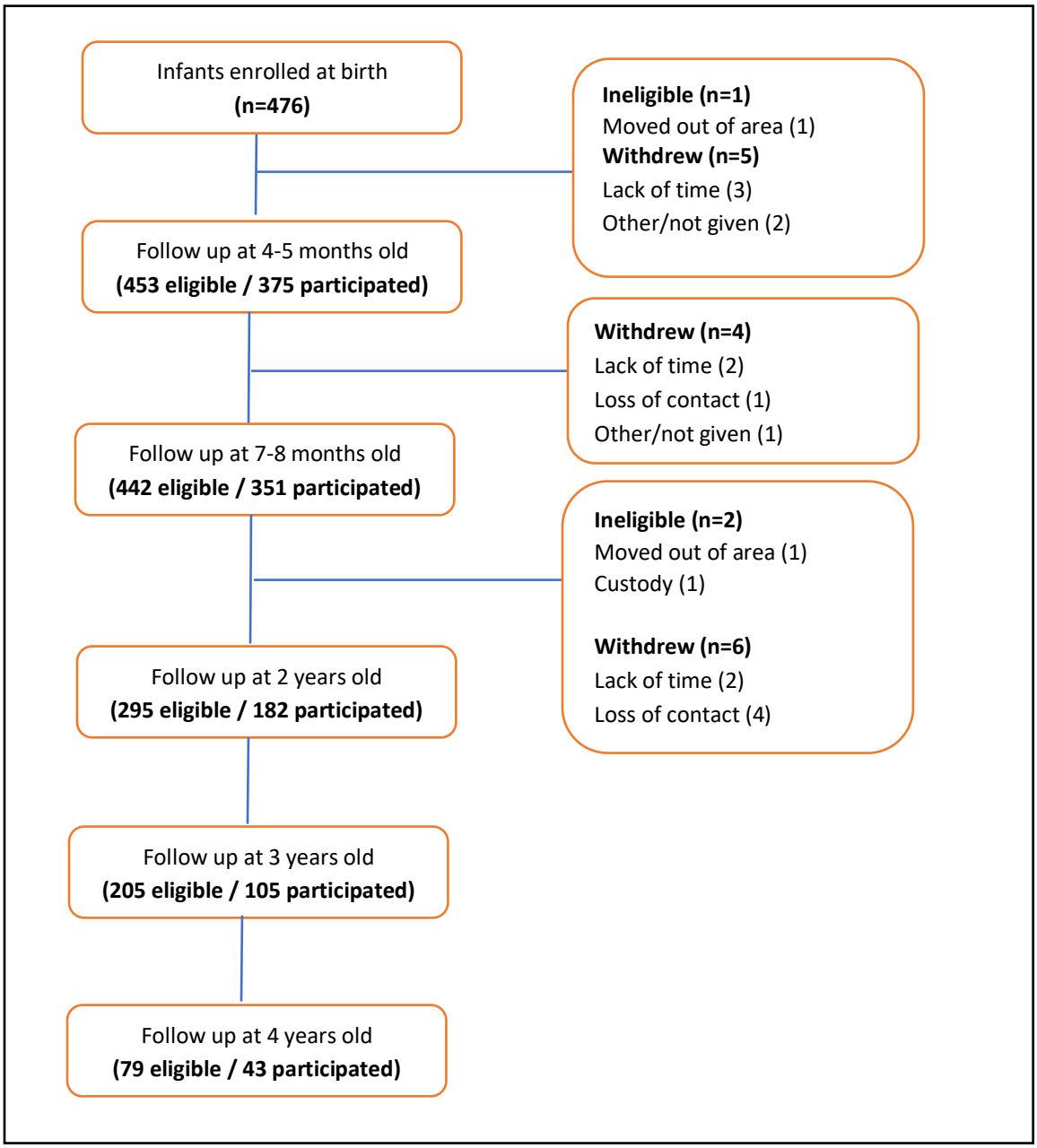

Figure 3.5. Flowchart of retention and participation during infancy and early childhood. "Eligible" refers to the number of children actively enrolled in the study who have reached a given age of assessment; data collection is ongoing as children meet each age of eligibility.

CHAPTER 4: ASSOCIATION OF PRENATAL MATERNAL PERCEIVED STRESS WITH SEXUALLY DIMORPHIC MEASURES OF COGNITION IN 4.5-MONTH-OLD INFANTS

4.1 INTRODUCTION

Recent literature has shown that maternal exposures to both chemical and non-chemical stressors during gestation are risk factors for adverse neurodevelopment. Maternal prenatal stress, a non-chemical stressor, is highly prevalent and has been associated with adverse physical and neurodevelopmental outcomes in children including lower birth weight, and impaired childhood motor and cognitive development (DiPietro, 2012; Barrett et al., 2013; Barrett et al., 2014; Kingston et al., 2015; Vehmeijer et al., 2018; Zhu et al., 2014).

When evaluating the impact of prenatal stress on cognitive development, previous studies have relied on global measures of cognition rather than measures of specific cognitive domains. The Bayley Scales of Infant Development (BSID) have been most often used, and a recent systematic review and meta-analysis of these studies found that higher maternal prenatal stress or anxiety was associated with lower scores for cognitive development on the BSID in early childhood (Kingston et al., 2015). The association of prenatal stress with child intelligence quotient (IQ) is more inconsistent, with some previous studies reporting associations of high maternal prenatal stress with poorer Full-Scale IQ scores at 7 and 11 years of age (Lamb et al., 2014) and other studies not finding consistent and statistically significant associations between IQ scores in early childhood and prenatal stress (Cortes Hidalgo et al., 2018; Laplante et al., 2008). A possible explanation for these differing results might be the different measures used to assess maternal prenatal stress and child overall cognition or the timing of cognitive assessment. For example, where IQ was assessed at older ages than the BSID, intervening experiences and development could alter the apparent association between prenatal stress an

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cognitive outcomes.

Maternal stress has been associated with disruptions in play behavior, an important aspect of development that is sexually dimorphic in humans (Alexander et al., 2009; Barrett et al., 2014). Barrett and colleagues (2014) showed that higher maternal stress during pregnancy was associated with a more masculinized pattern of play behavior in girls, but no significant change in boys' play behavior. These findings suggest that male and female fetuses may be differentially sensitive to maternal stress, and that prenatal stress may masculinize the play behavior of girls.

Very few human studies have assessed the effects of prenatal stress on early cognitive development and even fewer on sexually dimorphic aspects of cognition. Characterizing the impact of prenatal stress on early cognitive development can help inform interventions to help pregnant women cope with stress thus reducing the potential for adverse impacts on the child. Such research can also identify circumstances where intervening very early in development may help to avoid adverse outcomes associated with prenatal stress.

The goals of this research were to characterize the association of prenatal stress with a specific aspect of cognition measured at an earlier age than the outcomes measured in most previous studies of prenatal stress, and to examine potential sex differences in any observed associations. Our approach was unique in that it took advantage of research in developmental psychology showing that infants' looking behaviors can be used as reliable and stable measures to assess basic building blocks of cognition, in this case physical reasoning. Looking behaviors have been used to study infants' underlying cognitive processes (Aslin, 2007) by capitalizing on the fact that infants tend to look longer at stimuli that are novel or events that violate their expectations (reviewed by Baillargeon, 1995). To collect data efficiently from a large number of

infants, we created an automated version of a physical reasoning task, originally designed by Baillargeon (1995), that employed infrared eye-tracking. We used this automated task to assess young infants' abilities to reason about conditions in which objects should remain in place versus when they should fall.

4.2 METHODS

4.2.1. Study Population

The women included in this study were recruited as part of an ongoing prospective pregnancy and birth cohort study, the Illinois Kids Development Study (IKIDS) in Champaign-Urbana, IL. Women were recruited from 2014 to 2018, during their first trimester of pregnancy, from two local obstetric clinics. During their first prenatal visit, the women received a brochure with information about the study, and they filled out a card to indicate whether they were interested in learning more about the study. Women who expressed interest received a call from the IKIDS staff, during which the study was described in more detail and the woman's eligibility to participate was ascertained. Eligible women were between 18 and 40 years of age, fluent in English, not in a high-risk pregnancy or carrying multiples, living within a 30- minute drive of the University of Illinois, and not planning to move out of the area before their child reached one year of age. Women were enrolled at 8-14 weeks of gestation and provided written informed consent which included their child's participation after birth. Mothers participated in periodic study assessments across pregnancy and after the child's birth.

4.2.2. Prenatal Stress

The independent variable in this study was maternal perceived stress during pregnancy. Pregnant women completed the Perceived Stress Scale (PSS) (Cohen et al. 1988) two times during gestation, at 8-14 and 33-37 weeks.

The PSS is a 10-item questionnaire that is used to quantify cumulative perceived stress, by using a scale that evaluates how unpredictable, uncontrollable, and stressful respondents believe different situations in their life were during the previous month. It has been validated in multiple populations, including in pregnant women, and in a variety of countries (Baik et al., 2017; Cohen et al., 1988; Cohen et al., 2012; Khalili et al., 2017; Lee, 2012; Leung et al., 2010; Yokokura et al., 2017). Responses are rated on a five-point Likert scale ranging from 0 to 4 (0= never; 1= almost never; 2= sometimes; 3= fairly often; 4= very often). The possible total scores range from 0 to 40 with a higher total score indicating greater perceived stress. The median scores from women included in this analysis were used to classify the women into three stress categories: low, medium, and high. Women who scored below the cohort median at both time-points were classified as low stress. Those who scored above the median at one of the two time-points were classified as medium stress. Finally, those who scored above the median at both time-points were classified as high stress.

4.2.3. Physical Reasoning

To assess physical reasoning, we created an automated version of a task designed by Baillargeon (1995) in which infants watched events on a puppet stage, and their looking time was measured and recorded by hidden observers. In our automated version, infants watched computerized videos of the task on a large screen high-definition television, and their looking time was measured and recorded using an infrared eye tracker. By automating this task, it was feasible to administer it to a large sample of young infants. Study infants were assessed at 123 to 146 days of age while seated on a parent's lap in front of the television. Hereafter, we refer to this as the infant's 4.5-month visit. The testing area was surrounded by black curtains to reduce distractions. In addition, mothers wore darkened sunglasses to prevent their looking behavior or response to the videos from influencing the infant's looking behavior. Looking behaviors were

tracked using an EyeLink 1000 Plus infrared eye tracker (SR Research Ltd., Mississauga, Ontario, Canada).

To calibrate the eye tracker, the infant's attention was drawn to three specific calibration and validation points on the screen, using short animated clips with accompanying audio, prior to the start of the task. The infant's attention was then drawn to the center of the screen prior to each trial, to ensure that looking was not biased by gaze direction at the beginning of the trial.

The physical reasoning task assessed the infant's ability to reason about whether an object should stay in place (because it is supported), or fall (because it is not supported), and consisted of two events: a possible event and an impossible event (Figure 4.1). In the possible event, a gloved hand came into the scene holding a box. The hand slid the box along the floor and placed it against a wall. The hand released the box briefly, leaving it on the floor, then withdrew the box from the scene. In the impossible event, the hand placed the box against the wall, 11 inches from the floor (box height: 4.5 inches). The hand then released the box, leaving it suspended without support for 3.5 seconds before withdrawing the box. Each event lasted for about 8.5 seconds and was repeated 8 times. Thus, the impossible and possible display lasted for a total of 68 seconds each. Half of the infants saw the impossible event first and the other half saw the possible event first; the order in which infants saw the events was randomized in blocks of 48 infants within each sex using a computerized random number generator.

Since eye-tracking allows for a fine-grained analysis of looking responses, we were able to measure infants' looking to a more constrained area than in previous studies. The interest area dimensions were of 11 by 18 inches around the perimeter of the box (Figure 4.1). To ensure that infants were attending to the task, we established a minimum looking time in the interest area of 7.5 seconds, which was twice the length of time the box remained in the interest area each time the glove released it.

Physical reasoning ability was measured by calculating the difference in looking time between the impossible and possible events (impossible minus possible) wherein a higher value means the infant looked longer at the impossible than the possible event. At 4.5 months of age, girls typically look significantly longer at the impossible than at the possible event, suggesting that they expect the unsupported box to fall and are surprised when it does not. Boys tend to look equally at the two events suggesting that they do not share this expectation (Baillargeon, 1995). Girls' ability to detect the impossible event is believed to result from their earlier development of binocular vision allowing them to make better (more accurate) observations of events around them and learn about support relationships between objects at an earlier age, whereas binocular vision develops more slowly in males due to the actions of testosterone on the visual cortex during fetal development (Held, 1993; Held et al. 1996). Thus, this task is sensitive to sex differences in neurodevelopment.

4.2.4. Covariates

Study researchers met with the pregnant women three times during pregnancy. Covariate data from two of these visits to the participant's home (at 8-14 and again at 33-37 weeks) were used in this analysis and included information about key demographic, lifestyle, health, and diet variables collected via questionnaire and structured interviews. Maternal education was dichotomized to college educated versus not college educated. Household income was dichotomized at less than $60,000 per year versus $60,000 per year or more. Additional covariates collected included maternal smoking and alcohol consumption during the first trimester of pregnancy. At the time of the physical reasoning assessment, maternal postnatal stress was assessed with the PSS (dichotomized at the median to indicate high versus low postnatal stress), and a structured interview was used to determine whether the infant was exclusively breastfed.

4.2.5. Statistical Analysis

We examined descriptive and summary statistics for the relevant study variables. A Wilcoxon rank sum test was used to determine whether the expected sex difference in the outcome previously reported by Baillargeon (1995) (looking time difference between the impossible and the possible event) was replicated.

Our outcome measure was looking time difference (in seconds) defined as the impossible event looking time minus the possible event looking time. The distribution of this outcome was assessed to determine if it met the normality assumption for general linear models. General linear models were used to examine the association of prenatal maternal stress with looking time difference. All analyses were stratified by the sex of the child given the sex difference on the physical reasoning task and the expectation that the effect of stress on the task might differ between the sexes. Regression diagnostics were performed to identify influential points, defined as observations that had extreme Cook's Distance ($D>0.08$).

A *priori* knowledge and a directed acyclic graph (Hernan et al., 2000) were used to select the covariates included in the models. Socioeconomic indicators (maternal education, household income, mother's age at birth) were identified as potential confounders for inclusion in models. Infant's age at exam, and order of event presentation (impossible versus possible event shown first) were also included as covariates because they are known to be correlated with performance on the task. In addition, the interaction between order of presentation and stress was evaluated, with a criterion p-value of <0.15 to determine whether this interaction should stay in the final model.

A number of sensitivity analyses were performed to assess the robustness of any observed associations by considering adjustment for additional covariates. Among the women in our final analysis sample (n=107), there were few smokers (n=4) and relatively few women reporting alcohol consumption (n=25). We assessed potential confounding by smoking or

alcohol in secondary analyses removing women who reported any smoking and adjusting for first trimester alcohol intake. Because of the potential for breastfeeding to be on the causal pathway, additional adjustment for breastfeeding was done as a sensitivity analysis. Sensitivity analyses were also performed retaining influential observations (n=1 boy, n=1 girl). Lastly, because of the correlation between pre- and postnatal perceived stress (r_s = 0.65-0.73), adjustment for postnatal stress was also done as a sensitivity analysis.

4.3 RESULTS

4.3.1. Descriptive data

This analysis focuses on infants who participated and were age-eligible for the 4.5 month visit during two discrete periods between December of 2015 and May of 2018 when testing was conducted. A total of 193 infants were eligible to be tested. Of these infants, 162 (84%) participated in the 4.5-month visit, 123 (76%) of whom were tested; infants weren't tested if the program malfunctioned or the infant was not in the appropriate state (too fussy). A total of 114 (93% of tested infants) (57 girls and 57 boys) met criterion (minimum looking time of 7.5 seconds) on the task. Two observations identified as having high influence were removed from the analysis sample, and an additional 5 boys were missing at least one model covariate. Thus, our final adjusted analyses were performed on 56 girls and 51 boys. Table 4.1 details the characteristics for the 107 infants who were included in the final model. At the time of enrollment, about 86% of the mothers in the study had at least a college degree and about 69% had an annual household income of $60,000 per year or greater. At the time of birth, women were on average 31 years of age. This subsample did not differ from the full sample of the IKIDS cohort for any of these covariates (Table 4.2). Maternal smoking and alcohol consumption data were only available for the first trimester. Only 4% of our subsample reported any smoking during the first trimester and 22% reported any alcohol consumption. Women who reported drinking alcohol during the first trimester had a median of 2.5 drinks per week and

none reported more than 7 drinks per week. All infants in this subsample were born full-term (37 weeks of gestation or later). Around 64% of the infants were exclusively breastfed until the time of testing at 4.5 months of age. When stratified by sex, exclusive breastfeeding was less common in males than females. In addition, there was a tendency for mothers of male infants to be less educated (Table 4.1).

4.3.2. Maternal Stress

The median maternal PSS score at 8-14 weeks of gestation was 11 with a range from 0 to 36. At 33-37 weeks of gestation, the PSS median score was 10 with a range from 0 to 27. Prenatal stress scores at the two time points were moderately correlated (r_s= 0.64). Although, the prevalence of high maternal prenatal stress was similar in mothers of female and male infants, the distribution of low and medium level prenatal maternal stress varied by infant sex (Table 4.1). In addition, mothers of male infants had lower postnatal perceived stress compared with mothers of female infants (Table 4.1). The median postnatal PSS score was 9 with a range from 0 to 35 Prenatal stress scores at both time points were highly correlated with postnatal stress scores (r_s=0.73 and r_s=0.65, respectively).

4.3.3. Physical Reasoning Task

Among the 114 infants in the sex difference analysis, the mean (range) looking time for the impossible event in girls was 21.4 (9.8-44.1) seconds and in boys was 18.9 (7.9-36.7) seconds and for the possible event in girls was 17.4 (8.8-35.1) seconds and in boys was 17.8 (7.6-32.4) seconds. As expected, the Wilcoxon rank sum test showed that the difference in looking time between the two events (impossible minus possible) was higher among girls than boys. Girls, on average, looked at the impossible event 3.9 seconds longer than the possible event, whereas, in boys the difference in looking time was 1.0 second (p=0.05) (Figure 4.2).

There was no evidence of a significant (p<0.15) interaction between order of event presentation and stress so this interaction was not included in multivariable models. In covariate-adjusted general linear models stratified by sex, girls whose mothers had higher levels

of perceived stress during pregnancy had significantly shorter looking time differences than girls whose mothers had low levels of perceived stress (β= -7.1; 95% CI: -12.0, -2.2 seconds; p=0.006). There was a similar trend comparing girls whose mothers had medium versus low perceived stress (β= -3.4; 95% CI: -8.5, 1.6 seconds; p=0.18) The covariate-adjusted mean ± SE looking time difference for girls was 7.1±2.8 seconds in the low stress group (n=17), 3.7±2.4 seconds in the medium stress group (n=18), and 0.3±2.1 seconds in the high stress group (n=21) (Figure 4.3). In addition, there was a significant main effect of maternal age at birth, with girls looking slightly longer at the impossible event with each year of increasing maternal age (β= 0.7; 95% CI: 0.1, 1.3 seconds; p=0.02). In boys, there was no association of prenatal stress with looking time difference but there was a significant main effect of the order of events (p= 0.0007); boys looked longer at whichever event they saw first regardless of whether it was the possible or impossible event.

4.3.4. Sensitivity Analyses

Findings were essentially unchanged when we excluded infants whose mothers reported smoking during the first trimester (n=4), or when we adjusted models for maternal alcohol consumption during the first trimester or breastfeeding status at the time of exam. In addition, inclusion of the two influential observations in our models did not materially alter findings. When adjusting for postnatal maternal stress, associations with prenatal maternal stress were attenuated with wide confidence intervals, but girls whose mothers had higher levels of perceived stress during pregnancy still had shorter looking time differences than girls whose mothers had low levels of perceived stress (β= -3.9; 95% CI: -10.4, 2.6 seconds; p=0.23). The difference between girls whose mothers had medium versus low perceived stress was also attenuated and imprecise (β= -1.3; 95% CI: -7.1, 4.5 seconds; p=0.65). Similar to associations with prenatal stress, girls whose mothers had higher perceived stress postnatally showed shorter looking time differences than girls whose mothers had low levels of perceived stress

postnatally (β= -4.0; 95% CI: -9.5, 1.4 seconds; p=0.14). As was the case for prenatal stress, maternal postnatal stress was not associated with differences in looking time in boys.

4.4 DISCUSSION

Our results replicated the findings of sex difference in physical reasoning reported by Baillargeon (1995) among 4.5-month-old infants. Girls on average looked significantly longer at the impossible event, suggesting this event violated their expectations. Whereas boys did not have a significant difference in looking time, suggesting they do not share this expectation yet. This result demonstrates that an automated version of the task, using a computerized video display and an eye-tracking system, produced results very similar to those obtained with the live, puppet stage version that had been used previously. This automated approach has several advantages. It allows efficient testing of large numbers of infants, yields high quality quantitative data, and requires little training to administer, making this approach ideal for epidemiological settings.

Given previous literature on the association of maternal stress during pregnancy and sexually dimorphic outcomes, we hypothesized that higher prenatal maternal stress would be associated with sex-specific changes in girls' performance on the physical reasoning task. Our results show that the association between prenatal maternal perceived stress and physical reasoning differs by infant sex. Girls exposed to high levels of prenatal maternal perceived stress did not show the expected difference in looking time and looked at the impossible and possible events for about the same time, suggesting a delay in the development of physical reasoning regarding object support. Furthermore, this is reflective of slower cognitive development. In contrast, boys did not show an association between prenatal maternal perceived stress and looking time difference. This result could be due to a sex difference in susceptibility to prenatal stress, however, it could also be related to developmental timing. Boys are expected to develop an understanding of object support about a month later than girls.

Hence, we cannot rule out that there could be an effect of stress in infant boys if they had been assessed at a later age.

The results presented are consistent with previous studies examining the association between prenatal stress and sexually dimorphic aspects of development. Barrett et al. (2013 and 2014) studied the effects of prenatal maternal stress on both physical and behavioral aspects of development. They assessed stress using a Stressful Life Events scale, which asks about stressful events that might have happened during pregnancy, such as loss of a job or relationship difficulties. At 12.6 months of age the anogenital distance (AGD) was assessed in the offspring of enrolled mothers. This has been shown to be a good biomarker of androgen exposure. Normally, males have a longer AGD than females. Results showed that females whose mothers were exposed to more life event stressors had a longer AGD than females whose mothers had fewer stressful events during pregnancy. At 4 & 5 years of age, the same cohort of children were evaluated with the Preschool Activities Inventory (PSAI) to assess play behavior. Results showed that females whose mothers had higher life event stressors during pregnancy had more masculinized play behavior than females whose mothers who reported no stressors. These two findings are consistent with our results on the physical reasoning task. There is evidence to indicate that all three outcomes (AGD, play behavior, and our physical reasoning task) are mediated by exposure to androgens during development (Barrett et al., 2013; Barrett et al., 2014; Held, 1993; Held et al. 1996). Even though the underlying mechanisms of stress's impact on neurodevelopment are unknown, Barrett and colleagues (2013) suggest that maternal prenatal stress might induce increases in fetal cortisol and consequent increases in fetal testosterone (produced by either the placenta or the fetus).

Our findings support the possibility that postnatal stress might explain some of the observed difference in physical reasoning. Similar to prenatal stress models, girls exposed to higher levels of postnatal maternal perceived stress had a significantly shorter looking time difference than girls in the low postnatal stress category. However, in our analysis, it was

difficult to differentiate associations with pre- and postnatal stress since the two were highly correlated (r_s = 0.65-0.73). To differentiate prenatal and postnatal effects, a much larger sample size would be needed to identify sufficient numbers of women with high levels of prenatal stress only, high levels of postnatal stress only, or both.

It is important to mention that our study population had relatively low levels of stress overall compared to nationally representative scores in U.S. women reported by Cohen and colleagues in 2012. Our sample had mean (SD) PSS scores of 11.5(6.1) at 8-14 weeks of gestation and 10.6 (6.4) at 33-37 weeks of gestation. Cohen and colleagues (2012) reported that females in their sample in 1983 had a mean (SD) PSS score of 13.7 (6.6) (n=1,344), in 2006 of 16.1 (7.7) (n=1,034), and in 2009 of 16.1 (7.6) (n=1,032). They also reported that participants' PSS scores decreased with increasing education and income. The relatively low scores in our IKIDS sample may be related, at least in part, to the study population's high levels of education and income.

There are some limitations to the current study. One limitation is the small sample size, which limits the ability to address issues such as the impact of pre- versus postnatal maternal stress. Another limitation is that we only had smoking and alcohol data for the first trimester of pregnancy. However, only four women reported smoking during the first trimester, and it is unlikely women would start smoking later in pregnancy. The subset of women who reported consuming alcohol reported a median of 2.5 drinks per week and this alcohol intake was not correlated with stress nor with looking time differences; therefore, it is unlikely that alcohol intake during pregnancy would confound observed associations and sensitivity analyses adjusting for alcohol confirmed this conclusion. There are potential concerns that the present analysis relied on perceived stress and we did not have biological measures of stress. However, a positive correlation between PSS score and biomarkers of cortisol has been observed in some studies (Bowers et al., 2018; Kalra et al., 2017) thereby supporting the value of PSS as a proxy indicator of physiological stress response. There are two other concerns related to the PSS.

First, we categorized stress levels based on our sample scores on the PSS, this means our specific stress categories may not be generalizable to other populations. That said, finding changes in performance on an infant physical reasoning task associated with relatively low levels of maternal perceived stress (the average PSS score in our high stress group was 16.5) is still a broadly applicable observation. Also, the PSS does not capture other potentially relevant domains including depression, anxiety and life event stressors so there is likely some measurement error in our categorization of stress exposure.

In conclusion, our results suggest that maternal perceived stress is associated with poorer physical reasoning in girls at 4.5 months of age. Our findings provide additional evidence that maternal prenatal stress may adversely impact early neurodevelopment.

4.5 TABLES AND FIGURES

Table 4.1.
Overall and sex stratified demographic and lifestyle characteristics of IKIDS mother-infant pairs participating in infant physical reasoning assessments

	Subsample (n=107)		Girls (n=56)		Boys (n=51)	
	N (%)	Mean (SD)	n (%)	Mean (SD)	n (%)	Mean (SD)
Maternal race						
White, Non-Hispanic	88 (82)		44 (79)		44 (86)	
Other	19 (18)		12 (21)		7 (14)	
Maternal age (years)		30.9 (3.9)		30.7 (3.6)		31.2 (4.2)
Maternal education						
Some college or less	15 (14)		4 (7)		11 (22)	
College degree or higher	92 (86)		52 (93)		**40 (78)***	
Annual household income						
<$60,000	33 (31)		17 (30)		16 (31)	
>= $60,000	74 (69)		39 (70)		35 (69)	
Maternal smoking (first trimester)	4 (4)		1 (2)		3 (6)	
Maternal drinking (first trimester)	24 (22)		10 (18)		14 (27)	
Mode of delivery (% cesarean)	21 (20)		9 (16)		12 (24)	
Gestational age (weeks)		39.5 (1.2)		39.6 (1.2)		39.3 (1.1)
Infant age at time of testing (months)		4.4 (0.20)		4.4 (0.20)		4.4 (0.21)
Feeding status[1]						
Exclusively breastfed	68 (64)		46 (82)		**22 (43)***	
Other	39 (36)		10 (18)		29 (57)	
Prenatal Maternal Stress (category)[2]						
Low	39 (36)		17 (30)		21 (41)	
Medium	28 (26)		18 (32)		10 (20)	
High	41 (38)		21 (38)		20 (39)	
Postnatal Maternal Stress (category)[3]						
Low	52 (49)		22 (39)		30 (59)	
High	55 (51)		34 (61)		21 (41)	

[1]Feeding status at the time of the 4.5-month evaluation. Feeding status of "Other" includes infants that were exclusively formula fed and infants that had both formula and breastmilk.
[2]Prenatal maternal stress was assessed using the 10-item Perceived Stress Scale at 8-14 and 33-37 weeks of gestation. Categories were defined as: low (scores below the median at both times), medium (scores above the median at one of the two times), and high (scores above the median at both times).
[3]Postnatal maternal stress was assessed using the 10-item Perceived Stress Scale at the 4.5-month visit. Categories were defined as: low (scores below the median) and high (scores above the median).
**Bold values represent significant statistical difference between girls and boys.*

Table 4.2.

Maternal demographic and lifestyle information for participants included in the analysis and participants with an infant enrolled in the study as of June 2019

	Analysis sample (n=107)		IKIDS participants with infants enrolled (n=476)	
	n (%)	Mean (SD)	N (%)	Mean (SD)
Maternal race				
White, Non-Hispanic	88 (82)		385 (80.9)	
Other	19 (18)		90 (18.9)	
Missing/Unknown	0 (0)		1 (0.2)	
Maternal age (years)		30.9 (3.9)		30.3 (4.1)
Maternal education				
Some college or less	15 (14)		88 (18.5)	
College degree or higher	92 (86)		388 (81.5)	
Annual household income				
< $60,000	33 (31)		136 (29)	
>= $60,000	74 (69)		340 (71)	
Maternal smoking (first trimester)	4 (4)		24 (5)	
Maternal drinking (first trimester)	24 (22)		141 (30)	
Mode of delivery (% cesarean)	21 (20)		122 (26)	
Gestational age (weeks)		39.5 (1.2)		39.3 (1.5)
Prenatal Maternal Stress (category)				
Low	39 (36)		138 (29)	
Medium	28 (26)		134 (28)	
High	41 (38)		184 (39)	
Missing/Unknown	0 (0)		20 (4)	
Postnatal Maternal Stress (category)[1]				
Low	52 (49)		76 (47)	
High	55 (51)		86 (53)	

[1] *Postnatal Maternal Stress (n=162) for IKIDS participants with infants enrolled.*

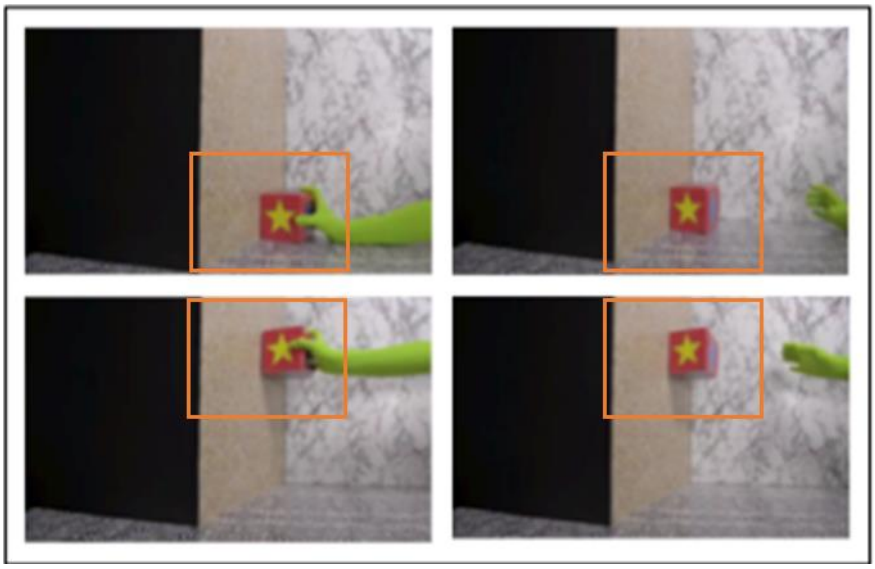

Figure 4.1. Physical Reasoning Task consists of two video events, the possible (top) and the impossible (bottom) event. The orange squares encompass the interest area used to identify infants who are looking at the box.

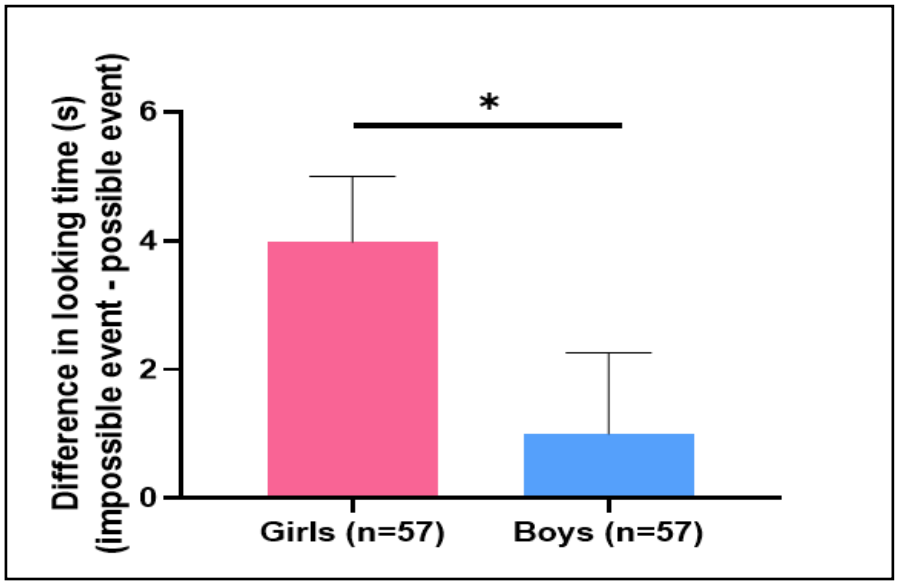

Figure 4.2. Sex stratified mean (SE) looking time differences on a physical reasoning task in IKIDS 4- to 5-month-olds (*p-value=0.05)

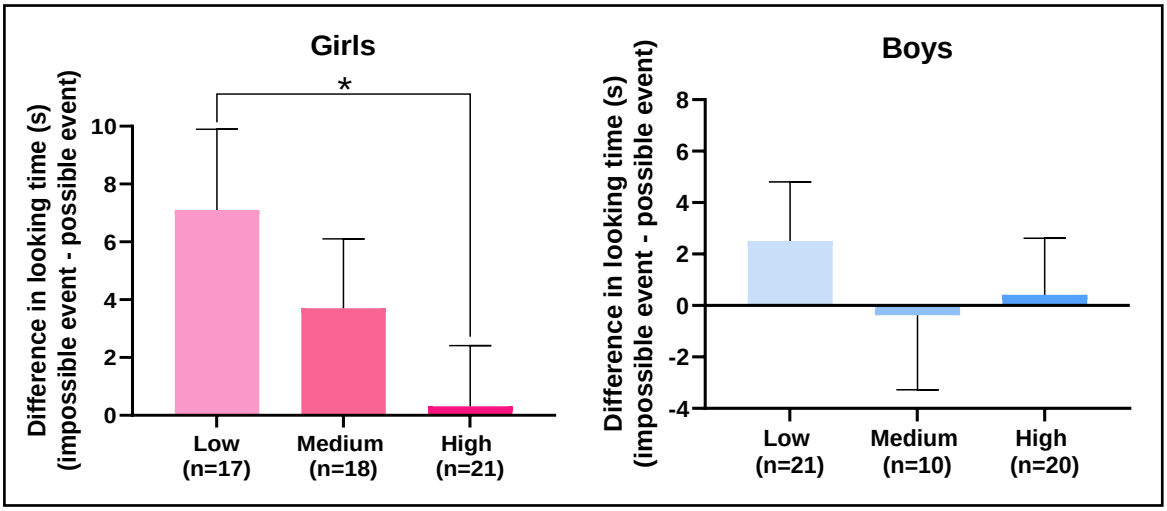

Figure 4.3. Adjusted least squares means of looking time differences (SE) on a physical reasoning task by prenatal stress exposure. Results stratified by sex in IKIDS 4.5-month-olds (n=107). There was a significant difference between girls in the low and high stress groups (p=0.02).

CHAPTER 5: ASSOCIATION OF PRENATAL MATERNAL STRESS AND PHTHALATES WITH MEASURES OF COGNITION IN 4.5-MONTH-OLD INFANTS

5.1 INTRODUCTION

Recent literature suggests, that many environmental factors (chemical and non-chemical) can affect the developing fetus by disrupting normal endocrine function (Davis and Sandman, 2010; Rovet, 2014; Toufexis et al. 2014). Both the hypothalamic-pituitary-adrenal (HPA) axis and hypothalamic-pituitary-gonadal (HPG) axis can be disrupted by these exposures and both hormonal pathways play an important role in neurodevelopment (Glover and Hill, 2012; Weiss, 2012; Moisiadis and Matthews, 2014; Toufexis et al. 2014). Prenatal exposures to maternal stress and phthalates have been individually associated with adverse effects on neurodevelopment in infants and children (Miodovnik et al. 2014; Kingston et al. 2015; Sutherland and Brunwasser, 2018; Vehmeijer et al. 2018), and these exposures are likely to co-occur.

As detailed in Chapters 1 and 4, maternal prenatal stress is highly prevalent and has been associated with adverse neurodevelopmental outcomes in the offspring (e.g. Kingston et al. 2015; Sutherland and Brunwasser, 2018; Vehmeijer et al. 2018). Stress, a non-chemical factor, can be measured multiple ways— objective measures (e.g. number or type of stressful life events in a certain period), subjective measures (a person's perception of their stress level), and biological measures (biomarkers of the stress response). Most studies of the impact of prenatal stress on neurodevelopmental outcomes have focused on objective and/or subjective measures of experienced stress. Very few have focused on biomarkers of chronic stress such as telomere length or hair cortisol concentrations. Furthermore, these previous studies have relied on global measures of cognition rather than measures of specific cognitive domains.

As detailed in Chapter 1, phthalates are endocrine disrupting chemicals found in a wide range of consumer products from food packaging to personal care products (Ejaredar et al. 2015). Due to their ubiquitous nature, daily exposure occurs via multiple routes including

ingestion, dermal contact, and inhalation (Sathyanarayana, 2008; Miodovnik et al. 2014). Previous studies have found associations between prenatal phthalate exposure and adverse outcomes in multiple aspects of neurodevelopment including cognition, behavior and motor development (e.g. Yolton et al. 2011; Kim et al. 2011; Miodovnik et al. 2014; Doherty et al. 2017). However, very few studies have assessed the potential impact of prenatal phthalate exposure on cognitive development very early in life, and to my knowledge there aren't any studies that have examined the potential for combined exposure to stress and phthalates during pregnancy to have a cumulative impact on neurodevelopment.

The goals of this research were to characterize the association of prenatal stress alone measured in multiple ways (perceived stress, stressful life events and maternal and cord blood telomere length), and phthalate exposure alone on physical reasoning in young infants; and additionally, to investigate the potential for cumulative effects of prenatal stress and phthalate exposure on physical reasoning.

5.2 METHODS

5.2.1. Study population

The IKIDS cohort is described in detail in Chapter 3. Pregnant women were recruited for participation from two local obstetric clinics (Carle Physician Group and Christie Clinic) beginning in December 2013. During their first prenatal visit, the women received a brochure with information about the study and they filled out a card to indicate whether they were interested in learning more about the study. Women who expressed interest received a call from the IKIDS staff, during which the study was described in more detail and the woman's eligibility to participate was ascertained. Eligible women were between 18 and 40 years of age, fluent in English, not in a high-risk pregnancy or carrying multiples, lived within a 30- minute drive of the University of Illinois, and were not planning to move out of the area before their child reached one year of age. Women provided written informed consent and were enrolled at 8 to 14 weeks

of gestation. Study mothers participated in periodic assessments across pregnancy and after the child's birth to collect health, diet, demographic, and lifestyle data as well as samples (urine, blood, hair) for exposure analysis.

5.2.2. Prenatal Stress

Multiple measures of prenatal maternal stress were used—a measure of perceived stress, a stressful life events scale, and telomere length in maternal blood and cord blood (see Chapter 3 tables). Maternal hair cortisol was also measured in a subset of the women (see Chapter 3), but it was available for too few women to use it as a measure of prenatal stress in the statistical models. As described in more detail in Chapter 4, the perceived stress scale (PSS) is a well-validated 10-item questionnaire that evaluates how unpredictable, uncontrollable, and stressful respondents believe different situations in their life were during the previous month (Cohen et al. 1988). Pregnant women completed the PSS two times during gestation, at 8-14 and 33-37 weeks. Stress was defined as: low (scores below the median at both times), medium (scores above the median at one of the two times), and high (scores above the median at both times). At birth women completed a brief stressful life events scale that asked about potential stressful events experienced during their pregnancy. The Stressful Life Events (SLE) scale is a 6-item questionnaire that includes a series of stressful events, including job loss or unemployment (self or partner), serious injury or illness (self or close family members), death in the family, divorce or serious relationship difficulties, legal or financial problems (self or partner), and any other major life event (open-ended). The possible total scores range from 0 to 6. SLE were dichotomized to women who reported no SLE (0) and women who reported one or more SLE (1+) during pregnancy.

Telomere length (TL) was assessed in two samples- maternal blood at 16-18 weeks of pregnancy and in cord blood at the time of delivery. The telomere length measurement assay was adapted from the published original method by Cawthon (2002) and represents a ratio of

two qPCR reactions: **T**elomere over **S**ingle copy gene, (T/S). DNA purification used the Qiagen QiaAmp DNA mini kit. The T/S ratio for each sample was measured twice, each with triplicate wells. When the duplicate T/S value and the initial value varied by more than 7% for any sample, it was run a third time and the two closest values were reported. The repeat plate was a mixture from both study plates and was used to make batch adjustments. The CV for telomere length measurement in this study is 2.1 +/- 1.5%. All assays for the entire study were performed using the same lots of reagents. Lab personnel lab who performed the assays were provided with de-identified samples and were blind to all demographic and exposure data.

5.2.3. Phthalates

The IKIDS protocol includes collection of a first morning urine sample at five time points across pregnancy (at 10-14, 16-18, 22-24, 28-30, and 34-36 weeks of gestation). Field blanks are obtained for ten percent of collections. These are analyzed along with the samples to monitor for environmental contamination. Samples are collected and aliquoted for storage at -80°C using BPA- and phthalate-free materials approved by the Centers for Disease Control and Prevention (CDC). In addition, a pooled sample of urine from all 5 time-points is archived for analysis.

Prenatal exposure to phthalates was measured via urine metabolites in the pooled sample and the individual sample collected at 16-18 weeks of pregnancy. The pooling of samples has been shown to be both cost effective and appropriate in research of endocrine disrupting chemicals. This approach was chosen for the IKIDS project because phthalates have very short half-lives and exposure varies greatly across time within individuals (Ejaredar et al. 2015). Given this variability, a single sample can be unreliable for making predictions about exposure levels across pregnancy, while a pooled sample is more representative of average exposure across pregnancy (Brookemeyer, 1999; Weinberg, 1999; Sham, 2002; Liu, 2003; Saha-Chaudhuri, 2013). The 16-18 weeks' time-point was assessed as it is the time of peak androgen production in male fetuses and thus may be an important window for the impacts of

endocrine disruptors (chemical and non-chemical). Urine samples were analyzed at the CDC Division of Laboratory Sciences using an online solid phase extraction-high performance liquid chromatography-isotope dilution tandem mass spectrometry using a modification of the methods described by Silva and colleagues (2007) and Ye and colleagues (2005). The CDC lab analyses have excellent sensitivity and reproducibility with coefficients of variation of 4-14%. The CDC analyzed 16 metabolites in approximately 1/3 of the samples, these were the first group of samples to be analyzed, with the development of new methods 3 extra metabolites have since been added. Hence, a total of 19 phthalate metabolites were measured in the remaining samples, which represent 10 parent compounds (see tables 3.7 and 3.8 in Chapter 3).

This study focused on diethyl phthalate (DEP) and di-(2-ethylhexyl) phthalate (DEHP) exposure because these two phthalates account for about 35% and 20%, respectively, of the total phthalate metabolites in maternal urine. In addition, diisononyl phthalate (DINP) was assessed as this is a common replacement for DEHP and exposure has been increasing (CDC, 2019). Exposure to DEP was measured via its urinary metabolite, monoethyl phthalate (MEP). While DEHP has four major urinary metabolites quantified by CDC. Thus, exposure to DEHP was measured via the molar sum of the following metabolites: mono-2-ethyl-5-carboxypentyl phthalate (MECPP), mono-2-ethyl-5-hydroxyhexyl phthalate (MEHHP), mono-2-ethyl-5-oxohexyl phthalate (MEOHP), and mono-2-ethylhexyl phthalate (MEHP). Not all urine samples were sent to the CDC at the same time and between shipments new methods for phthalate metabolite measurements were developed, thus DINP was assessed two ways. As the sum of two urinary metabolites: mono-(2,6- dimethyl-7-carboxyheptyl) phthalate (MCOP) and mono-isononyl phthalate (mNP) which were measured in all of the women or as the sum of three metabolites: mono-(2,6- dimethyl-7-carboxyheptyl) phthalate (MCOP), mono-isononyl phthalate (mNP), and mono-oxononyl phthalate (MONP) which were measured in roughly 2/3's of the

women. Women are never exposed to a single parent phthalate; hence, the following sums of multiple phthalates were also considered: the sum of anti-androgenic phthalates and the sum of all phthalates. The anti-androgenic (AA) sum is composed of eleven urine metabolites and is based on mechanistic studies in animal models. Exposure to the AA sum was measured via the molar sum of the metabolites: monoisobutyl phthalates (miBP), monobenzyl phthalate (mBzP2), mono-2-ethylhexyl phthalate (MEHP), mono-2-ethyl-5-hydroxyhexyl phthalate (MEHHP), mono-2-ethyl-5-oxohexyl phthalate (MEOHP), mono-2-ethyl-5-carboxypentyl phthalate (MECPP), mono-(2,6- dimethyl-7-carboxyheptyl) phthalate (MCOP), mono-n-butyl phthalate (mBP), mono-hydroxyisobutyl phthalate (mHiBP), and mono-hydroxybutyl phthalate (mHBP). For the sum of all phthalates, the molar sum of 16 metabolites measured in all of the women was used. Potential interactions between these various phthalate exposure measures and prenatal maternal stress also were explored for the measures of stress that were found to be associated with the cognitive outcome.

5.2.4. Physical Reasoning Task

As described in Chapter 4, we created an automated version of the physical reasoning task designed by Baillargeon (1995). In our automated version, infants watched computerized videos of the task on a large screen high-definition television, and their looking time was measured and recorded using an infrared eye tracker. Study infants were assessed at 123 to 146 days of age while seated on a parent's lap in front of the television. The testing area was surrounded by black curtains to reduce distractions. In addition, mothers wore darkened sunglasses to prevent their looking behavior or response to the videos from influencing the infant's looking behavior. Looking behaviors were tracked using an EyeLink 1000 Plus infrared eye tracker (SR Research Ltd., Mississauga, Ontario, Canada). To calibrate the eye tracker, the infant's attention was drawn to three specific calibration and validation points on the screen using short animated clips with accompanying audio prior to the start of the task. The infant's attention was then drawn to the center of the screen prior each trial, to ensure that looking was

not biased by gaze direction at the beginning of the trial. The physical reasoning task consisted of two video events: a possible event and an impossible event. In the possible event, a gloved hand came into the scene holding a box. The hand slid the box along the floor and placed it against a wall and released it briefly before withdrawing the box out of the scene. In the impossible event, the same gloved hand placed the box against the wall midair, leaving the box suspended without support underneath. Each event lasted for about 8.5 seconds and was repeated 8 times. Thus, each event display lasted for a total of 68 seconds each. To ensure that infants were attending to the task, we established a minimum looking time in the interest area of 7.5 seconds, which was twice the length of time the box remained in the interest area each time the glove released it. Half of the infants saw the impossible event first and the other half saw the possible event first; the order in which infants saw the events was randomized in blocks of 48 infants within each sex using a computerized random number generator. Physical reasoning ability was measured by calculating the difference in looking time between the impossible and possible events (impossible minus possible) wherein a higher number means the infant looked longer at the impossible than the possible event.

5.2.5. Covariates

Study researchers met with the pregnant women three times during pregnancy. Covariate data from one of these visits to the participant's home (at 8-14 weeks) were used in this analysis and included information about key demographic, lifestyle, health, and diet variables collected via questionnaire and structured interviews. Maternal education and household income were collected during the first maternal visit at 8 to 14 weeks of gestation. Maternal education was dichotomized to college educated versus not college educated. Household income was dichotomized at less than $60,000 per year versus $60,000 per year or more. Additional covariates collected during study visits included maternal smoking and alcohol consumption during the first trimester of pregnancy.

5.2.6. Statistical Analysis

All statistical analyses were performed using SAS 9.4 software (SAS Institute Inc., Cary, NC, USA). Similar to Chapter 4, all analyses were stratified by the sex of the child given the sex difference on the physical reasoning task and the expectation that the effect of stress on the task might differ between the sexes. First the association of prenatal stress with difference in looking time to the two events was evaluated. General linear models were used to examine the association of each prenatal maternal stress assessment (perceived stress, stressful life events, maternal blood TL, cord blood TL) with looking time difference. Then the association of phthalate exposure with the outcome was evaluated. Again, general lineal models were used to examine the association between each maternal urinary biomarker of exposure (sum of DEHP metabolites, sum of DINP metabolites, MEP, Anti-Androgenic sum, and the sum of all phthalates) and looking time difference. Finally, for measures of prenatal stress that were significantly associated with looking time difference, the possible interactions between the stress measure and each of the phthalate exposure measures were assessed.

Regression diagnostics were performed to identify influential points, defined as observations that had extreme Cook's Distance. In the models that included phthalate exposure, observations with exposure biomarker concentrations greater than 2 standard deviations above the mean were also removed from the models. Main effects of exposure were considered significant if p-values were less than 0.05. For all models, the interaction between the exposure and order of event presentation (possible first or impossible first) was evaluated. A criterion p-value of <0.15 was used to determine whether this interaction should stay in the final model.

A priori knowledge and a directed acyclic graph (Hernan et al., 2000) were used to select the covariates included in the models. Socioeconomic indicators (maternal education, household income, mother's age at birth) were identified as potential confounders for inclusion in models. Infant's age at exam and order of event presentation (impossible event first versus

possible event first) were also included as covariates because they are known to be correlated with performance on the task.

A number of sensitivity analyses were performed to assess the robustness of any observed associations by considering adjustment for additional covariates. Among the women in our final analyses, there were few smokers (n=3 or 4, it varied by model, see table 5.1) and relatively few women reporting alcohol consumption (n= approximately 30, it varied by model). We assessed potential confounding by smoking or alcohol in secondary analyses removing women who reported any smoking and adjusting for first trimester alcohol intake.

5.3 RESULTS

5.3.1. Descriptive Data

As of May 2019, a total of 159 infants (78 girls and 81 boys) met criterion (minimum looking time of 7.5 seconds) on the task and were included in these analyses. Adjusted models for the different stress measures had 118, 111, and 94 infants, respectively, due to missing covariate or exposure information for some infants. Adjusted models for the phthalate exposure had 158 infants. Table 5.1 details the characteristics of the sub-sample of mothers whose infants were included in each analysis. The demographics did not significantly differ across the sub-samples used in these analyses nor with the demographics of the full IKIDS cohort (described in Chapter 3, Table3.1. At the time of enrollment, more than 80% of the mothers had at least a college degree and about 70% had an annual household income of $60,000 per year or greater. Maternal smoking and alcohol consumption data were only available for the first trimester. Only 4% of the subsample included in these analyses reported any smoking during the first trimester and about 25% reported any alcohol consumption. As described in more detail in Chapter 3, alcohol consumption included recollections from weeks 0 to 14 meaning some of the drinking reported was before knowledge of the pregnancy. In addition, most women reported

less than 4 drinks per week. Infants had an average gestational age of 39 weeks and about 20% were delivered via cesarean section.

5.3.2. Stress Measures

In covariate-adjusted general linear models stratified by sex, there were no significant associations of SLE or cord blood telomere length with performance on the physical reasoning task. However, as described in Chapter 4, there was a significant association between prenatal maternal perceived stress and performance on the physical reasoning task. Girls whose mothers had higher levels of perceived stress during pregnancy had significantly shorter looking time differences than girls whose mothers had low levels of perceived stress (β= -7.1; 95% CI: -12.0, -2.2 seconds; p=0.006) (Figure 3.4). There also was a significant association between maternal TL and looking time difference in girls (β=13.2; 95% CI: 1.6, 24.8; p-value=0.0269), but not boys, and an interaction between maternal TL and event order in girls (p-value=0.0574). In girls who saw the impossible event first TL was associated with smaller looking time differences (poorer performance) (Figure 5.1).

5.3.3. Phthalates

Results of covariate-adjusted general linear models stratified by sex, for each phthalate of interest at 16-18 weeks gestation sample and pooled sample are presented in Table 5.3. There wasn't a significant association between ΣDEHP in the pooled sample and performance on the physical reasoning task in either sex. However, for ΣDEHP measured at 16-18 weeks of gestation, a significant association was found in girls (Table 5.3). An increase in prenatal ΣDEHP (16-18 weeks of gestation) was associated with a decrease in looking time difference in girls, but not boys (Figure 5.2). There was a significant association between ΣDINP (sum that contained only MCOP and MINP) for both samples (16-18 weeks and pooled) and performance on the physical reasoning task in boys. Boys with higher exposure looked longer at the possible event (Figure 5.3 and 5.4). In addition, there were significant associations between the Anti-Androgenic sum in the pooled sample and the sum of all phthalates at 16-18 weeks of gestation

and performance in the physical reasoning task in boys, in which boys with higher exposure looked longer at the possible event (Figures 5.5 and 5.6, respectively).

5.3.4. Interactions

As described previously, there were associations between both maternal perceived stress and maternal TL and performance on the physical reasoning task in girls. Thus, the potential interaction of stress with phthalate exposure was explored only for these stress measures and only in girls. Results showed there was only one significant interaction between prenatal maternal stress and prenatal exposure to phthalates. Specifically, there was a significant interaction between prenatal perceived stress and ΣDINP (sum that contained all three metabolites: MCOP, MINP, and MONP) at 16-18 weeks of gestation in girls (Figure 5.7). Specifically, girls whose mothers were in the medium stress group and had higher exposure to ΣDINP had a greater difference in looking time (β= 23.1; 95%CI: 3.7, 45.8; p-value=0.0466) than those whose mothers were in the low stress group.

5.3.5. Sensitivity Analyses

Findings were essentially unchanged when we excluded infants whose mothers reported smoking during the first trimester (n= 3 or 4, it varied by model, see table 5.1), or when we adjusted models for maternal alcohol consumption during the first trimester.

5.4 DISCUSSION

The results showed an association between maternal TL and physical reasoning in girls. Shorter telomere length was associated with shorter looking time difference in the physical reasoning task. This result is consistent with the association between higher maternal perceived stress and shorter looking time differences in girls presented in Chapter 4.There was an interaction with event order, with girls who saw the impossible event first showing a stronger association with telomere length. This is an important factor, as the effect of event order might explain part of the association observed. Event order was counterbalanced across infants with

some seeing possible first and some seeing impossible first because infants tend to look longer at a stimulus that is novel regardless of whether it defies expectations (Aslin, 2007). Since this association was only statistically significant in the girls who saw the impossible event first, it is difficult to differentiate between the contribution of the novelty of seeing something for the first time versus the impossible nature of the event. Interestingly, as described in Chapter 3, there wasn't a significant correlation between perceived stress scores and maternal TL in our sample. As suggested in Chapter 3, this might be due to the fact that these are measuring different aspects of the women's stress. Maternal TL is likely a measure of longer-term chronic stress, with the shortening of TL likely happening as a result of oxidative stress (Feiler et al. 2018; Mathur et al. 2016; Oliveira et al. 2016; Wojcicki et al. 2015). Whereas, PSS assessed perceived stress during the month before completing the survey (Cohen et al. 1988). Although these measures are likely measuring longer term vs more recent stress levels, it is striking that both are associated with a sex-specific negative impact on this cognitive task.

No associations were observed between SLE during pregnancy or cord blood telomere length and looking time difference. These results contrast with the associations observed by Barrett et al. (2013 and 2014) and Feiler et al. 2018, where they saw associations of each measure with differences in anogenital distance (Barrett et al. 2013), play behavior (Barrett et al. 2013), and BSID scores (Feiler et al. 2018). A possible explanation for these differences is the outcome assessed. Barrett et al. (2013) focused on physical development, Barrett et al. (2014) focused on play behavior, and Feiler et al. (2018) focused on cognitive developments as evaluated by the BSID. Another possible explanation for the difference between Barrett et al. findings and those presented in this chapter is that a very small sub-group of our women reported SLE during pregnancy (described in Chapter 3). In addition, the women in the IKIDS cohort tend to have higher socioeconomic status. This in combination with few reported SLE suggests that they potentially have more resources to cope with the stress of unexpected life events when the do occur. Interestingly, as described in Chapter 3, there was a significant

correlation between perceived stress scores and SLE in our sample. However, this correlation wasn't a strong one and the women's perceived stress could be related to other day to day factors that do not rise to the level of a SLE as defined in the scale. These results together with those presented in Chapter 4, suggest that maternal perceived stress seems to be a better predictor of outcomes in the physical reasoning task than the SLE scale.

When evaluating the effects of prenatal exposure to phthalates significant associations were found between ΣDEHP at 16-18 weeks of gestation and difference in looking time in girls, between ΣDINP2 in both samples and difference in looking time in boys, between Anti-Androgenic sum in the pooled sample and difference in looking time in boys, and between the sum of all phthalates at 16-18 weeks of gestation and difference in looking time in boys. Interestingly, the association in boys is consistent across of all these phthalate exposure measures. Together these findings suggest the potential adverse impacts of prenatal exposure of phthalates on physical reasoning in boys. Regardless, of the timing or order of event presentation, boys with higher exposure to ΣDINP2, Anti-Androgenic sum, and the sum of all phthalates were looking longer at possible event than the impossible event. The finding that the impact of DEHP, where girls look longer at the impossible event with higher exposure, was only seen when exposure was assessed at the 16-18 time point (in girls) and not in the pooled sample may suggest that the impact of this exposure is stronger during early to mid-pregnancy, but further analyses are needed to understand this association. Another important aspect of this association is that we see greater difference in looking time with greater exposure to DEHP suggesting an improvement in girls. There are multiple possible reasons for the positive associations with DEHP. First, it is possible that this effect might stem from residual uncontrolled confounding. For example, this model did not control for infant birth weight or other possible confounders that could be related to both prenatal exposure to phthalates and performance on the task. Another possible reason is the relatively small number of observations in this analysis. This result needs to be replicated in a larger sample to draw better conclusions

about this apparent positive relationship between DEHP exposure and physical reasoning in girls. Finally, although most studies show a negative impact, there are other studies in the literature that have found associations between higher phthalate exposure and better neurodevelopmental outcomes (e.g. Braun, 2017). Endocrine disrupting chemicals often have non-monotonic dose response curves so it is conceivable that there could be a range of DEHP exposure that is associated with better outcomes (Vandenberg et al. 2012).

There was little indication of interactive effects between maternal stress and prenatal phthalate exposure. Analyses for each significant stress measure (perceived stress and maternal telomere length) with each phthalate biomarker of interest were assessed, however, only one significant interaction was observed. An interaction between prenatal maternal perceived stress and ΣDINP in girls was observed. Interestingly, this interaction was guided by the difference between girls in the low and medium stress group. The findings did not suggest a cumulative negative impact of maternal stress and phthalates, it rather they suggested a positive effect of DINP exposure in girls exposed to moderate prenatal stress. There was no significant association of DINP with difference in looking time in girls whose mothers had low or high levels of perceived stress, but there was a significant positive association of difference in looking time with DINP in the medium stress group in which girls with higher exposure to DINP had greater looking time differences. Again, we have a suggestion that higher exposure to a phthalate, specifically DINP, might improve girls' performance on this task. However, as already discussed this result should be interpreted cautiously given the small number of observations in each group.

Finally, there are some strengths and limitations to this study that should be considered. This study took advantage of data regarding key demographic factors and maternal lifestyle that were available. However, evaluation of additional factors like clinical diagnosis of anxiety or depression, medication use, breastfeeding status, and infant birth weight as potential covariates will be needed to draw more complete conclusions. These data have been collected but are not

yet available for use in analyses due to the ongoing nature of this cohort study. This study took advantage of state-of-the-art eye tracking technology allowed automated collection of precise looking behavior data. This allowed for assessment of specific cognitive domains in a large sample of infants and much earlier in life than cognition has typically been assessed in other epidemiological studies. The use of multiple measures of stress is a major strength of this study, as most studies focus on stressful life events or perceived stress and few have included biomarkers of chronic stress or included both biomarkers and self-reported measures of stress. A limitation is that another important biological stress measure (maternal hair cortisol) was not yet available for all of the women in the sample. Another major strength of this study is that, to our knowledge, this is the first study to assess the potential for cumulative effects of stress and phthalates on neurodevelopment during the first year of life. Interestingly there was no indication of a cumulative negative impact. That said, because of the small sample size of the current study, power to detect interactive effects of multiple exposures was relatively low and future studies will be needed to confirm the findings.

In conclusion, the findings presented here suggest that maternal perceived stress and maternal telomere length are associated with impaired physical reasoning in girls. Our findings provide additional evidence that maternal stress may adversely impact early neurodevelopment.

5.5 TABLES AND FIGURES

Table 5.1.

Demographic and lifestyle characteristics of mother-infant pairs included in analyses

	Stressful Life Event analysis (n=118)		Maternal Blood TL analysis (n=111)		Cord blood TL analysis (n= 94)		Perceived Stress Scale analysis (n=107)		Phthalates analyses (n=158)	
	N (%)	Mean (SD)	N (%)	Mean (SD)	N (%)	Mean (SD)	N (%)	Mean (SD)	N (%)	Mean (SD)
Maternal race										
White, Non-Hispanic	94 (80)		91 (82)		75 (80)		88 (82)		127 (80)	
Other	20 (20)		20 (18)		19 (20)		19 (18)		32 (20)	
Maternal age (years)		30.6 (3.8)		30.3 (3.7)		30.4 (3.5)		30.9 (3.9)		30.4 (3.9)
Maternal education										
Some college or less	15 (13)		14 (13)		10 (11)		15 (14)		29 (18)	
College degree or higher	103 (87)		97 (87)		84 (89)		92 (86)		130 (82)	
Annual household income										
<$60,000	36 (31)		35 (32)		26 (28)		33 (31)		48 (31)	
≥$60,000	82 (69)		76 (68)		68 (72)		74 (69)		109 (69)	
Maternal smoking	3 (3)		4 (4)		3 (3)		4 (4)		6 (4)	
Maternal drinking	28 (24)		30 (27)		27 (29)		24 (22)		37 (23)	
Mode of delivery (% cesarean)	23 (19)		20 (18)		16 (17)		21 (20)		34 (22)	
Gestational age (weeks)		39.5 (1.1)		39.5 (1.2)		39.5 (1.2)		39.5 (1.2)		39.5 (1.1)
Infant sex										
Female	57 (48)		55 (49.5)		42 (45)		56 (52)		78 (49)	
Male	61 (52)		56 (50.5)		52 (55)		51 (48)		81 (51)	

Table 5.2.

Maternal urinary metabolite exposure biomarker characterization for infants included in current analysis

		16-18 weeks gestation sample				Pooled sample			
Parent compound	**Urinary metabolite measured**	**N**	**Median (µg\L)**	**Minimum (µg\L)**	**Maximum (µg\L)**	**N**	**Median (µg\L)**	**Minimum (µg\L)**	**Maximum (µg\L)**
DEP	MEP	158	24.34	0	576.4	159	29.05	4.8	672.8
DEHP	MECPP	158	6.91	1.4	96.7	159	8.67	2.9	65.5
	MEHHP	158	4.5	0	72.9	159	5.69	1.96	49.7
	MEOHP	158	3.9	0	48.3	159	4.34	1.6	36.7
	MEHP	158	1	0	8.4	159	1.26	0	10.9
DINP	MINP	158	0.43	0	96.9	159	0.7	0.1	30.3
	MCOP	158	5.79	0.6	867.9	159	9.04	1.4	235.4
	MONP	118	1.76	0.3	367.5	118	2.36	0.7	106.9
Anti-Androgenic Sum	MIBP	158	7.84	1.2	622.2	159	8.75	3.1	883.8
	MBZP2	158	4.38	0	121.8	159	5.6	0.86	70.7
	MEHP	158	1	0	8.4	159	1.26	0	10.9
	MEHHP	158	4.5	0	72.9	159	5.69	1.96	49.7
	MEOHP	158	3.9	0	48.3	159	4.34	1.6	36.7
	MCOP	158	5.79	0.6	867.9	159	9.04	1.4	235.4
	MBP	158	10.7	1.8	186.4	159	12.48	3.1	145.1
	MHIBP	158	2.63	0	196.1	159	3.2	0.85	283.4
	MHBP	158	1	0	19.4	159	1.22	0	13.7
Sum of All Phthalates	MHINCH	158	0.56	0	53.9	159	0.9	0.1	32.6
	MCNP	158	1.73	0.1	58.9	159	1.9	0.6	20.3
	MCOP	158	5.79	0.6	867.9	159	9	1.4	235.4
	MECPP	158	6.91	1.4	96.7	159	8.7	2.9	65.5
	MEHHP	158	4.5	0	72.9	159	5.69	1.96	49.7
	MEOHP	158	3.9	0	48.3	159	4.34	1.6	36.7
	MEHP	158	1	0	8.4	159	1.26	0	10.9
	MCPP	158	1.06	0	65.8	159	1.37	0.3	24.4
	MHBP	158	1	0	19.4	159	1.22	0	13.7
	MHIBP	158	2.63	0	196.1	159	3.2	0.85	283.4
	MIBP	158	7.8	1.2	622.2	159	8.75	3.1	883.8
	MINP	158	0.43	0	96.9	159	0.7	0.1	30.3
	MBP	158	10.7	1.8	186.4	159	12.48	3.1	145.1
	MBZP2	158	4.4	0	121.8	159	5.6	0.86	70.7
	MEP	158	24.34	0	576.4	159	29.05	4.8	672.8
	MCOCH	158	0.43	0	19.3	159	0.53	0	10.5

Table 5.3.

Findings for models examining main effects associations between prenatal exposure and cognitive outcome measured

	Females			Males		
Exposure measure	**p-value**	**β estimate**	**95% confidence interval**	**p-value**	**β estimate**	**95% confidence interval**
Prenatal Stress Measures						
Perceived Stress Scale (PSS)						
Low	ref	-	-	Ref	-	-
Medium	0.18	-3.4	-8.5, 1.6	0.43	-2.9	-10.0, 4.3
High	0.006	-7.1	-12.0, -2.2	0.46	-2.1	-7.8, 3.6
Stressful Life Event (SLE)	0.95	0.13	-4.2, 4.5	0.31	2.6	-2.5, 7.7
Maternal Telomere Length (MTL)	0.03	13.3	1.6, 24.7	0.16	-13.4	-32.1, 5.3
Cord blood Telomere Length (CBTL)	0.43	3.9	-5.9, 13.7	0.92	-0.63	-13.4, 12.1
Phthalates (Pooled sample)						
ΣDEHP	0.26	14.4	-10.9, 39.7	0.28	-16.8	-47.4, 13.8
MEP	0.97	-1.1	-51.7, 49.6	0.93	-3.4	-83.4, 75.5
ΣDINP (two metabolites)	0.33	-14.9	-45.5, 15.7	0.04	-32.9	-64.3, -1.5
ΣDINP (three metabolites)	0.93	1.9	-40.6, 44.3	0.31	44.5	-42.9, 13.2
ΣAnti-Androgenic	0.82	-1.2	-11.9, 9.5	0.005	7.8	-38.4, -7.1
ΣAll Phthalates	0.62	1.4	-4.3, 7.1	0.11	-6.2	-14.0, 1.6
Phthalates (16-18 weeks gestation sample)						
ΣDEHP	0.02	30.8	4.0, 57.6	0.45	-8.9	-32.5, 14.6
MEP	0.62	14.6	-44.6, 73.9	0.21	-37.3	-96.5, 22.0
ΣDINP (two metabolites)	0.35	-8.3	-26.0,9.5	0.003	-32.2	-53.2, -11.2
ΣDINP (three metabolites)	0.12	54.5	-13.9, 123	0.75	9.9	-51.7, 71.6
ΣAnti-Androgenic	0.43	2.7	-4.0, 9.3	0.08	-8.4	-17.7, 1.0
ΣAll Phthalates	0.51	1.5	-29.8, 5.9	0.006	-8.2	-14.1, -2.4

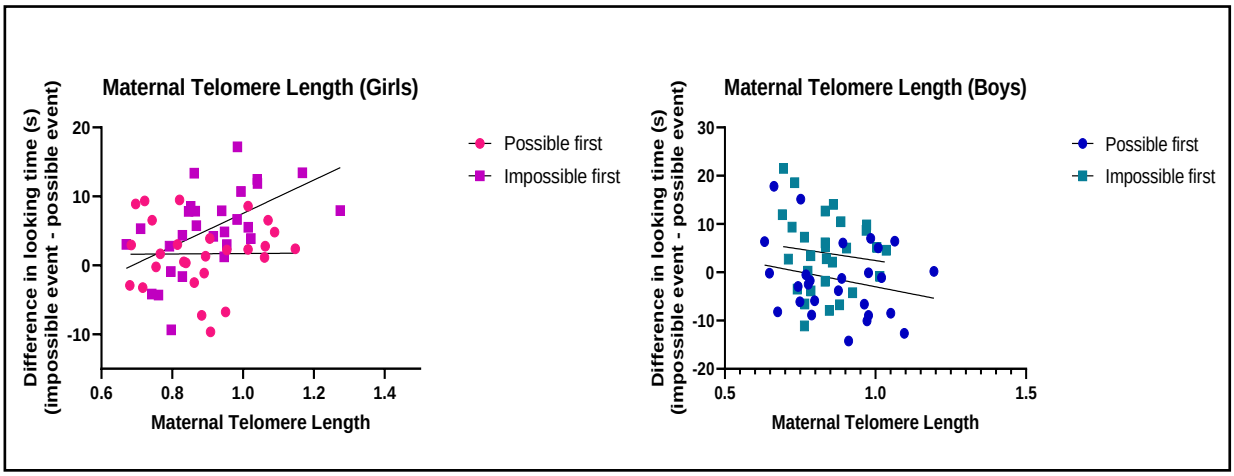

Figure 5.1. Interaction of maternal telomere length and order event with difference in looking time.

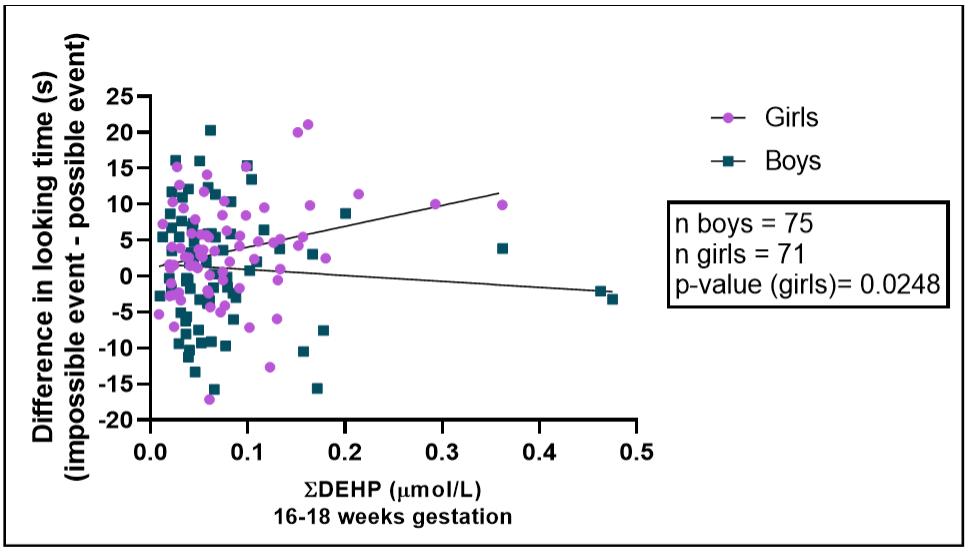

Figure 5.2. Association in girls and boys of ΣDEHP concentration at 16-18 weeks of gestation and difference in looking time.

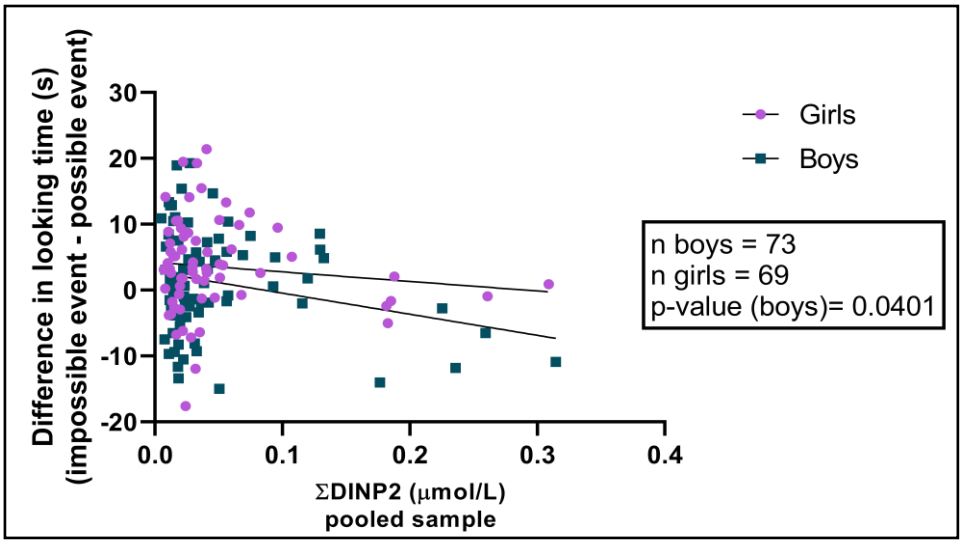

Figure 5.3. Association in girls and boys of ΣDINP at pooled sample and difference in looking time.

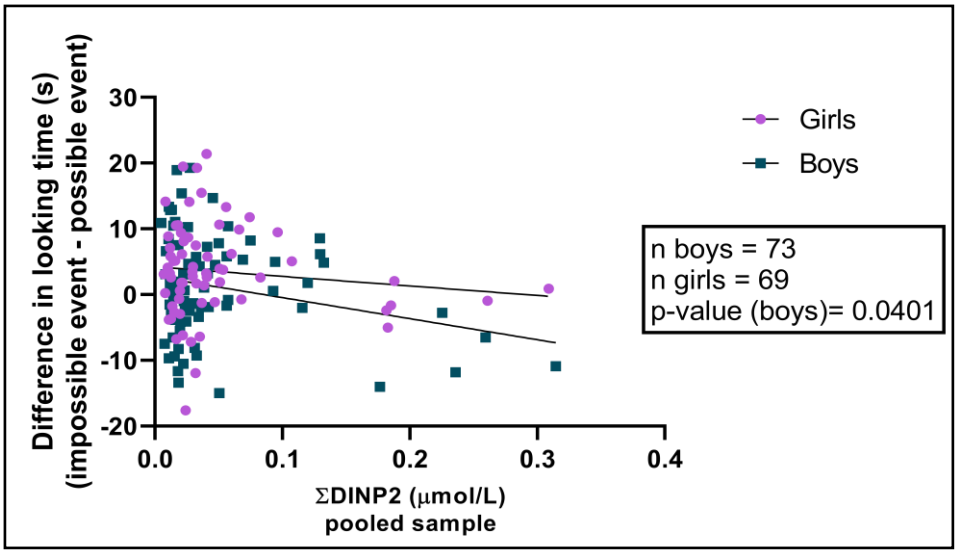

Figure 5.4. Association in girls and boys of ΣDINP at 16-18 weeks of gestation and difference in looking time.

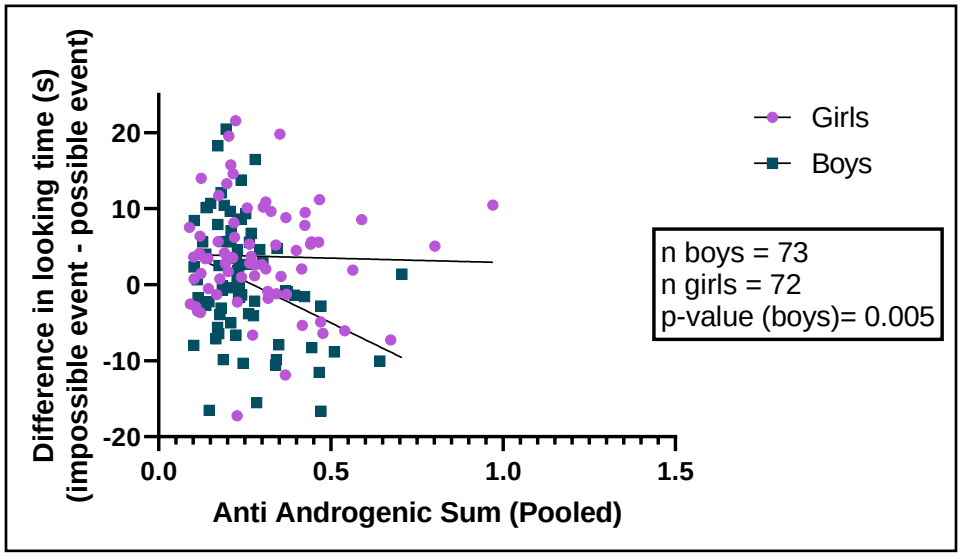

Figure 5.5. Association in girls and boys of Anti Androgenic Sum in pooled sample and difference in looking time.

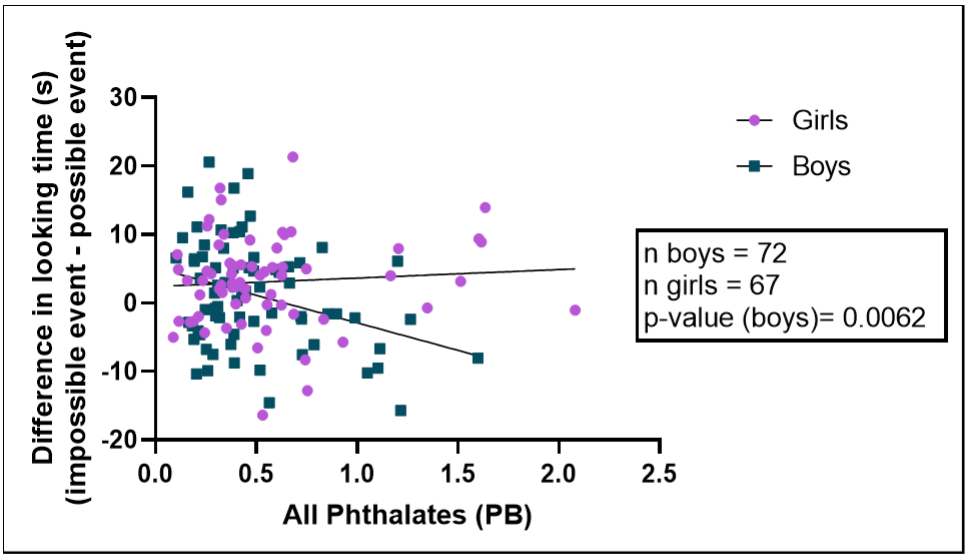

Figure 5.6. Association in girls and boys of the sum of all phthalates at 16-18 weeks of gestation sample and difference in looking time.

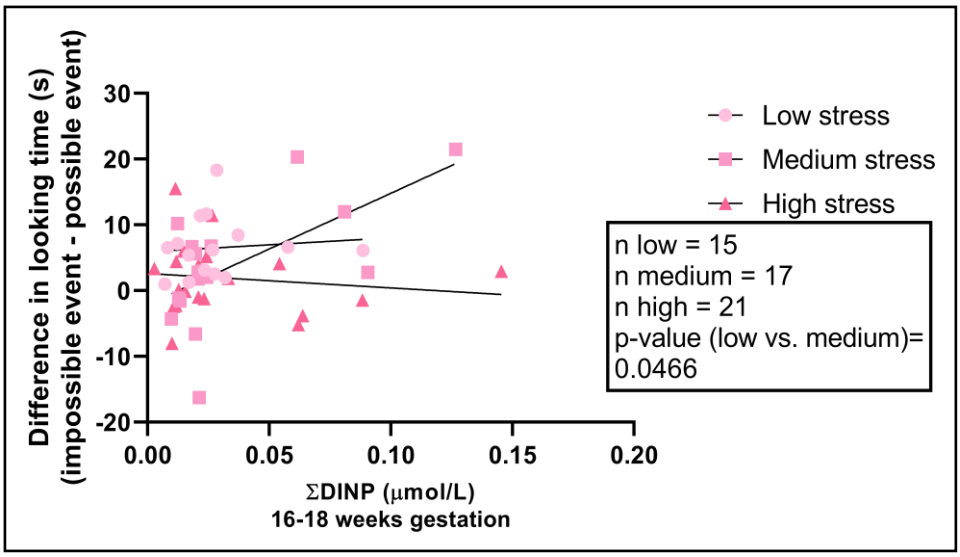

Figure 5.7. Interaction in girls between prenatal maternal perceived stress and ΣDINP at 16-18 weeks of gestation and difference in looking time.

CHAPTER 6: ASSOCIATION OF PRENATAL MATERNAL STRESS WITH MEASURES OF COGNITION IN 7.5-MONTH-OLD INFANTS AND POTENTIAL INTERACTIONS WITH PHTHALATE EXPOSURE

6.1 INTRODUCTION

As detailed in previous chapters, prenatal exposure to chemical and non-chemical environmental factors has the potential to affect neurodevelopment. A number of studies have reported negative impacts of prenatal stress on cognitive, behavioral, social, and biological outcomes, but most assessments have been during childhood and adolescence. Furthermore, previous studies have largely relied on global measures of cognition, rather than measures of specific cognitive domains. The Bayley Scales of Infant Development (BSID) has been most often used, and a recent systematic review and meta-analysis of these studies found that higher maternal prenatal stress or anxiety was associated with lower scores on the BSID in early childhood (Kingston et al. 2015). However, the BSID has been shown to generally be a poor predictor of long-term cognitive outcomes (Anderson et al. 2010, 2017; McCall et al. 1993).

Research in developmental psychology has shown that that infant looking behaviors can be used as reliable and stable measures of basic cognitive processes such as memory, attention, and information processing (Fagan et al. 2007; McCall et al. 1993; Rose et al. 1995). Importantly, these measures have been shown to be predictive of cognitive abilities later in childhood (Rose et al. 1995, 1997, 2001) and tend to be stable within individuals across time (Aslin 2007). Thus, these measures may serve as early indicators of long-term neurodevelopmental risk. Looking behaviors on visual recognition memory (VRM) tasks have been studied extensively across the first year of life. In VRM tasks, infants are shown a pair of identical two-dimensional stimuli side-by-side and allowed to study them for a set amount of time. Then the familiar stimulus is paired with a novel stimulus and this sequence is repeated with several different pairs of stimuli. The IKIDS research group automated this task with the use of infrared eye tracking technology that captures very precise real time looking behavior

data and uses those data to control the stimulus display. Three looking time variables were assessed. The proportion of time the infant spent looking at a novel stimulus, novelty preference, was used to measure recognition memory. Average fixation time during the familiarization trials, the average time the infant spent looking at a stimulus before looking away, was used to measure information processing speed, and time to reach familiarization, the time the infant took to reach looking time criteria on the familiarization trials, was used as a measure of attention, as off-task behavior prolonged time to reach criterion.

Previous unpublished studies in our cohort by Dzwilewski examined the association between prenatal exposure to phthalates (DEP, DEHP, and DINP) and performance on the VRM. Results showed significant negative associations of prenatal phthalate exposure with performance on the VRM task. Specifically, Dzwilewski observed longer trial durations and decreased novelty preference in males with higher prenatal exposure to DEHP and DINP, respectively.

Hence, the goal of the current study was to assess the potential association of prenatal maternal stress with basic building blocks of cognition including recognition memory, processing speed and attention as assessed on the visual recognition memory task. In addition, for outcomes where a significant impact of prenatal stress was observed, we assessed whether there were cumulative effects of maternal stress and phthalate exposure.

6.2 METHODS

6.2.1. Study population

The IKIDS cohort is described in detail in Chapter 3. Pregnant women were recruited for participation from two local obstetric clinics (Carle Physician Group and Christie Clinic) beginning in December 2013. During their first prenatal visit, the women received a brochure with information about the study and they filled out a card to indicate whether they were interested in learning more about the study. Women who expressed interest received a call from

the IKIDS staff, during which the study was described in more detail and the woman's eligibility to participate was ascertained. Study design, eligibility criteria and cohort characteristics have been covered in previous chapters.

6.2.2. Prenatal Stress

Details regarding the various measures used to assess maternal stress are presented in Chapter 3. Multiple measures of prenatal maternal stress were used—a measure of perceived stress, a stressful life events scale (SLE), and telomere length (TL) in maternal blood and cord blood (see Chapter 3 tables). As described in previous chapters, pregnant women completed the Perceived Stress Scale (Cohen et al. 1988) two times during gestation, at 8-14 and 34-37 weeks. The median scores from women included in this analysis were used to classify the women into three stress categories: low, medium, and high. Women who scored below the cohort median at both time-points were classified as low stress. Those who scored above the median at one of the two time-points were classified as medium stress. Finally, those who scored above the median at both time-points were classified as high stress. At birth women also complete a brief stressful life event scale that asked about potential stressful life events (SLE) experienced during their pregnancy. The possible total scores range from 0 to 6. SLEs were relatively rare in this sample, thus women were dichotomized to those who reported no SLEs (0) and those who reported one or more SLEs (1+) during pregnancy.

TL was assessed in two samples- maternal blood at 16-18 weeks of pregnancy and in cord blood at the time of delivery. The TL measurement assay was adapted from the published original method by Cawthon (2002) and represents a ratio of two qPCR reactions: **T**elomere over **S**ingle copy gene, (T/S).

6.2.3. Phthalates

As described in previous chapters, the IKIDS protocol includes collection of a first morning urine sample at five time points across pregnancy (at 10-14, 16-18, 22-24, 28-30, and

34-36 weeks of gestation). A pooled sample of urine including aliquots from all 5 time-points is archived for analysis. Prenatal exposure to phthalates was measured via urine metabolites in the pooled sample and also in the individual sample collected at 16-18 weeks of pregnancy. Urine samples were analyzed at the CDC Division of Laboratory Sciences using an online solid phase extraction-high performance liquid chromatography-isotope dilution tandem mass spectrometry method described by Silva and colleagues (2007) and Ye and colleagues (2005). The CDC analyzed 16 metabolites in approximately 1/3 of the samples. These were the first group of samples to be analyzed. With the development of new methods 3 extra metabolites have since been added. Hence, a total of 19 phthalate metabolites were measured in the remaining samples, which represent 10 parent compounds (see tables 3.7 and 3.8 in Chapter 3).

As described in Chapter 5, this study focused on DEP and DEHP exposure because these two phthalates account for about 35% and 20%, respectively, of the total phthalate metabolites in maternal urine. In addition, DINP was assessed as this is a common replacement for DEHP and exposure has been increasing (CDC, 2019). Exposure to DEP was measured via its urinary metabolite, MEP. While DEHP has four major urinary metabolites quantified by CDC. Thus, exposure to DEHP was measured via the molar sum of the following metabolites: MECPP, MEHHP, MEOHP, and MEHP. Not all urine samples were sent to the CDC at the same time and, as described above, between shipments new methods for phthalate metabolite measurements were developed, thus DINP was assessed two ways. As the sum of two urinary metabolites: MCOP and mNP which were available on all women, or as the sum of MCOP, mNP, and MONP which was only available for approximately 2/3's of the women. Women are never exposed to a single parent phthalate; hence, the following sums of multiple phthalates were also considered: the sum of anti-androgenic phthalates and the sum of all phthalates. The anti-androgenic (AA) sum was composed of the molar sum of eleven urine metabolites and was

based on mechanistic studies in animal models (sum described in Chapter 5). The sum of all phthalates was composed of sixteen urine metabolites, that were measured in all of the women in our sample. Interactions between phthalates and prenatal maternal stress were explored, were there was clear and consistent evidence of an association of stress with a particular cognitive outcome.

6.2.4. Visual Recognition Memory

Infants were assessed using a computerized paired-comparison visual recognition memory task based on the one developed by Rose and colleagues (Rose et al. 1992). Study infants were assessed at 224 to 259 days of age while seated on a parent's lap in front of the television. Hereafter, this is referred to as the infant's 7.5-month visit. The testing area was surrounded by black curtains to reduce distractions. In addition, mothers were instructed to remain as neutral as possible in their demeanor and to look downward as testing took place to prevent their looking behavior or response to the videos from influencing the infant's looking behavior. Looking behaviors were tracked using an EyeLink 1000 Plus infrared eye tracker (SR Research Ltd., Mississauga, Ontario, Canada). To calibrate the eye tracker, the infant's attention was drawn to three specific calibration and validation points on the screen using short animated clips with accompanying audio prior to the start of the task. The infant's attention was then drawn to the center of the screen prior each trial, to ensure that looking was not biased by gaze direction at the beginning of the trial.

The Visual Recognition task consisted of nine blocks of three trials each – one familiarization trial followed by two test trials (Figure 6.1). The first five blocks consisted of human faces and the last four blocks consisted of images of geometrical shapes (Figure 6.2). During the familiarization trial, the infant was shown identical black-and-white photographs of human faces or identical colored images of shapes displayed side-by-side. The stimuli remained until the infant had accumulated 20 seconds looking at them, during the blocks of human faces.

In the geometrical shapes familiarization, the stimuli remained until the infant had accumulated 10 seconds of looking at them. During each test trial, the face/shape seen in the familiarization trial was paired with a novel face/shape. The novel face/shape was presented on one side during the first test trial, and the opposite side during the second test trial, to ensure that any looking preferences were stimulus-based rather than side-based. Results from the first and second trial were averaged for analysis. Each test trial lasted approximately five seconds, with a minimum cumulative looking time of one second required. Infants included in the statistical analyses completed the full set of fact or shape trials. For the shape trials, block 8 was not included in the analyses. Unpublished work (Dzwilewski et al., under review) showed that infants had a strong preference for one of the two stimuli shown in block 8 (pink and blue stimulus; see Figure 6.2).

Each infant was assessed in one of four conditions. Within each sex, condition was assigned randomly within blocks of 48 infants (with blocking used to allow approximate balance of conditions within each sex and across time). In conditions A and B, the same stimuli were familiar, and these conditions were considered set 1; in conditions C and D, the other stimulus in each stimulus pair was the familiar stimulus and these conditions were considered set 2. In conditions A and C, the novel stimulus was initially presented on the right side (test trial 1) and then on the left (test trial 2); whereas in conditions B and D, the novel stimulus was presented on the left side first and then the right. Data from the EyeLink eye tracking system were reduced and extracted using DataViewer software (SR Research Ltd., Mississauga, Ontario, Canada), which allows the user to define areas of interest on the stimuli, and provides information on fixations, saccades, and blinks within each defined area of interest. In the visual recognition memory task, multiple assessments were made.

During the familiarization trials these outcomes were considered: average fixation duration, the average time an infant spent looking at stimuli before looking away which is a

measure of processing speed, and time to reach familiarization, the time an infant took to reach looking time criteria which is a measure of attention. During the test trial the outcome considered was novelty preference, the proportion of time spent looking at the novel stimulus which is a measure of recognition memory.

6.2.5. Covariates

Study researchers met with the pregnant women three times during pregnancy. Covariate data from one of these visits to the participant's home (at 8-14 weeks) were used in this analysis and included information about key demographic, lifestyle, health, and diet variables collected via questionnaire and structured interviews. Maternal covariates included age, education, and household income. Maternal verbal IQ was assessed using the Peabody Picture Vocabulary Test, 4th edition (PPVT-IV; Dunn et al. 2007) at a follow-up assessment during the child's first year of life. Additional covariates collected during study visits included maternal smoking and alcohol consumption during the first trimester of pregnancy. Infants sex was obtained at the time of birth and infant age at assessment was calculated as the number of days between date of birth and assessment day. As mentioned previously, infants were assessed in one of four conditions that can be grouped in two stimulus sets (set 1 vs set 2). Unpublished work (Dzwilewski et al., under review) showed that averaging across the two test trials for each stimulus set, eliminates the need to account for which side the novel image appeared on first. However, which stimulus set was novel did impact novelty preference scores and thus does need to be included as a covariate in statistical models.

6.2.6. Statistical Analysis

All statistical analyses were performed using SAS 9.4 software (SAS Institute Inc., Cary, NC, USA). General linear models were used to examine the association of each prenatal maternal stress assessment (perceived stress, SLE, maternal TL, cord blood TL) with performance on the visual recognition memory task (i.e. average fixation duration, time to reach

familiarization, and novelty preference). Based on previous findings interactions between infants' sex, stress, and which set of stimuli were novel (set 1 vs set 2) were assessed for each stress exposure-outcome model. For models where the interaction was found to have a p-value > 0.15, the interaction was removed from the model, sex was retained as a covariate, and main effects of the stress measure on the outcome across both sexes was assessed. For models where the interaction was found to have a p-value <0.15, models were stratified by sex or set of stimuli as appropriate. The same approach was used to examine the potential interaction between prenatal maternal stress (perceived stress, SLE, and maternal TL) and phthalates (DEP, DEHP, DINP, anti-androgenic sum, and sum of all phthalates). Dunnett's comparisons were applied for perceived stress post-hoc analyses with the low stress group as the reference group.

A priori knowledge and a directed acyclic graph (Hernan et al., 2000) were used to select the covariates included in the models. Socioeconomic indicators (maternal education, household income, mother's age at birth) were identified as potential confounders for inclusion in models. Maternal education was dichotomized to college educated versus not college educated. Household income was dichotomized at less than $60,000 per year versus $60,000 per year or more. Infant's age at exam and which set of stimuli was novel (set 1 vs set 2) were also included as covariates because they are known to be correlated with performance on the task.

Regression diagnostics were performed to identify influential data points, defined as observations that had extreme Cook's Distance. In the models that included phthalate exposure, observations with exposure biomarker concentrations greater than 2 standard deviations above the mean were also removed from the models. Main effects were considered significant if p-values were less than 0.05.

A number of sensitivity analyses were performed to assess the robustness of any observed associations by considering adjustment for additional covariates. Among the women

in our final analyses, there were few smokers (varied by model, see Table 6.1) and relatively few women reporting alcohol consumption (varied by model, see Table 6.1). We assessed potential confounding by smoking or alcohol in secondary analyses removing women who reported any smoking or adjusting for first trimester alcohol intake. Lastly, because of the correlation between pre- and postnatal perceived stress (r= 0.57-0.60), adjustment for postnatal stress was also done as a sensitivity analysis.

6.3 RESULTS

6.3.1. Descriptive Data

As of June 2019, a total of 351 mother-infant pairs participated in the 7.5-month visit at the IKIDS lab. Of those infants, eye tracking data were available for 326; data were not collected for the other 32 due to eye tracking failure (software malfunction or inability to track infant's gaze; n=10) or infant's state (fussiness, sleepiness, inattentiveness; n=22). Furthermore, there were 215 infants that had a complete cognitive dataset for the face trials analyses and 158 for the shape trials. There was available information on perceived stress and SLE for 219 mothers, maternal telomere length for 185 mothers, and cord blood telomere length for 169. In addition, there were 207 infants that had prenatal phthalate information and cognitive data. Each adjusted model had fewer observations due to incomplete data. Specifically, this was due to either incomplete cognitive information, missing covariates, or missing stress measures (see Table 6.1).

Table 6.1 details the characteristics of the sub-sample of mothers whose infants were included in each analysis. The demographics did not significantly differ across the sub-samples in these analyses nor in comparison with the demographics of the full IKIDS cohort (described in Chapter 3, Table 3.1). At the time of enrollment, more than 80% of the mothers had at least a college degree and about 70% had an annual household income of $60,000 per year or greater. Maternal smoking and alcohol consumption data were only available for the first trimester. Only 4% of the subsample included in these analyses reported any smoking during the first trimester

and about 30% reported any alcohol consumption. As described in more detail in Chapter 3, alcohol consumption included recollections from weeks 0 to 14 meaning some of the drinking reported was before knowledge of the pregnancy. In addition, most women reported less than 4 drinks per week. Infants in these analyses had an average gestational age of 39 weeks and about 20% were delivered via cesarean section.

6.3.2. Maternal Stress

For women included in the models described below, the median maternal PSS score at 8-14 weeks was 11 with a range from 0 to 26. At 34-37 weeks of gestation, the median PSS score was 10 with a range of 0 to 32. Prenatal stress scores at the two time points were moderately correlated (r_s= 0.60). The median postnatal PSS score (survey administered at the 7-month visit) was 9 with a range from 0 to 27. Prenatal stress scores at both time points were moderately correlated with the postnatal stress score (r_s= 0.57 and 0.60, respectively). A total of 54 (36%) of women reported at least one SLE during pregnancy. The median of SLE reported was of 0 with a range of 0 to 4. The average maternal TL was 0.88 T/S with a range of 0.61 to 1.28 T/S. The average cord blood TL was of 1.34 T/S with a range of 0.87 to 1.92 T/S. These distributions do not significantly differ from those presented in Chapter 3 for the full IKIDS cohort.

6.3.3. Associations of Perceived Stress with Cognitive Outcomes
6.3.3.1. Novelty Preference

In covariate-adjusted general linear models, there were no significant associations of perceived stress with novelty preference in the face trials. In the shapes trials, there was trend for an interaction between perceived stress and sex (p=0.0701). In models stratified by sex, there was a significant effect of perceived stress in boys, but not girls (Figure 6.3). Boys whose mothers had medium levels of perceived stress during pregnancy had significantly smaller proportion of looking time to the novel stimulus than boys whose mothers had low levels of perceived stress (β= -0.1; 95% CI: -0.17, -0.02; p=0.02). The covariate-adjusted mean ± SE

proportion of time looking at the novel stimulus for boys was 0.58±0.03 in the low stress group (n=14), 0.48±0.03 in the medium stress group (n=13), and 0.62±0.02 in the high stress group (n=19) (Figure 6.3).

6.3.3.2. Fixation Duration

In covariate-adjusted general linear models for fixation duration in the face trials, there was a trend for an interaction between perceived stress and condition (p-value=0.0915). In models stratified by condition, there was a significant effect of perceived stress in infants tested with set 1 as the familiar stimulus, but not those tested with set 2 (Figure 6.4). Infants whose mothers had high levels of perceived stress during pregnancy had significantly shorter fixation durations than infants whose mothers had low levels of perceived stress (β= -1.2; 95% CI: -2.1, -0.43 seconds; p=0.0063). There was a similar trend for infants whose mothers had medium levels of perceived stress during pregnancy compared to infants whose mothers had low levels of perceived stress (β= -0.75; 95% CI: -1.7, 0.14 seconds; p=0.097). The covariate-adjusted mean ± SE fixation duration in faces trials for set 1 was 4.67±0.4 in the low stress group (n=19), 3.91±0.4 in the medium stress group (n=22), and 3.41±0.3 in the high stress group (n=27) (Figure 6.4). Interestingly, in the shapes trials there was a trend for an interaction between perceived stress and sex (p=0.1168). In models stratified by sex, there was a significant effect of perceived stress in girls, but not boys (Figure 6.5). Girls whose mothers had high levels of perceived stress during pregnancy had significantly shorter fixation durations than girls whose mothers had low levels of perceived stress (β= -1.1; 95% CI: -1.8, -0.35 seconds; p=0.0084). There was also a trend for shorter fixation durations in girls whose mothers had medium levels of perceived stress during pregnancy compared to girls whose mothers had low levels of perceived stress (β= -0.87; 95% CI: -1.7, 0.07 seconds; p=0.065). The covariate-adjusted mean ± SE fixation duration for girls was 4.0±0.4 in the low stress group (n=23), 3.2±0.5 in the medium stress group (n=13), and 3.0±0.4 in the high stress group (n=21) (Figure 6.5).

6.3.3.3. Trial Duration

In covariate-adjusted general linear models for trial duration in the face trials, there was a main effect of perceived stress (p=0.002). Infants whose mothers had high levels of perceived stress during pregnancy had longer trial durations than those whose mothers had low levels of perceived stress (β= 10.8; 95% CI: 4.4, 17.2 seconds; p=0.002). The covariate-adjusted mean ± SE trial duration for infants was 48.1±2.9 in the low stress group (n=51), 47.8±3.4 in the medium stress group (n=38), and 58.9±2.8 in the high stress group (n=54) (Figure 6.6). Similarly, there was a main effect of perceived stress on trial duration in the shapes trials (p=0.002). Infants whose mothers had high levels of perceived stress during pregnancy had longer trial durations than those whose mothers had low levels of perceived stress (β= 9.6; 95% CI: 4.0, 15.3 seconds; p=0.002). The covariate-adjusted mean ± SE trial duration for infants was 23.3±2.9 in the low stress group (n=36), 27.6±3.3 in the medium stress group (n=26), and 32.9±2.6 in the high stress group (n=41) (Figure 6.7).

6.3.4. Associations of Stressful Life Events with Cognitive Outcomes
6.3.4.1. Novelty Preference

In covariate-adjusted general linear models for novelty preference, there were no significant associations of SLE in the face trials. In the shapes trials, there was a significant interaction between SLE and condition (p=0.0240). In models stratified by condition, there was a significant effect of SLE for set 1, but not set 2 (Figure 6.8). Infants whose mothers had one or more SLE during pregnancy had a greater proportion of looking time to the novel stimulus than those whose mothers did not experience SLE during pregnancy (β= -0.09; 95% CI: -0.15, -0.02; p=0.0089). The covariate-adjusted mean ± SE proportion of looking time for infants in set 1 was 0.55±0.03 in the no SLE group (n=32) and 0.64±0.03 in the at least one SLE group (n=13) (Figure 6.8).

6.3.4.2. Fixation Duration

In covariate-adjusted general linear models for fixation duration, there were no significant associations of SLE in the face trials. There was a main effect of SLE on fixation duration in the shapes trials (Figure 6.9). Infants whose mothers had one or more SLE during pregnancy had shorter average fixation times than those whose mothers did not experience SLE during pregnancy (β= 0.77; 95% CI: 0.21, 1.33; p=0.0075). The covariate-adjusted mean ± SE fixation duration for the shapes trials was 3.6±0.25 in the no SLE group (n=76) and 2.8±0.29 in the at least one SLE group (n=29) (Figure 6.9).

6.3.4.3. Trial Duration

In covariate-adjusted general linear models for trial duration, there was a trend for an interaction between SLE and condition in the face trials (p-value=0.0575). In models stratified by condition, there was a significant effect of SLE for set 1, but not set 2 (Figure 6.10). Infants whose mothers had more SLE during pregnancy had longer trial durations than those whose mothers did not experience SLE during pregnancy (β= -14.7; 95% CI: -24.4, -4.9; p=0.0036). The covariate-adjusted mean ± SE trial duration for infants in set 1 was 43.8±4.8 in the no SLE group (n=37) and 58.5±4.3 in the at least one SLE group (n=30) (Figure 6.10). In the shapes trials, there also was a significant interaction between SLE and condition (p=0.0011). In models stratified by condition, there was a significant effect of SLE in set 1, but not set 2 (Figure 6.11). Infants whose mothers had more SLE during pregnancy had longer trial durations than those whose mothers did not experience SLE during pregnancy (β= -17.1; 95% CI: -24.7, -9.5; p=<.0001). The covariate-adjusted mean ± SE trial duration for infants who saw set 1 was 19.2±3.3 in the no SLE group (n=32) and 36.3±3.6 in the at least one SLE group (n=14) (Figure 6.11).

6.3.5. Associations of Maternal Telomere Length with Cognitive Outcomes
6.3.5.1. Novelty Preference

In covariate-adjusted general linear models for novelty preference, there was a significant three-way interaction between maternal TL, sex, and condition in the face trials (p=0.04). When stratified by sex, there was a significant two-way interaction between maternal TL and condition in girls, but not boys (Figure 6.12) (β=-0.32; 95% CI: -0.55, -0.10; p-value=0.006). As shown in Figure 6.12, girls in set 1 showed an increased novelty preference with shorter maternal TL. There were no significant associations between maternal TL and novelty preference in shapes trials.

6.3.5.2. Fixation Duration

In covariate-adjusted general linear models for fixation duration, there was a main effect of maternal TL on fixation duration in the face trials (β=1.81; 95% CI: 0.18, 3.44; p-value=0.0298). Shorter maternal TL was associated with shorter fixation duration (Figure 6.13). There was no significant association between maternal TL and fixation duration in shapes trials.

6.3.5.3. Trial Duration

In covariate-adjusted general linear models for trial duration, there was a main effect of maternal TL on trial duration in the face trials (β=-23.9; 95% CI: -43.3, -4.5; p-value=0.0163). Shorter maternal TL was associated with longer trial duration (Figure 6.14). There was no significant association between maternal TL and trial duration in shapes trials.

6.3.6. Cord Blood Telomere Length and Visual Recognition Memory

There was no association between cord blood TL and novelty preference, trial duration, or fixation duration in the face trials. The only significant association was a significant three-way interaction between cord blood TL, sex, and condition (p=0.0206) for fixation duration in the shapes trials. In models stratified by sex, there was a significant effect of cord blood TL in boys, but not girls (Figure 6.15) (β=-7.2; 95% CI: -13.3, -1.1; p-value=0.0224). As shown in Figure

6.15, boys in set 1 showed longer fixation durations with shorter cord blood TL. Whereas, boys in set 2 showed shorter fixation duration with shorter cord blood TL.

6.3.7. Interactions

The most consistent associations of maternal stress with performance on the VRM task were associations of multiple measures of stress with trial duration, where more stress was associated with longer trial duration. Therefore, interactions between the stress measures and phthalate exposure were examined for this specific outcome as a starting point to begin to address whether stress and phthalate exposure could interact to increase risk. All phthalates of interest were examined; however, significant interactions between stress and phthalate exposure were only observed for DINP exposure at 16-18 weeks of gestation.

There was a significant interaction between maternal perceived stress and prenatal exposure to DINP (MCOP and mNP) at 16-18 weeks of gestation (Figure 6.16) (β=70.9; 95% CI: 3.9, 137.8 seconds; p-value=0.0381). There was an increase in trial duration with higher exposure to DINP at 16-18 weeks of gestation in infants whose mothers had high perceived stress during pregnancy.

There was a significant three-way interaction between SLE, condition and prenatal exposure to DINP (MCOP, mNP, and MONP) at 16-18 weeks of gestation (p=0.0154). In models stratified by condition, there was significant interaction between SLE and DINP in set 1, but not set 2 (Figure 6.17; β=326.6; 95% CI: 33.5, 619.7 seconds; p-value=0.03). There was an increase in trial duration with higher exposure to DINP in infants whose mothers did experience SLE during pregnancy.

There was a significant three-way interaction between maternal TL, sex, and prenatal exposure to DINP (MCOP and mNP) at 16-18 weeks of gestation (p=0.0093). In models stratified by sex, there was a significant interaction between maternal TL and prenatal exposure to DINP in boys, but not girls (p=0.0195). Both maternal TL and exposure to DINP are continuous variables. However, longer TL is identified as a positive measure of health but higher

levels of DINP are identified as a negative measure of exposure. For ease of interpretation, and to further investigate the interaction of TL and DINP exposure in boys, DINP exposure was dichotomized into the top 25th percentile of exposure and the bottom 75th percentile of exposure. In this model, there was a significant interaction between maternal TL and DINP in boys in the top 25 of DINP exposure (β=117.6; 95% CI: 15.0, 220.2 seconds; p-value=0.0254). Boys in the top 25 percent of DINP exposure had longer trial durations with increasing maternal TL (Figure 6.17).

6.3.8. Sensitivity Analyses

Findings were essentially unchanged when we excluded infants whose mothers reported smoking during the first trimester, or when we adjusted models for maternal alcohol consumption during the first trimester. Associations of postnatal perceived stress with trial duration were similar to those observed with prenatal perceived stress. There was a trend for higher postnatal perceived stress to be associated with longer trial duration in the face trials (β=5.1; 95% CI: -0.52, 10.6 seconds; p-value=0.0764), and there was a significant association between higher maternal postnatal perceived stress and longer trial duration in the shape trials (β=6.6; 95% CI: 1.6, 11.5 seconds; p-value=0.0101).

6.4 DISCUSSION

6.4.1. Overview of Results

This study showed that prenatal maternal stress is associated with changes in performance on the VRM task at 7.5 months of age. As summarized in table 6.3, in covariate-adjusted models significant associations were observed between perceived stress and decreased novelty preference in boys, shorter fixation duration, but only for set 1 in girls, and longer trial duration across sexes and conditions. Stressful life event models showed significant associations of SLE with greater novelty preference but in set 1 only, shorter fixation duration, and longer trial duration, but again in set 1 only. Maternal TL models showed significant

associations of shorter maternal TL with greater novelty preference in girls in set 1 only, shorter fixation durations across sexes and conditions, and longer trial duration across sexes and conditions. Finally, shorter cord blood TL was significantly associated with longer fixation duration in boys that saw set 1.

6.4.2. Trial Duration

Trial duration, a measure of attention, was associated with perceived stress, SLE, and maternal TL. In all cases longer trial durations where associated with great prenatal stress. When assessing the association of perceived stress with trial duration, results showed longer trial duration with higher levels of perceived stress. This was consistent across face and shape trials and there were no interactions with condition or sex. When assessing the association of SLE with trial duration, results showed longer trial duration, again across both face and shape trials, with exposure to at least one SLE during pregnancy, but only in infants who saw set 1. When assessing the association of maternal TL with trial duration, shorter TL was associated with longer trial duration in both sexes and conditions, but only on the face trials. In summary, the results summarized here and in Table 6.3 showed that infants of both sexes whose mothers had higher stress took longer to reach the criterion looking time in familiarization trials. The amount of time it takes an infant to the reach looking time criterion reflects attention, as more time spent looking away or off task extends the amount of time to reach criterion (20 seconds in face trials and 10 seconds in shape trials). Thus, together these findings suggest a potential adverse impact of maternal stress on visual attention.

6.4.3. Fixation Duration

Fixation duration, considered to be a measure of processing speed, was associated with perceived stress, stressful life events, maternal TL, and cord blood TL. When assessing the effects on perceived stress on fixation duration, results showed shorter fixation durations with higher levels of perceived stress. Interestingly, in the face trials this effect was only seen in set 1. Whereas, in the shapes trials this effect was only seen in girls, regardless of the condition.

Similar to these results, fixation duration was also shorter in women who reported SLE during pregnancy. However, in contrast to the results seen with perceived stress, this result was only seen in the shapes trials and there was no interaction with condition or sex. Similar to the results of perceived stress and SLE, shorter maternal TL also was associated with shorter fixation duration, but in contrast with the previous results, this was seen in the face trials and across sexes and conditions. When assessing the effects of cord blood TL, results showed that shorter cord blood TL was associated with longer fixation duration in set 1 and shorter fixation duration in set 2. This association was only significant in boys. It is important to note that when stratifying by both sex and condition there were a small number of observations (15 and 28, respectively) per group. Thus, this finding for cord blood TL should be interpreted cautiously. A greater number of observations will be needed to corroborate these results that suggest the association is both stimulus set and sex specific.

Interestingly, there were associations between maternal stress and shorter fixation duration across three different measures of stress. Although, the results varied in terms of the sex, type of trial (faces vs. shapes), and condition (set 1 vs. 2) where associations were seen. Previous studies (e.g. Sigman et al. 1991), have interpreted shorter fixations to indicate faster information processing speed. Hence, together these results suggest a positive impact of prenatal stress on information processing. However, in this sample there were significant negative correlations between fixation duration and trial duration (r_s= -0.62 and -0.54 [p-value= <.0001]; face and shapes trials, respectively), with shorter fixations associated with longer trial durations. A possible interpretation is that the infants having shorter fixations (“faster processors”) are spending more time off task since they need less time to familiarize themselves with the stimuli and thus, are taking longer to reach the familiarization criterion. Another possible interpretation is that shorter fixations may be indicative of attentional problems. That is, infants that are more easily distracted may look away more frequently resulting in shorter fixation durations. However, the association between stress and fixation duration

sometimes was only seen in infants who saw stimulus set 1 and/or in a specific sex. It is important to keep in mind that when interactions with condition and sex are present the number of observations per group becomes quite small. Therefore, these results should be interpreted with caution until they can be replicated in a larger sample.

6.4.4. Novelty Preference

Novelty preference was associated with perceived stress, SLE, and maternal TL, but not in a consistent manor across the three stress measures. Moderate levels of perceived stress were associated with decreased novelty preference but only in the shape trials and only in boys, suggesting a negative impact of perceived stress on working memory in boys. Conversely, SLE were associated with greater novelty preference on the shape trials, but only in infants who saw set 1. Finally, when assessing the effects of maternal telomere length, results showed that shorter TL was associated with higher novelty preference but only on the face trials and only in girls who saw set 1. The associations between SLE and maternal TL are in contrast with the effects seen with perceived stress, as SLE and maternal TL results suggest a positive effect of stress on novelty preference, whereas, association with perceived stress suggest a negative effect. A possible reason for these contrasting results, is that the associations differed in which stimulus set, sex, and type of trial (faces or shapes) was related to stress. Results suggested a decreased novelty preference in boys, but an improvement in novelty preference in girls. As mentioned in previous chapters, there is evidence to suggest that girls and boys are differentially sensitive to maternal stress. However, as mentioned previously, interactions with sex and stimulus set decrease the number of observations per group, hindering our ability to draw accurate conclusions.

6.4.5. Sensitivity Analyses

Sensitivity analyses revealed that the increase in trial duration with higher prenatal perceived stress could potentially be explained by postnatal levels of perceived stress. Similar to prenatal stress models, higher postnatal stress levels were associated with longer trial

duration. A similar relationship was also observed in Chapter 4, where pre- and postnatal perceived stress had similar associations with performance in the physical reasoning task. However, as mentioned previously in Chapter 4, it is difficult to differentiate the impact of pre- and postnatal stress since the two are correlated (r_s= 0.57-0.60). To tease out the impact of pre- vs postnatal maternal stress, a larger sample size would be needed which included sufficient numbers of women with high levels of prenatal stress only, high levels of postnatal stress only, or both.

6.4.6. Sex differences

A number of sex differences were observed in the results presented in this chapter. There was a significant decrease of novelty preference in boys whose mothers were in the medium maternal perceived stress group, whereas in girls, higher maternal perceived stress was associated with shorter fixation duration. Shorter maternal TL was associated with greater novelty preference in girls in set 1. Shorter cord blood TL was associated with decreased fixation duration in boys in set 2. Previous literature suggest that males and females may be differentially sensitive to prenatal maternal stress (refer to Chapter 1). However, the results presented here did not show a clear pattern to suggest that one sex was more sensitive to stress than the other.

6.4.7. Conditions

A number of interactions were observed with condition (set 1 vs. set 2). When analyzed further, there were consistently significant associations in set 1, but not set 2. Unpublished work (Dzwilewski et al., under review) showed that infants viewing set 1 had shorter average run duration (average time spent on-task before looking way; another measure of information processing speed) than those in set 2. In addition, Dzwilewski showed that the infants in set 1 had greater novelty preference and longer trial duration than those in set 2. Clearly there are differences in how infants respond to the two sets of stimuli. However, at this time it is not clear how these differences relate to stress.

6.4.8. Stress Measures

Perceived stress, SLE, and maternal TL were all associated with the cognitive outcomes assessed. Findings were mostly consistent across trial duration and fixation duration with all three stress measures predicting longer trial durations and shorter fixations with higher maternal stress. This was despite the lack of correlation between maternal stress measures (refer to Chapter 3); except for perceived stress and SLE, however, these were only weakly correlated. As mentioned previously, this could be due to the fact the different stress measures are tapping different aspects of stress (e.g. maternal TL measures longer term chronic stress versus perceived stress measures stress in the previous month). In contrast to these three measures, cord blood TL did not seem to be a good predictor of the infant cognitive outcomes assessed.

6.4.9. Interactions

Interactions between prenatal stress and phthalates, were only assessed for trial duration in the face trials, since the effect of prenatal stress on trial duration was generally consistent across stress measures. There was a significant interaction of perceived stress and prenatal exposure to DINP (2 metabolites) at 16-18 weeks of gestation. Results showed that infants whose mothers had higher perceived stress levels and higher prenatal exposure to DINP had longer trial duration, suggesting a cumulative effect of perceived stress and DINP exposure. There was also a significant interaction of SLE and exposure to DINP (3 metabolites) at 16-18 weeks of gestation. Results showed that infants whose mothers did not experience SLE showed longer trial duration with higher exposure to DINP, but the same pattern was not seen for infants whose mothers did experience stressful events. This is contrary to the relationship observed for perceived stress, but there were only 11 observations in the group whose mothers did experience SLE, hence, these results should be interpreted with caution until they are replicated in a larger sample. Finally, there was an interaction between maternal TL, prenatal exposure to DINP (two metabolites) and sex at 16-18 weeks of gestation Boys whose mothers had shorter maternal TL and were in the top 25% of DINP exposure had shorter trial duration, a finding that

was contrary to expectations. Whereas, boys whose mothers had shorter TL and where in the bottom, 75% percentile of exposure to DINP showed the previously reported result were shorter TL was associated with longer trial duration. It is important to note that there were only 19 boys in the top 25% of DINP exposure, so a greater sample will be needed to corroborate these unexpected results.

Finally, it is important to note that even though all phthalates of interest were assessed, we only saw interactions of stress with DINP which is used as a common replacement for DEHP. This adds to the growing body of evidence suggesting for regrettable replacements, where the chemical replacing the original one might be having even more detrimental effects than the original. Finally, although these interaction effects were not consistent across stress measures these analyses show the importance of accounting for multiple environmental factors, as pregnant women are exposed to a myriad of environmental factors and the response to one factor may be different when the other is also present.

6.4.10. Strengths and Limitations

There are several strengths and some limitations of this study that should be taken into account when considering its findings and implications. As with the physical reasoning task discussed in chapters 4 and 5, the visual recognition memory task took advantage of state-of-the-art eye tracking technology that allowed automated collection of precise looking behavior data. In addition, this task assessed basic components of cognitive function that tend to be stable within individuals across time (Aslin 2007) and have been shown to be predictive of cognitive abilities later in childhood (Rose et al. 1995, 1997, 2001). Thus, the outcomes measured here may serve as early indicators of long-term neurodevelopmental risk. As mentioned in previous chapters, this study considered a wide-range of demographic and lifestyle variables that could potentially be related to the outcomes of interest. However, due to the ongoing nature of the study other potential covariates that will be needed to draw more complete conclusions were not available at this time. As stated in previous chapters, the use of

multiple measures of stress including perceived stress, SLE, maternal and cord blood TL is a strength of this study. However, a limitation is that another important biological stress measure (maternal hair cortisol) was not yet available for all of the women in the sample and could not be used as a stress biomarker in the statistical models. Also, the fact that a multiple measures of stress were investigated in relation to multiple measures of cognitive outcomes increases the likelihood of spurious findings. Despite this problem, consistent patterns of results such as the tendency for increases in trial duration and decreases in fixation duration to be associated with higher stress across multiple measures of stress suggests that these are not spurious findings. Another major strength of this study is that, to our knowledge, this is the first study to assess the potential for cumulative effects of stress and phthalates on neurodevelopment during the first year of life. Interestingly the only interaction that indicated of a cumulative negative impact was with perceived stress, the interactions with SLE and maternal TL did not follow this pattern. That said, because of the small sample size of the current study, power to detect interactive effects of multiple exposures was relatively low and future studies will be needed to confirm the findings.

In conclusion, the findings presented here suggest that maternal stress, regardless of the measure, is associated with changes in performance on the visual recognition memory task. Specifically, findings suggest a negative impact on infants' attention and a potential for interaction with other environmental factors to worsen the outcome. Our findings provide additional evidence that maternal stress can impact early neurodevelopment.

6.5 TABLES AND FIGURES

Table 6.1.

Demographic and lifestyle characteristics of mother-infant pairs included in analyses

	Perceived Stress Scale analyses (n=152)		Stressful Life Events analyses (n=152)		Maternal Blood TL analyses (n=132)		Cord blood TL analyses (n=116)		Phthalates analyses (n=207)	
	N (%)	Mean (SD)	N (%)	Mean (SD)	N (%)	Mean (SD)	N (%)	Mean (SD)	N (%)	Mean (SD)
Maternal race										
White, Non-Hispanic	132 (87)		133 (87)		115 (87)		103 (89)		176 (85)	
Other	20 (13)		19 (13)		17 (13)		13 (11)		30 (15)	
Maternal age (years)		30.6 (3.8)		30.7 (3.8)		30.7 (3.8)		30.7 (3.5)		30.7 (3.9)
Maternal education										
Some college or less	21 (14)		21 (14)		19 (14)		15 (13)		29 (18)	
College degree or higher	131 (86)		131 (86)		113 (86)		101 (87)		130 (82)	
Annual household income										
<$60,000	42 (28)		42 (28)		39 (30)		31 (27)		48 (31)	
≥$60,000	110 (72)		110 (72)		93 (70)		85 (73)		109 (69)	
Maternal smoking	6 (4)		6 (4)		5 (4)		4 (3)		6 (4)	
Maternal drinking	47 (31)		48 (32)		38 (29)		36 (31)		37 (23)	
Mode of delivery (% cesarean)	36 (24)		37 (25)		27 (21)		24 (21)		34 (22)	
Gestational age (weeks)		39.5 (1.2)		39.5 (1.2)		39.6 (1.2)		39.6 (1.2)		39.5 (1.1)
Infant sex										
Female	79 (52)		80 (53)		67 (51)		56 (48)		105 (51)	
Male	73 (48)		72 (47)		65 (49)		60 (52)		102 (49)	

Table 6.2.

Maternal urinary metabolite exposure biomarker characterization for infants included in current analysis

		16-18 weeks gestation sample				Pooled sample			
Parent compound	**Urinary metabolite measured**	**N**	**Median (µg\L)**	**Minimum (µg\L)**	**Maximum (µg\L)**	**N**	**Median (µg\L)**	**Minimum (µg\L)**	**Maximum (µg\L)**
DEP	MEP	214	23.2	0	666.4	215	26.4	4.8	445.5
DEHP	MECPP	214	8	1.3	937.5	215	9.3	2.6	296
	MEHHP	214	4.7	0	750	215	6.2	1.2	250.7
	MEOHP	214	4.1	0	472.5	215	4.8	1	161.5
	MEHP	214	1.1	0	153	215	1.5	0	46.5
DINP	MINP	214	0.5	0	80.9	215	0.8	0.1	24.9
	MCOP	214	7.5	1.1	867.9	215	11.3	1.4	370.5
	MONP	127	1.7	0	367.5	128	2.5	1	99.8
Anti-Androgenic Sum	MIBP	214	7.5	1.3	622.2	215	8.8	1.8	883.8
	MBZP2	214	4.6	0	226.9	215	5.4	0.7	172.8
	MEHP	214	1.1	0	153	215	1.5	0	46.5
	MEHHP	214	4.7	0	750	215	6.2	1.2	250.7
	MEOHP	214	4.1	0	472.5	215	4.8	1	161.5
	MCOP	214	7.5	1.1	867.9	215	11.3	1.4	370.5
	MBP	214	11.5	1.1	321.4	215	12.7	3.1	221.8
	MHIBP	214	3	0	196.1	215	3.1	0.85	283.4
	MHBP	214	1	0	19.4	215	1.3	0	15.5
Sum of All Phthalates	MHINCH	214	0.45	0	17.3	215	0.8	0	30.2
	MCNP	214	1.8	0.1	58.9	215	2.1	0.4	128.8
	MCOP	214	7.5	1.1	867.9	215	11.3	1.4	370.5
	MECPP	214	8	1.3	937.5	215	9.3	2.6	296
	MEHHP	214	4.7	0	750	215	6.2	1.2	250.7
	MEOHP	214	4.1	0	472.5	215	4.8	1	161.5
	MEHP	214	1.1	0	153	215	1.5	0	46.5
	MCPP	214	1.1	0	52.1	215	1.4	0.4	23.1
	MHBP	214	1	0	19.4	215	1.3	0	15.5
	MHIBP	214	3	0	196.1	215	3.1	0.85	283.4
	MIBP	214	7.5	1.3	622.2	215	8.8	1.8	883.8
	MINP	214	0.5	0	80.9	215	0.8	0.1	24.9
	MBP	214	11.5	1.1	321.4	215	12.7	3.1	221.8
	MBZP2	214	4.6	0	226.9	215	5.4	0.7	172.8
	MEP	214	23.2	0	666.4	215	26.4	4.8	445.5
	MCOCH	214	0.38	0	7.8	215	0.51	0	17.1

Table 6.3.
Summary of maternal stress results on each outcome of the visual recognition memory task

Perceived Stress				
Outcomes	**Girls**	**Boys**	**Set 1**	**Set 2**
Novelty Preference (Faces)	--	--	--	--
Novelty Preference (Shapes)	--	↓	--	--
Fixation Duration (Faces)	--	--	↓	--
Fixation Duration (Shapes)	↓	--	--	--
Trial Duration (Faces)	↑	↑	↑	↑
Trial Duration (Shapes)	↑	↑	↑	↑
Stressful Life Events				
Outcomes	**Girls**	**Boys**	**Set 1**	**Set 2**
Novelty Preference (Faces)	--	--	--	--
Novelty Preference (Shapes)	--	--	↑	--
Fixation Duration (Faces)	--	--	--	--
Fixation Duration (Shapes)	↓	↓	↓	↓
Trial Duration (Faces)	--	--	↑	--
Trial Duration (Shapes)	--	--	↑	--
Maternal Telomere Length				
Outcomes	**Girls**	**Boys**	**Set 1**	**Set 2**
Novelty Preference (Faces)	↓	--	↓	--
Novelty Preference (Shapes)	--	--	--	--
Fixation Duration (Faces)	↑	↑	↑	↑
Fixation Duration (Shapes)	--	--	--	--
Trial Duration (Faces)	↓	↓	↓	↓
Trial Duration (Shapes)	--	--	--	--
Cord blood Telomere Length				
Outcomes	**Girls**	**Boys**	**Set 1**	**Set 2**
Novelty Preference (Faces)	--	--	--	--
Novelty Preference (Shapes)	--	--	--	--
Fixation Duration (Faces)	--	--	--	--
Fixation Duration (Shapes)	--	↓	↓	--
Trial Duration (Faces)	--	--	--	--
Trial Duration (Shapes)	--	--	--	--

*Upward arrows represent a positive association between the stress measure and the outcome presented. For example, higher levels of perceived stress were associated with longer trial durations. Downward arrows represent a negative association. For example, longer maternal telomere length was associated with a decrease in trial duration (which also reflects that shorter telomere length is associated with longer trial duration). Outcomes with arrows in every field, the association was a main effect (no interaction with condition or sex).

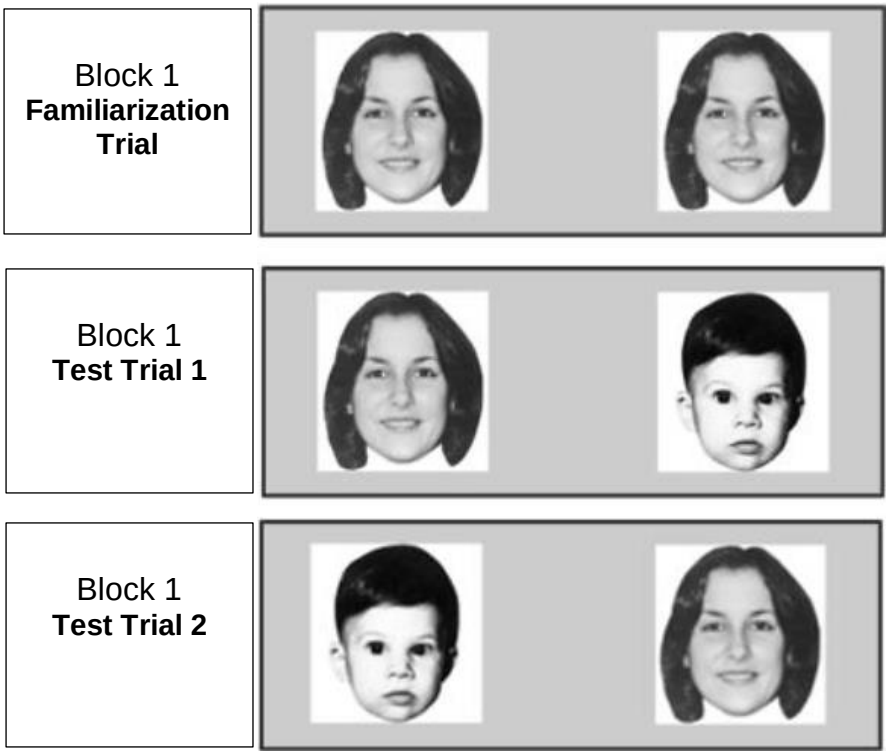

Figure 6.1. Example of stimulus displayed across block 1 of assessment paradigm.

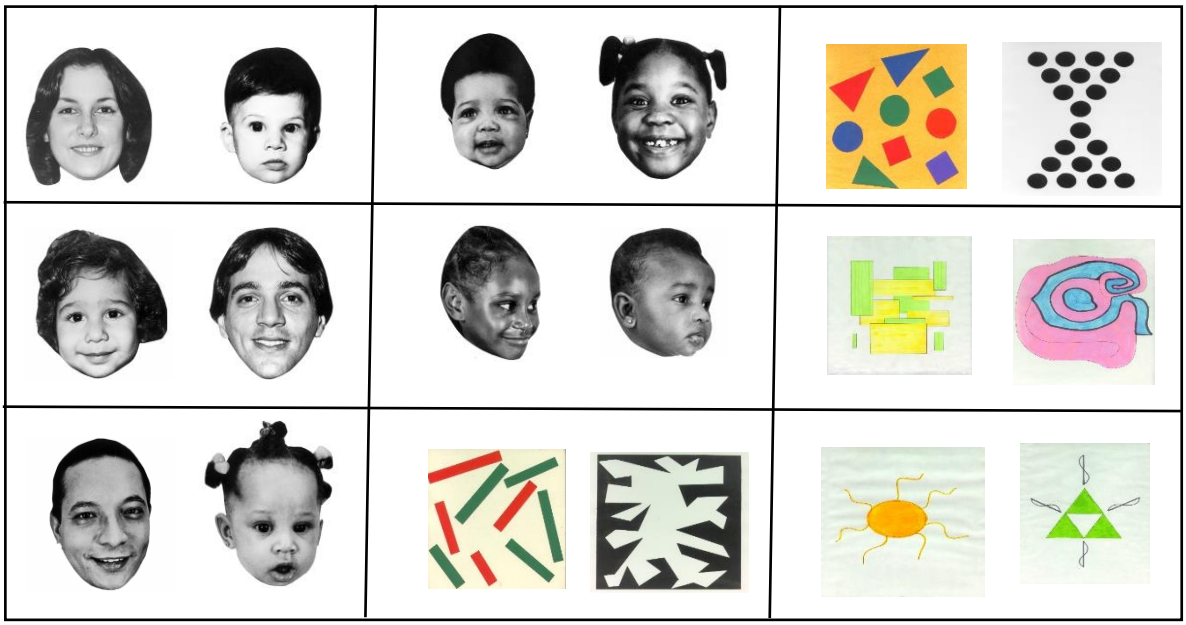

Figure 6.2. Stimulus pairs used for VRM assessment. Stimuli on the left side of each pair were the familiar stimuli in conditions for which set 1 were the familiar stimuli; stimuli on the right side of each pair were the familiar stimuli for which set 2 were the familiar stimuli. Pairs were presented in the same order in all assessment conditions, starting with the pair at the top left of this figure, through 5 pairs of faces, then 4 pairs of shapes, ending with the pair at the bottom right.

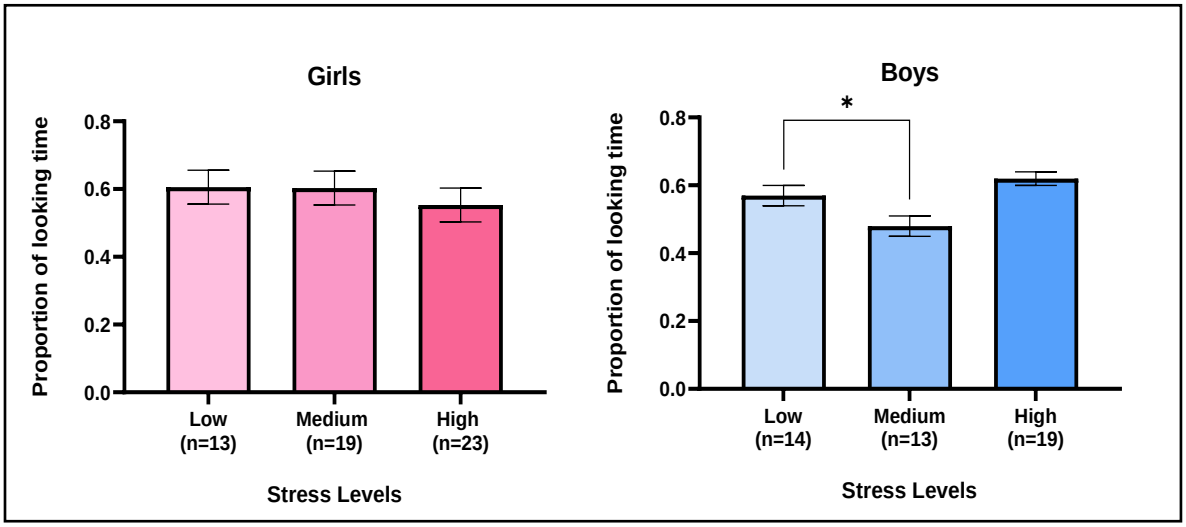

Figure 6.3. Association of maternal perceived stress with novelty preference in shapes trials in girls and boys. There was a significant difference between boys in the low stress group and those in the medium stress group (*p-value=0.02).

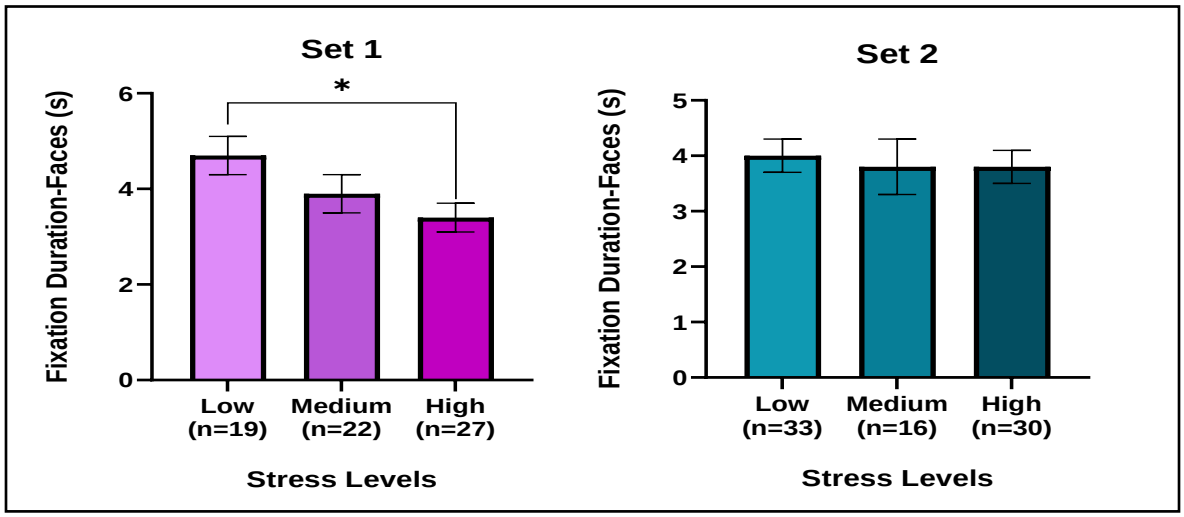

Figure 6.4. Association of maternal perceived stress with fixation duration in faces trials in set 1 and 2. There was a significant difference between infants in the low stress group and those in the high stress group in set 1 (*p-value=0.0063).

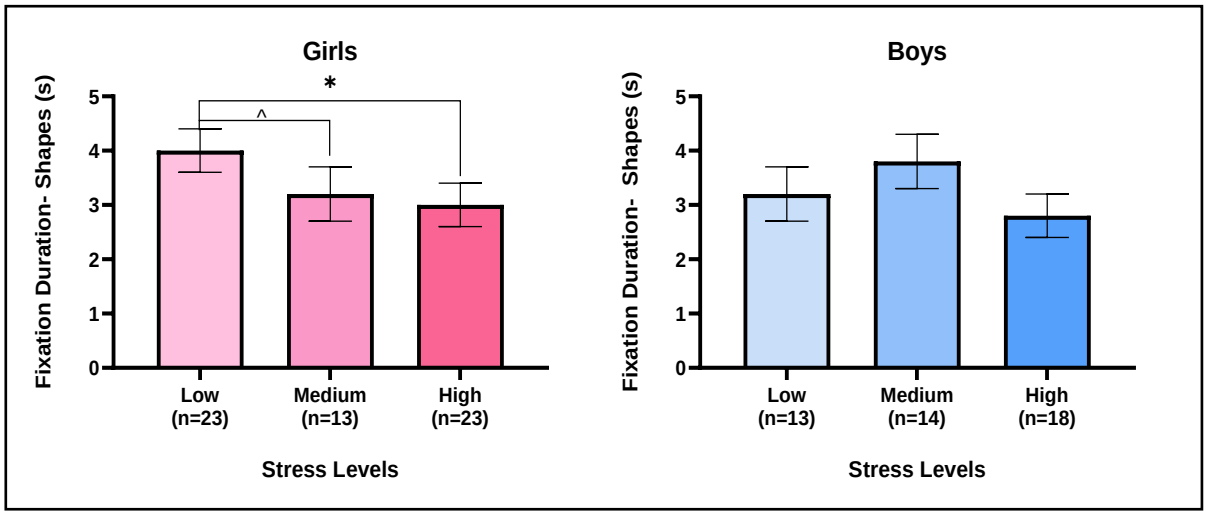

Figure 6.5. Association of maternal perceived stress with fixation duration in shapes trials in girls and boys. There was a significant difference between girls in the low stress group and the girls in the high stress group (*p-value=0.0084). There was a similar trend between girls in the low stress group and the girls in the medium stress group (^p-value=0.0655).

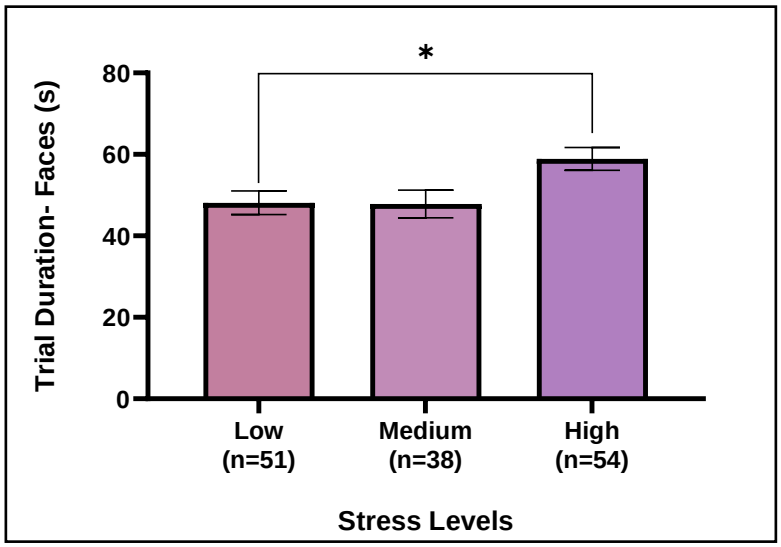

Figure 6.6. Association between maternal perceived stress and trial duration in faces trials. There was a significant difference between infants' in the low stress group and those in the high stress group (*p-value=0.002).

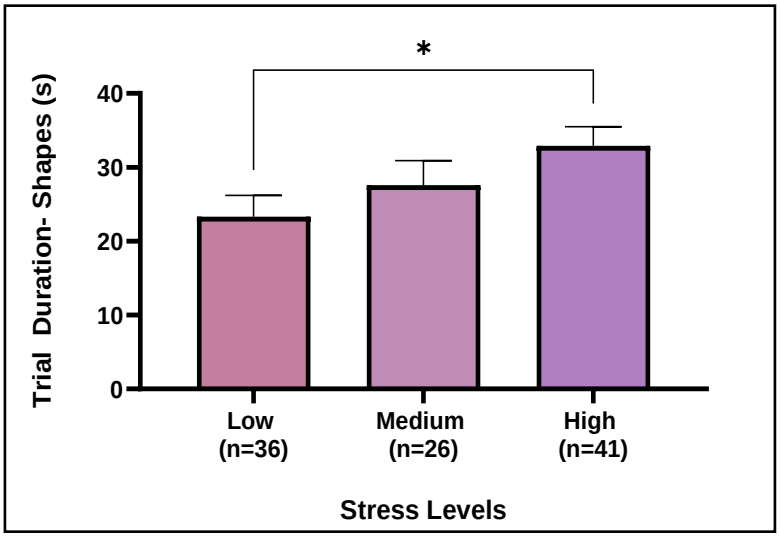

Figure 6.7. Association between maternal perceived stress and trial duration in shapes trials. There was a significant difference between infants' in the low stress group and those in the high stress group (*p-value=0.002).

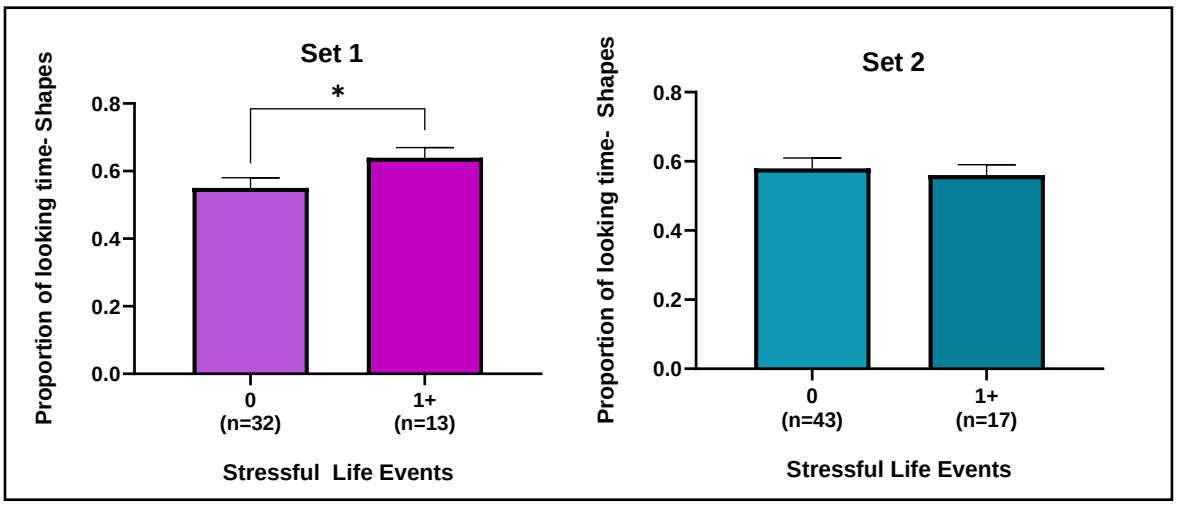

Figure 6.8. Association of stressful life event with novelty preference in shapes trials in set 1 and 2. There was a significant difference between infants in the low stress group and those in the high stress group in set 1 (*p-value=0.0089).

Figure 6.9. Association of stressful life events with fixation duration in shapes trials. There was a significant difference between the infants whose mothers didn't experience stressful life events during pregnancy and those who did (*p-value=0.0075).

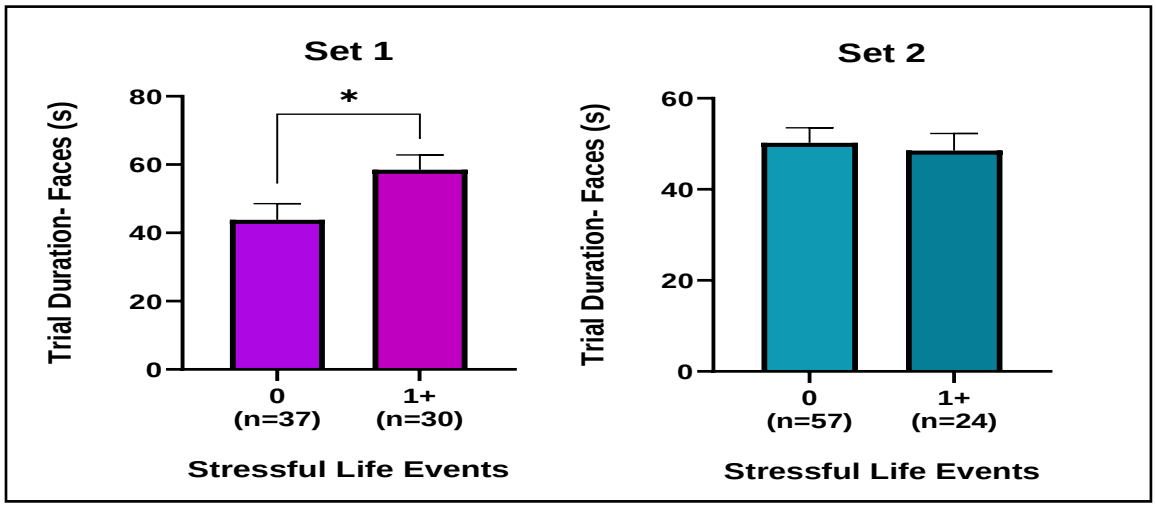

Figure 6.10. Association of stressful live events with trial duration in faces trials. There was a significant difference the infants whose mothers didn't experience stressful life events during pregnancy and those who did in set 1 (p-value=0.0036).

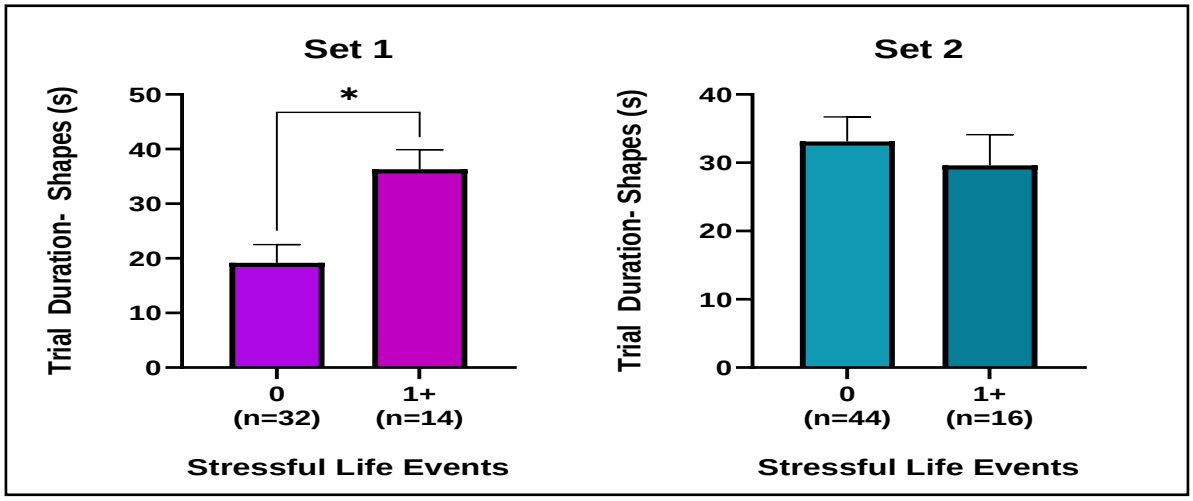

Figure 6.11. Association of stressful live events with trial duration in shapes trials. There was a significant difference the infants whose mothers didn't experience stressful life events during pregnancy and those who did in set 1 (*p-value<.0001).

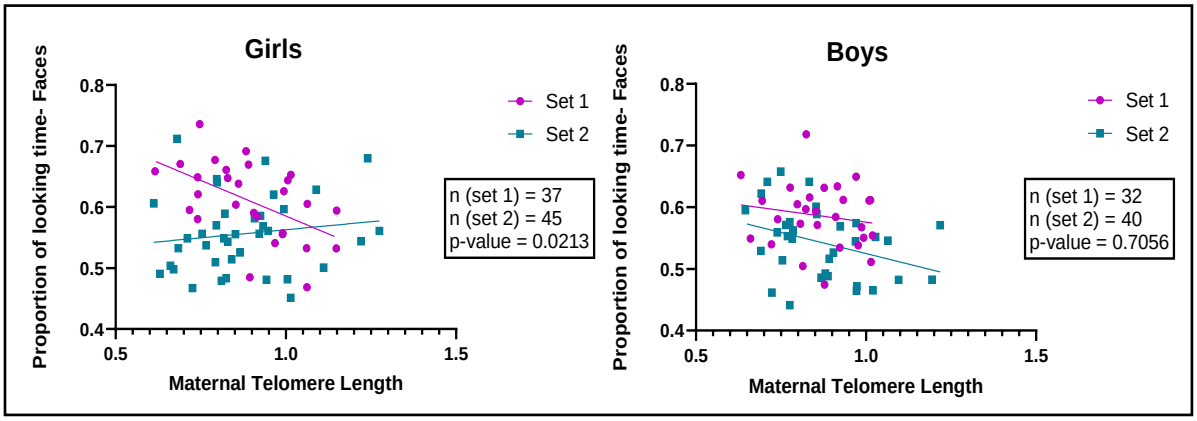

Figure 6.12. Association of maternal telomere length with novelty preference in faces trials. There was significant interaction of maternal telomere length with condition in girls.

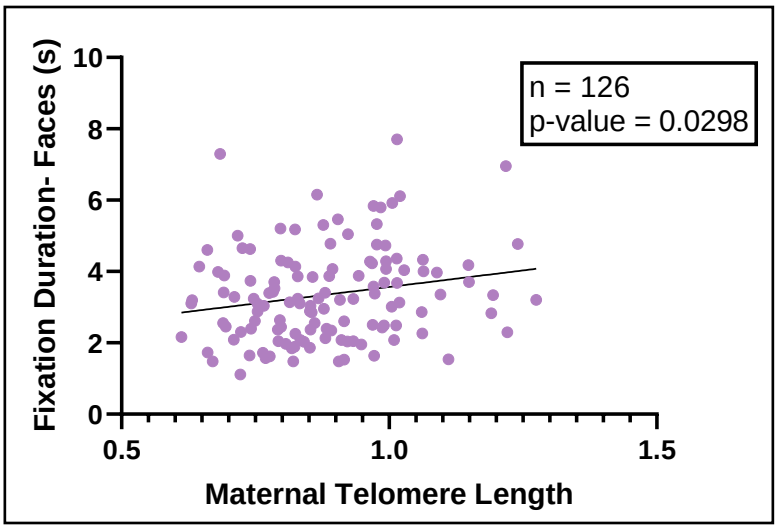

Figure 6.13. Association of maternal telomere length with fixation duration in faces trials.

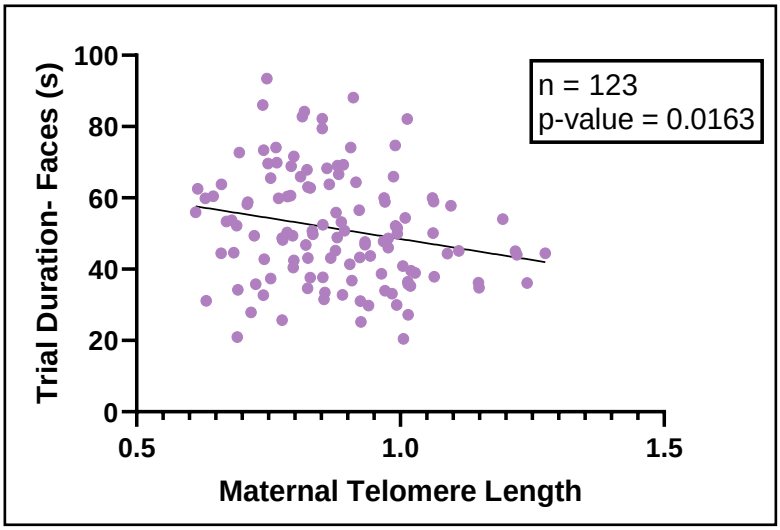

Figure 6.14. Association of maternal telomere length with trial duration in faces trials.

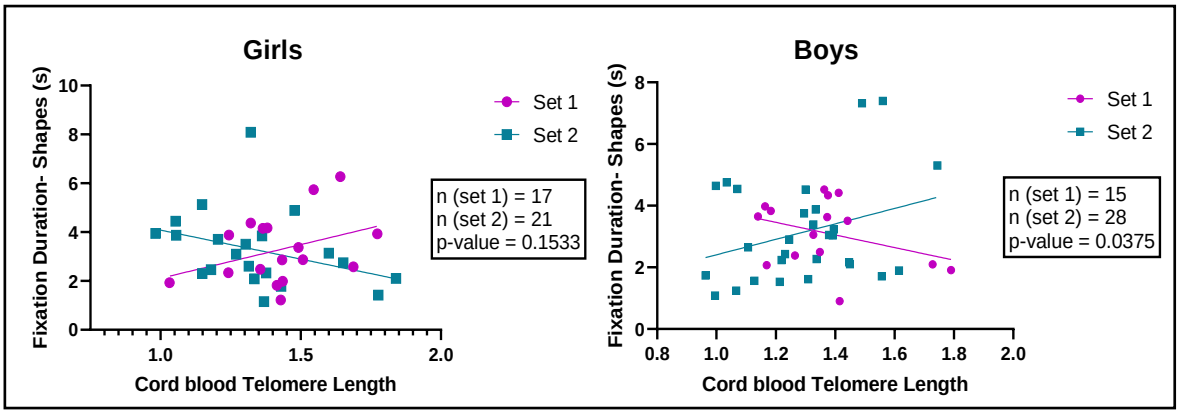

Figure 6.15. Association of cord blood telomere length with fixation duration in shapes trials. There was significant interaction of cord blood telomere length with condition in boys.

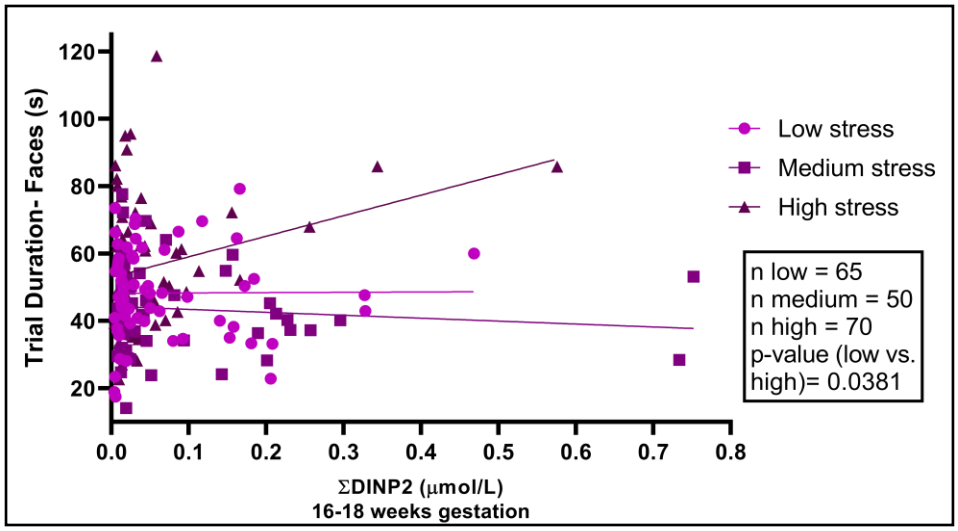

Figure 6.16. Interaction between perceived stress levels and prenatal exposure to DINP with trial duration in faces trials.

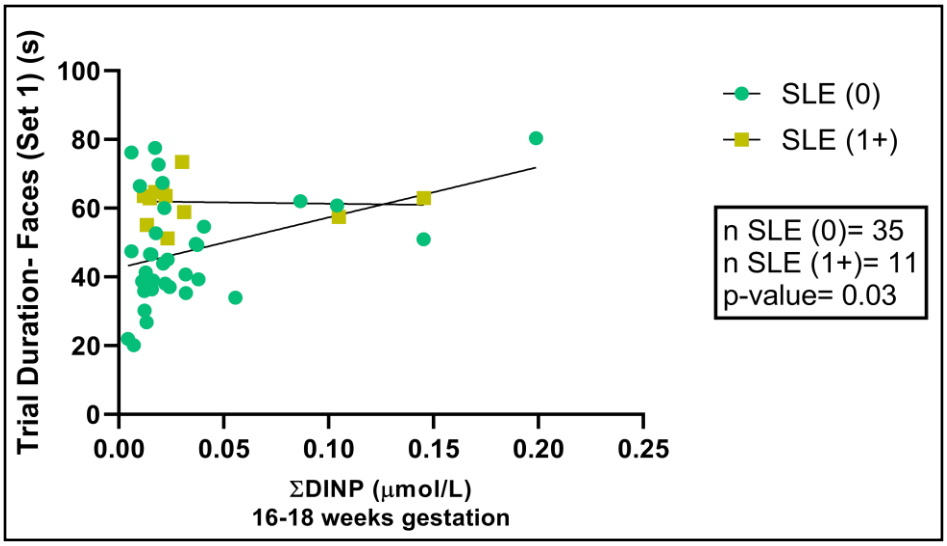

Figure 6.17. Interaction between stressful life events and prenatal exposure to DINP with trial duration in set one of the faces trials.

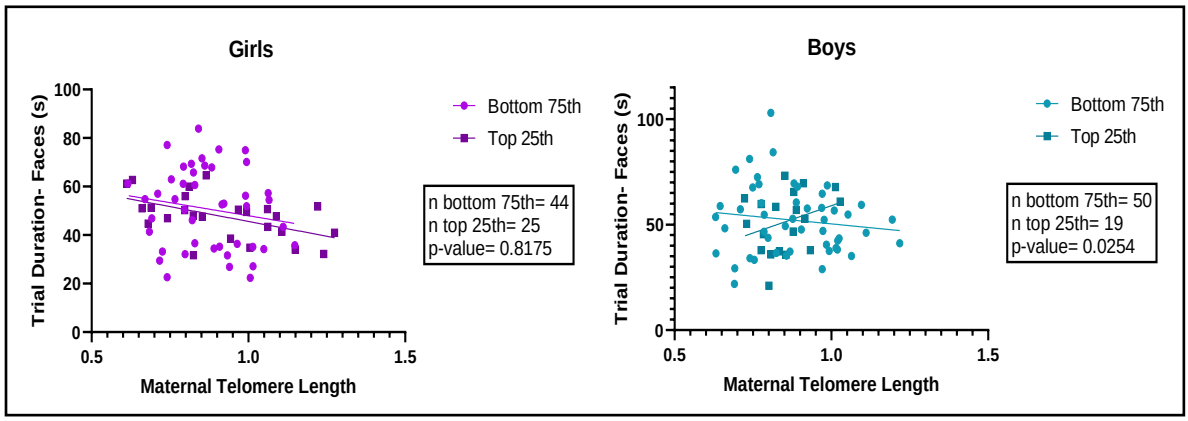

Figure 6.18. Interaction between maternal telomere length and prenatal exposure to DINP (dichotomized) with trial duration in faces trials.

CHAPTER 7: INTEGRATIVE DISCUSSION

The neurodevelopment impacts of prenatal maternal stress and the possible interactions of maternal stress with other environmental factors are a growing public health concern. Previous studies have found associations between maternal stress and adverse impacts on play behavior, scores on the Bayley Scales of Infant Development (BSID), language development, and intelligence quotient (IQ) (Laplante et al. 2008; Barrett et al. 2014; Lamb et al. 2014; Kingston et al. 2015). There is also evidence that phthalates may have adverse impacts on behavior, scores on the BSID, IQ, and language development (Kim et al. 2011; Miodovnik et al. 2014; Doherty et al. 2017; Ipapo et al. 2017; Bornehag et al. 2018; Olesen et al. 2018). There are fewer studies that have assessed the potential for interactive effects of maternal stress and environmental factors on infants' development (Cowell et al. 2015; Barrett et al. 2016; Tamayo y Ortiz et al. 2017; Zhou et al. 2017; Arbuckle et al. 2019). In addition, to our knowledge no study has looked at the potential for cumulative effects of stress and phthalates on neurodevelopment during the first year of life.

The goals of this dissertation were to characterize the associations between maternal prenatal stress and infant cognitive outcomes at 4.5 and 7.5 months of age (physical reasoning, novelty preference, information processing speed, and visual attention) and to examine potential sex differences in any observed associations (Chapter 4, 5, and 6). This dissertation also aimed to examine multiple measures of maternal stress and their ability to predict infants' performance on the outcomes of interest. Another goal was to examine the potential cumulative effects of stress and phthalates on these cognitive outcomes (Chapter 5 and 6).

Results presented in Chapters 4 and 5 suggest an adverse impact of prenatal maternal stress (higher maternal perceived stress or shorter maternal telomere length) on girls' performance on a physical reasoning task. Overall, women in the study reported having relatively low levels of perceived stress yet higher perceived stress relative to other women in the sample was associated with poorer physical reasoning in girls. These results are important

as they not only add to the body of evidence that prenatal maternal stress can have a negative impact on neurodevelopment, but also that an impact of stress can be measured as early as 4 months of age and even with relatively low levels of stress. The other measure that predicted physical reasoning was maternal telomere length. Shorter maternal telomere length was significantly associated with poorer physical reasoning, but only in girls who saw the impossible event first. The importance of this result is two-fold. First, it is consistent with the results of prenatal maternal perceived stress, showing that both measures of stress have a negative impact in girls. Secondly, it suggests that different aspects of maternal stress may be contributing to similar cognitive impairments in the offspring. As mentioned in previous chapters, maternal perceived stress and telomere length were not significantly correlated. This could be due to the fact that the perceived stress scale asked about stress in the previous month (Cohen et al. 1988), whereas maternal telomere length is a measure of longer-term chronic stress over the mother's lifetime (Feiler et al. 2018; Mathur et al. 2016; Oliveira et al. 2016; Wojcicki et al. 2015). Despite the fact that these two measures are likely measuring different aspects of maternal stress they resulted in a similar delay in the development of physical reasoning in girls.

Results presented in Chapter 6 suggest there are impacts of prenatal maternal stress on infants' novelty preference, information processing speed, and visual attention. Although all outcomes studied at 7.5 months of ages were impacted by prenatal maternal stress in some way, the most consistent finding was an association with trial duration which is a measure of attention. Three stress measures (perceived stress, stressful life events, and maternal telomere length) were all associated with longer trial durations, which suggests that maternal stress impairs infants' visual attention. The results for the other two cognitive outcomes were not as consistent. However, together with the results reported in Chapter 4 and 5, these results provide evidence to suggest that maternal stress affects multiple domains of cognition in the developing infant and that multiple measures of stress were associated with these cognitive outcomes. As mentioned in Chapter 1, there are several possible mechanisms through which

prenatal stress may act to impact fetal brain development. It is possible that there is a dysregulation of the placental enzyme 11β-HSD2 with maternal stress, which leads to greater exposure of the fetus to cortisol and, in consequence, the fetal limbic system, the hypothalamus, and the cortex (Moisiadis and Matthews, 2014) may be overstimulated. These are areas that have been shown to regulate the HPA axis, behavior, and memory acquisition. Another important aspect of Chapter 6 is that the outcomes measured have been shown to be stable over time within an individual and to reliably predict later cognitive ability (Rose et al. 1995, 1997, 2001; Aslin, 2007). IKIDS research is ongoing and the children are being followed prospectively. Assessments of similar cognitive domains will take place through early and middle childhood. Therefore, the research group will have the unique opportunity in the future to assess whether the impacts of prenatal stress (including some additional measures like maternal hair cortisol) on early cognitive outcomes found here predict continued risk for adverse impacts on cognition at later ages.

This study also assessed the potential impact of prenatal exposure to phthalates on physical reasoning (Chapter 5) and the potential for interactions between maternal stress and phthalates (Chapters 5 and 6). Results presented in Chapter 5 suggest a sex specific adverse impact of multiple biomarkers of prenatal phthalate exposure (DINP, Anti-Androgenic sum, and sum of all phthalates) on performance on the physical reasoning task in boys. Interestingly, boys with higher phthalate exposure looked longer at the possible event suggesting they were taking longer to study an event that might be more familiar to them. Previously it has been suggested that boys show the expected looking time difference in the physical reasoning task (looking longer at the impossible event), 3-4 weeks later girls do (Baillargeon, 1995). Hence, these findings suggest phthalate exposure may further delay the development of this understanding in boys.

Although a greater sample size is needed to make firm conclusions about interactive effects of stress and phthalates, in the initial analyses conducted here to assess the potential for

interactions between stress and phthalates (Chapters 5 and 6) significant interactions were only observed with DINP exposure. This is important because DINP is being used as a replacement for DEHP in many products and exposure to this phthalate is increasing. Furthermore, interactions were only seen with exposure at 16-18 weeks of gestation and not with the pooled sample which represented average exposure across pregnancy. There are multiple implications of these results. First, the results demonstrated that there are interactions between two environmental factors, although the effects of the two were not always cumulative. This is important as maternal psychological stress is high prevalent in the US, with up to 25% of women experiencing clinically relevant levels during pregnancy and/or postpartum (Kingston et al. 2015), and phthalate exposure is also widespread among pregnant women (Ejaredar et al. 2015). Second, the fact that effects were observed with a replacement chemical, DINP, both alone and together with maternal stress adds to a growing body of evidence for "regrettable replacements". That is replacement chemicals are often similar in structure and mechanism of action to the chemicals they replace and are thus likely to cause similar or even more detrimental effects. Finally, it is important to note that 16-18 weeks of gestation—which appears to be an important time point for phthalate effects—is during a period that has been identified as important window of brain development during which sexual differentiation of the brain is occurring. Testosterone which plays a key role in this process peaks in the male fetus at about 16 weeks of gestation (Auyeung et al. 2013).

An important aspect of this study is that the work presented relied on a novel approach that made use of infrared eye tracking. This state-of-the-art approach allowed creation of automated versions of cognitive tasks used in developmental psychology. There are multiple implications for the use of this approach in the environmental epidemiology field. It provides more detailed and accurate information on infant looking behavior than has been feasible in previous studies. For example, the ability to identify a specific area of interest in the physical reasoning task around the perimeter of the box. In addition, the automated data collection and

data processing made possible through use of the eye-tracker allowed for efficient assessment of a large sample of infants, which is needed in epidemiological studies.

Another important aspect of this work is that it identified brief and easily administrable measures of maternal stress that predict cognitive outcomes in the offspring, this could help the public health goal of primary prevention. Simple surveys, like the PSS, would allow for early identification of women who that are at a higher risk of poor cognitive outcomes in their infants. This could lead to interventions during pregnancy to help mothers cope with stress and potentially alleviate or prevent adverse impacts on the child. Alternatively, understanding which groups of women are at greater risk due to chronic stress would help healthcare providers to anticipate which children may need intervention services and be ready to implement them before the adverse outcomes become evident.

Finally, there are ways in which future research could build on these findings. The findings presented in this dissertation support the idea that maternal perceived stress is an important aspect to assess when examining psychological distress. In a low stress sample like ours, perceived stress seems to be a better measure than stressful life events for predicting outcomes, likely because a low number of women reported stressful life events and because in this relatively well-educated and high income sample women may have a good social support network that helps them cope with these events when they do occur. Hence, future research should consider not only the occurrence of stressful events, but also the women's perception of how stressful the events she experienced were. Future studies should also assess the impact of postnatal maternal stress and postnatal phthalate exposure on these cognitive outcomes, as the prenatal period is not the only vulnerable period for exposure to maternal stress or chemicals. This study included sensitivity analyses to assess the potential impact of postnatal maternal stress. Results suggested that postnatal stress might contribute to some of the effects observed. However, the study was not designed to assess the relative impact of prenatal vs postnatal maternal stress on infant cognition. As mentioned previously, pre- and postnatal

stress were correlated, so a relatively large sample will be needed differentiate the separate impact of stress during these each of these periods.

Finally, IKIDS is now part of the national Environmental Influences on Child Health Outcomes (ECHO) consortium funded through the National Institutes of Health (NIH). ECHO aims to collect similar data from 70 cohort studies throughout the US (50,000 children total) to assess impacts of many environmental factors on child development. Importantly, the infant cognition assessment paradigm used in the 7.5-month visit has been implemented in a subset of the cohorts that are part of the consortium. Thus, future research will be able to combine data across multiple cohorts to form a larger sample that is more diverse in terms of sociodemographic characteristics providing more power to address questions such as the impact of pre- vs postnatal exposures to stress or chemicals as well as the impact of combined exposure to maternal stress and chemicals.

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APPENDIX A: INSTITUTIONAL REVIEW BOARD APPROVAL LETTER

Research contained herein took place as part of the Illinois Kids Development Study- the continuation of a pilot study of the same name. This Institutional Review Board letter notes approval of the updated protocol before participant recruitment initiation for this continuing phase of the study. Annual review occurred throughout the duration of this research.

UNIVERSITY OF ILLINOIS
AT URBANA-CHAMPAIGN

Office of the Vice Chancellor for Research
Institutional Review Board
528 East Green Street
Suite 203
Champaign, IL 61820

October 4, 2013

Susan Schantz
Beckman Institute
2347 Beckman
405 N Mathews
M/C 251

RE: *I Kids - Illinois Kids Development Study*
IRB Protocol Number: 09498

Dear Dr. Schantz:

Thank you very much for forwarding the modifications to the University of Illinois at Urbana-Champaign Institutional Review Board (IRB) office for your project entitled *I Kids - Illinois Kids Development Study*. I will officially note for the record that these major modifications to the original project, as noted in your correspondence received September 16, 2013, new NIEHS/EPA grant proposal, revised summary of research, testing location changes, additional performance sites Christie Clinic and Presence-Covenant Medical Center, change length of study to 2 years, new measures, optional EEG/ERP testing, additional tests for blood/urine samples, revised recruitment materials, revised instrument measures, addition of 16 & removal of 3 study team members, and HIPAA authorizations documents , have been approved. The expiration date for this IRB protocol, UIUC number 09798, is 01/27/2014. The risk designation applied to your project is *No More than Minimal*.

As your modifications involved changes to consent form(s), I am attaching the revised form(s) with date-stamp approval. Please note that copies of date-stamped consent forms must be used in obtaining informed consent. If modification of the consent form(s) is needed, please submit the revised consent form(s) for IRB review and approval. Upon approval, a date-stamped copy will be returned to you for your use.

Please note that additional modifications to your project need to be submitted to the IRB for review and approval before the modifications are initiated. To submit modifications to your protocol, please complete the IRB Research Amendment Form (see http://irb.illinois.edu/?q=forms-and-instructions/research-amendments.html). Unless modifications are made to this project, no further submittals are required to the IRB.

We appreciate your conscientious adherence to the requirements of human subjects research. If you have any questions about the IRB process, or if you need assistance at any time, please feel free to contact me or the IRB Office, or visit our Web site at http://www.irb.illinois.edu.

Sincerely,

Anita Balgopal, Director, Institutional Review Board

Attachment(s)

c: Jodi Flaws Andrea Aguiar Carle IRB Barbara Hall
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