PERINATAL DETERMINANTS OF MENTAL DISORDERS

PERINATAL DETERMINANTS OF MENTAL DISORDERS IDENTIFYING RISK FACTORS AND TESTING THE EFFECTIVENESS OF EARLY INTERVENTIONS ON INFANT AND CHILD EMOTION REGULATION

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Lay Abstract

Healthy brain development is important for health and success in life. However, risk factors such as the mother's poor physical and mental health during pregnancy and in the first postnatal year can increase the risk of emotion and behaviour problems in offspring. Therefore, the objectives of this thesis were to i) identify links between modifiable pre and postnatal risk factors and poorer offspring brain development and ii) determine if intervening on one of these risk factors might improve offspring brain development. Results from this thesis show that an unhealthy maternal diet in pregnancy was linked to more offspring emotion, behaviour, and brain development problems and that treating postpartum depression in mothers may improve offspring brain development. This work suggests that identifying and intervening on modifiable risk factors is important to improve early brain development and may prevent the development of mental disorders later in life.

Abstract

Objectives: To investigate the preventive potential of the Developmental Origins of Health and Disease (DOHaD) hypothesis as it pertains to emotion dysregulation and psychopathology by: i) elucidating the impact of modifiable perinatal risk factors, and ii) examining whether a postnatal intervention can improve infant emotion regulation.

Methods: Studies 1 and 2 used data from the Canadian Healthy Infant Longitudinal Development (CHILD) cohort and the Maternal-Infant Research on Environmental Chemicals (MIREC) cohort to examine if modifiable perinatal risk factors (including prenatal diet quality) confounded the link between prenatal metabolic complications and offspring psychopathology. Study 3 used MIREC data to examine if prenatal diet quality was linked to a biomarker of emotion regulation in infants (autonomic nervous system (ANS) function). Studies 4 and 5 used data from 40 infants of mothers diagnosed with postpartum depression (PPD) and 40 healthy control infants matched on infant age sex and socioeconomic status. These studies examined if infant emotion regulation (Study 4) and mother-infant physiological synchrony (a marker of dyadic emotion regulation-Study 5) improved following maternal cognitive behavioral therapy (CBT) for PPD.

Results: In Studies 1 and 2, prenatal diet quality accounted for significant variance in the links between prenatal metabolic complications and offspring psychopathology. In Study 3, poor prenatal diet quality was associated with adverse ANS development in offspring. In Studies 4 and 5, infants exhibited more adaptive emotion regulation and mother-infant synchrony improved following maternal receipt of CBT for PPD.

Conclusions: Elucidating the impact of modifiable perinatal risk factors on offspring psychopathology provides meaningful targets for intervention, and postnatal interventions may improve offspring emotion regulation and could reduce the risk of psychopathology. This work highlights the importance of the perinatal period as a time during which modifiable risk factors can be identified and intervened upon to reduce mental disorder risk across the lifespan.

Keywords: Developmental Origins of Health and Disease, Emotion regulation, Infancy, Childhood, Psychopathology, Prenatal diet, Postpartum depression

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List of Abbreviations

μV: Microvolts
ADHD: Attention deficit hyperactivity disorder
ANS: Autonomic nervous system
BASC-II: Behaviour assessment scale for children
BMI: Body mass index
CBCL: Child behaviour checklist
CBT: Cognitive behavioural therapy
CES-D10: Centre for epidemiological studies depression scale short form
CHILD: Canadian Healthy Infant Longitudinal Development cohort
CI: Confidence interval
DAT: Dopamine transporter
DFT: Discrete Fourier transform
DOHaD: Developmental Origins of Health and Disease
ECG: Electrocardiogram
EEG: Electroencephalography
EGI: Electrical geodesics incorporated
EPDS: Edinburg Postnatal depression scale
FAA: Frontal alpha asymmetry
FFQ: Food frequency questionnaire
FFT: Fast Fourier transform
fNIRS: Functional near infrared spectroscopy
GDM: Gestational diabetes mellitus
HEI-2010: Healthy eating index 2010
HRV: Heart rate variability
IBQ-R: Infant behavior questionnaire short form revised
IGT: Impaired glucose tolerance
IQ: Intelligence quotient
MATLAB: Matrix Laboratory
MIREC: Mother infant research on environmental chemicals
MIREC-ID: Mother infant research on environmental chemicals-Infant development
mPFC: Medial prefrontal cortex
N: Number
OFC: Orbitofrontal cortex
p: Probability
PAR: Predictive adaptive response
PM MW: Peak matched multitaper window
PPD: Postpartum depression
PSNS: Parasympathetic nervous system
RMSSD: Root mean square of successive differences
r_{part}: Semi-partial correlation

RSA: Respiratory sinus arrhythmia
SAS: Statistical analysis software
SD: Standard deviation
SDNN: Standard deviation of N-N intervals
SES: Socioeconomic status
SPSS: Statistical package for the social sciences
STFT: Short time Fourier transform
VIF: Variance inflation factor
WPPSI: Weschler preschool and primary scale for intelligence
β: unstandardized beta

Declaration of Academic Achievement

This sandwich thesis is comprised of five studies, each written by the student. He led all formal data acquisition procedures (for Studies 1 2 and 3), collected data from each participant (for studies 4 and 5). For each study, the student helped conceive the objectives and hypotheses, cleaned all physiological data, conducted all analyses, prepared the initial draft of each manuscript, and incorporated subsequent edits from co-authors. This work was completed between September 2015 and May 2020. Therefore, the studies that comprise this thesis meet requirements for inclusion in the text. Finally, in accordance with the McMaster School of Graduate Studies requirements, I outline the contributions made by each co-author on each study.

Study 1 examines the link between exposure to prenatal maternal metabolic complications and offspring behaviour problems before and after adjusting for overall prenatal diet quality. John E. Krzeczkowski conceptualized the idea for this study, analyzed data and interpreted findings, wrote the first draft of the manuscript and approved the final manuscript as submitted. Drs Lau, Fitzpatrick, Tamana, Smithson, Lefebvre, Subbarao, Turvey, de Souza, Schmidt, Sears, Becker, Schmidt, Kozyrskyj and Mandhane contributed to design of the CHILD cohort study, selected data collection instruments, aided in interpretation of findings, reviewed and revised the manuscript, and approved the final manuscript as submitted. Dr. Van Leishout helped conceive the idea for this study and provided guidance on data analysis and interpretation of the data. Dr.

Van Lieshout critically evaluated and edited of drafts of the manuscript, and approved the final manuscript as submitted.

Study 2 aimed to replicate the findings from Study 1 by investigating links between prenatal maternal metabolic complications and offspring cognitive and behaviour problems before and after adjusting for maternal prenatal diet quality. John E. Krzeczkowski conceptualized the study objectives, acquired the data, analyzed all data, interpreted findings, wrote the first draft of the manuscript and incorporated subsequent edits and revisions. Dr. Khrsita Boylan aided in conceptualizing the idea for the study, supported data extraction procedures, provided feedback and interpretation of the data. Dr. Boylan reviewed and critically evaluated the intellectual content of the manuscript Drs Arbuckle, Dodds, Muckel Fraiser and Ms. Favotto support for the intellectual content of the article, supported edits and subsequent drafts of the manuscript. Dr. Van Lieshout helped conceive the idea for this study and provided guidance on data analysis and interpretation of the data. Dr. Van Lieshout critically evaluated and edited of drafts of the manuscript, and approved the final manuscript as submitted.

In study 3, we investigated the link between prenatal maternal diet quality and offspring autonomic nervous system functioning. John E. Krzeczkowski conceptualized the idea for the study and its objectives, analyzed data and interpreted findings, wrote the first draft of the manuscript, incorporated subsequent edits and revisions to the manuscript. Drs. Boylan, Arbuckle, Muckle, Poliakova, Seguin, and Ms. Favotto contributed to data interpretation and to the intellectual content of the article and provided feedback on subsequent versions of the article. C. Savoy, B. Amani and N. Mortaji

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Studies 4 and 5 investigated whether treating maternal PPD was capable of optimizing offspring neurodevelopment and altering the physiological mechanisms underlying mother-infant interaction patterns. John E. Krzeczkowski collected data from each participant, cleaned and analyzed all data, interpreted the findings, wrote the first draft of both manuscripts and incorporated subsequent edits. Both papers were coauthored by the thesis supervisor Dr. Van Lieshout and committee member Louis A. Schmidt. Both supported data interpretation, provided intellectual support for the content of the article and critically evaluated subsequent drafts of the article. Study 5 was also co-authored by committee member Dr. Ferro, who provided support and feedback on statistical analyses plans and on the intellectual content of the article.

Chapter 1: Background

Prevalence and origins of psychopathology

One in five Canadians experiences a mental disorder in any given year (Smetain et al., 2011). While most chronic diseases arise in adulthood, over half of all mental disorders first emerge in childhood (Kessler et al., 2005). Therefore, investigating factors that affect the development of mental disorders early in life is critical. Exposure to adverse prenatal and early postnatal environmental conditions can increase the risk for mental disorders, and physiological and behavioural antecedents of mental disorders are detectable even in infancy (Wakschlag et al., 2018, 2019). Therefore, identifying the prenatal and early postnatal conditions linked to increased risk for mental disorders and designing interventions that effectively target these conditions may be our best chance to reduce the impact of, or even prevent the development of mental disorders.

Emotion regulation and psychopathology

Emotion regulation is defined as the voluntary or automatic processes involved in monitoring, evaluating and modifying the valence and/or intensity of emotions to accomplish one's goals (Thompson, 1994). Recognition of the importance of emotion regulation development began in part with the publishing of a series of papers in the *Monograph of the Society for Research in Child Development* (Fox, 1994), in which researchers argued that adaptive emotion regulatory capacity in infancy and early childhood provides the foundation for complex socioemotional, behavioural, and cognitive functioning across the lifespan (Calkins, 1994; Cole, Michel, & O'Donnell,

1994; Cole, Ram, & English, 2019; Fox, 1998). While primitive emotion regulatory processes are observable infancy (e.g., gaze aversion, initiation of tactile stimulation) these processes diversify in scope and complexity across the lifespan, allowing the individual to regulate emotions in response to a wide range of environmental challenges and stressors (Cole, Martin, & Dennis, 2004; Cole et al., 1994). Given its importance across contexts, it is not surprising that problems with emotion regulation in infancy and in early childhood have significant detrimental effects on health and success across the lifespan (Wakschlag et al., 2019). These problems are thought to underlie the development of almost all forms of psychopathology and increase the risk for cognitive dysfunction (Beauchaine, 2015). Further, problems with emotion regulation in childhood are associated with a tripling of the risk of income rates below the poverty line, criminal convictions and substance abuse (Moffitt et al., 2011). Therefore, understanding the prenatal and early postnatal factors that affect the development of emotion regulation is critical if we hope to prevent the development of mental disorders, as well as a host of other adverse outcomes later in life.

Factors that affect the development of emotion regulation

While genetics establish the foundation for emotion regulatory systems, optimal development and fine-tuning of these systems depends heavily on the quality of prenatal and early postnatal environmental factors (Beauchaine, 2015; Gabard-Durnam et al., 2018; Tottenham, 2019). Examples of environmental factors associated with optimal development of emotion regulation include healthy maternal prenatal nutrition, the maintenance of a healthy gestational weight and adequate weight gain in pregnancy, low

levels of pre and postnatal maternal stress, predictable and sensitive maternal caregiving patterns and a stimulating home environment (Nyaradi, Li, Hickling, Foster, & Oddy, 2013; Räikkönen, Pesonen, Roseboom, & Eriksson, 2012; Rivera, Christiansen, & Sullivan, 2015; Tottenham, 2019). Conversely, exposure to adverse prenatal and early postnatal environmental conditions are associated with atypical deviations within early developing emotion regulatory systems and therefore, may play a prominent role in the developmental origins of psychopathology (Feldman, 2015; Kim, Bale, & Epperson, 2015; Räikkönen et al., 2012; Schlotz & Phillips, 2009).

The Developmental Origins of Health and Disease Hypothesis: An explanation and a brief history

Decades of evidence have observed links between adverse prenatal and early postnatal environmental conditions and an increased risk for adverse outcomes across organ systems, including the nervous system, in offspring later in life. The developmental origins of health and disease DOHaD hypothesis posits that exposure to adverse perinatal (pre and early postnatal) environmental conditions can alter physiological development and increase susceptibility for disease across the lifespan (Gluckman, Hanson, & Buklijas, 2010; Gluckman & Hanson, 2004). Evidence supporting the DOHaD hypothesis has accrued over the past century (Gluckman et al., 2010). The origins of the DOHaD hypothesis can be traced back to early 20th century experiments that aimed to determine whether environmental conditions were capable of altering behaviour patterns across generations. For example, in the early 1920s, Paul Kammerer used a midwife toad (*Alytes obstetricians*) model to examine if characteristics acquired through altered environmental

conditions could be passed on to subsequent generations. Under normal conditions, midwife toads tend to mate on land; however, by exposing toads to hot and dry conditions, Kammerer showed that these toads altered their behaviour and mated in water (Vargas, 2009). This change in mating behaviour persisted through six generations, even when the environment was returned to the previous normal conditions (Vargas, 2009). Given that genes were thought to be the only mechanism though which heritable information could be transmitted mother to offspring, Kammerer's findings were contested to the point of data falsification accusations (Gluckman, Hanson, & Buklijas, 2010; Vargas, 2009).

Nonetheless, the idea that prenatal and early postnatal environmental conditions can play a role in altering behaviour across generations was gaining traction. In the 1930s, recognition of the importance of prenatal environmental conditions for offspring development was bolstered by increasing interest in the study of teratology. While this field focused exclusively on factors linked to severe morphological deviations in development, emerging epidemiological evidence was beginning to report on associations between exposure to adverse perinatal conditions that are *not* considered teratogenic and increased morbidity and mortality risk later in life (Gluckman et al., 2010; Wadhwa, Buss, Entringer, & Swanson, 2009).

The idea that adverse perinatal conditions can increase the risk for non-communicable diseases garnered more attention in the 1970s. In a series of papers, German endocrinologists reported associations between adverse perinatal conditions and increased risk for cardiometabolic problems, particularly obesity and arteriosclerosis in

offspring (Gluckman et al., 2010). In the 1980s, Norbert Freinkel hypothesized that "fuel-mediated teratogenesis" could explain how maternal prenatal metabolic complications may impair offspring development (Freinkel, 1980). Freinkel proposed that the fetus is sensitive to subtle changes in maternal nutrition and metabolism, and that fetal alterations occurring in response to these factors could impact postnatal health and development (Freinkel, 1980; Friedman, 2015). Further, in the late 1970s and 1980s, scientists around the world began to utilize large national birth registries to examine links between perinatal conditions and cardiometabolic outcomes. British epidemiologist David Barker published the most famous of these studies in *the Lancet* in 1986 (Barker, 1986). These studies reported strong associations between birthweight and cardiovascular disease later in life (Barker, 1986). Based on this evidence, Barker proposed the 'fetal origins hypothesis' (also known as the "the Barker Hypothesis"), which posited that exposure to prenatal adversity increased non-communicable disease risk in adulthood (Barker, 2007). The majority of the research designed to test this hypothesis focused on examining associations between proxies of adverse prenatal conditions, such as birthweight, and offspring cardiometabolic outcomes in adulthood. Stemming in part from these studies, the increasing interest in the associations between perinatal conditions and later health and development led to the creation of the World Congress of Fetal Origins of Adult Disease. However, researchers testing this hypothesis began to report links between a range of adverse prenatal and postnatal conditions and increased disease risk across organ systems. As a result, following the 2003 World Congress meeting, the Fetal Origins of Disease hypothesis was formally changed to the Developmental Origins of Health and

Disease hypothesis ‘‘to recognize the broader scope of developmental cues, extending from the oocyte to the infant and beyond, and the concept that the early life environment has widespread consequences for later health’’ (Gillman et al., 2007, p. 625).

DOHaD and mental disorders

The broadening of the DOHaD concept saw more studies establish links between exposure to adverse perinatal conditions and offspring cognitive, emotional and behavioural problems across the lifespan (Kim et al., 2015; O’Donnell & Meaney, 2016; Van Den Bergh, 2011; van den Bergh et al., 2017). Similar to the earlier DOHaD research investigating cardiometabolic outcomes, proxies for early life adversity such as pre-term birth and birthweight were examined as predictor variables to investigate offspring risk for psychopathology. Pre-term birth, as well as variation at the extreme ends of the birthweight spectrum, were linked to lower intelligence, attention deficit hyperactivity disorder, mood disorders, and psychotic and personality disorders across the lifespan (Colman, Ataullahjan, Naicker, & Van Lieshout, 2012; Pappas et al., 2017; Saigal, Szatmari, Rosenbaum, Campbell, & King, 1991; Schlotz & Phillips, 2009). Further, studies have linked extremely low birth weight to neurophysiological activity associated with risk for mental disorders in adulthood (Krzeczkowski, Schmidt, Savoy, Saroj, & Van Lieshout, 2018; Miskovic, Schmidt, Boyle, & Saigal, 2009). Additionally, researchers testing the DOHaD hypothesis as it pertains to mental disorders have utilized more advanced observational designs, including negative controls, mendelian randomization natural experiments to attempt to establish causal links between prenatal and early postnatal adverse conditions and offspring psychopathology (see Gage, Munafò, & Davey

Smith, 2016 for review). For example, natural experiments using data from individuals prenatally exposed to the Dutch Hunger Winter of 1944-1995 at the end of World War II provided important evidence on the impact of prenatal adversity on risk for later mental disorders. During this time, a Nazi embargo caused a food shortage resulting in rations of less than 500 calories per day per person. Individuals born to women that were pregnant during this time exhibited an increased risk for schizophrenia, cognitive problems, poor stress regulation and mood disorders in adulthood relative to those not prenatally exposed to the embargo (Brown, Van Os, Driessens, Hoek, & Susser, 2000; Susser & St Clair, 2013). These results were replicated in studies examining offspring outcomes following prenatal exposure to widespread famine occurring during Mao's Great Leap Forward Transition in China (Brown et al., 2000; Susser & St Clair, 2013).

Although this evidence is useful in establishing links between perinatal adversity and risk for psychopathology, the exposures examined in most of these studies are either ethically impossible or very difficult to modify via intervention. Therefore, recent research explored the potential impact of more specific prenatal and early postnatal exposures and mechanisms linked to increased risk for psychopathology in offspring. Indeed, prenatal exposure to maternal factors such as unhealthy prenatal diet, obesity, excessive gestational weight gain, lack of exercise as well as pre and postnatal exposure to maternal mood disorders are all associated with adverse neurodevelopment and increased risk for psychopathology in offspring across the lifespan (Meaney, 2018; Rivera et al., 2015; van den Bergh et al., 2017; Van Lieshout, 2013). Taken together, these

studies have provided strong evidence suggesting that prenatal and early postnatal adverse conditions increase the risk for mental disorders later in life.

Mechanisms of the DOHaD hypothesis

Developmental plasticity

Given the strong support for the DOHaD hypothesis as it pertains to mental disorders, researchers have attempted to elucidate the mechanisms through which prenatal and early postnatal conditions might increase the risk for psychopathology later in life. The concept of developmental plasticity is key to explaining the mechanisms underlying the DOHaD hypothesis (Bateson et al., 2004). Developmental plasticity is defined as the phenomenon by which a range of different phenotypes can result from a single genotype depending on the environmental conditions present during particular windows of development (Bateson, Gluckman, & Hanson, 2014). These developmental windows are referred to as 'sensitive periods,' and two of the most important of these occur in the prenatal and early postnatal periods (Nelson & Gabard-Durnam, 2020; Tottenham, 2019). Since substantial physiological changes are metabolically costly after the phenotype has developed, the ability to alter the development and circuitry of physiological systems is generally restricted to these sensitive periods (Duckworth, 2015). As a result, adverse environmental conditions during these sensitive periods can have a particularly potent and lasting impact on the trajectory of neurodevelopment and could increase psychopathology risk later in life (Schlotz & Phillips, 2009; Tottenham, 2019).

Characterizing adverse exposures

Adverse environmental conditions that alter neurodevelopment during sensitive prenatal and early postnatal periods are organized on a continuum (Gluckman & Hanson, 2004a). This continuum ranges from teratogens (e.g., radiation, certain pharmaceuticals) which severely damage neurodevelopment, to conditions that may lead to sub-optimal neurodevelopment, but are not considered teratogenic (e.g., maternal obesity, elevated maternal stress) (Burton, Fowden, & Thornburg, 2016; Gluckman & Hanson, 2004b). Perinatal exposure to conditions at the less severe end of the spectrum are thought to initiate the predictive adaptive response (PAR) in offspring (Bateson et al., 2004, 2014). The PAR is considered an adaptive mechanism whereby the alterations that occur in the fetal and/or infant brain in response to adversity aim to increase the chances of survival later in life (Bateson et al., 2014). However, except for exposure to known teratogens, the demarcation is less clear as to whether an adverse exposure may alter physiology by the PAR, or if exposures lead to functional damage within certain neurophysiological circuits (e.g., see Rutter, 2004; Swanson & Wadhwa, 2008; Hanson & Gluckman, 2014 for debates). The following sections describe the two of the most widely cited mechanisms underlying DOHaD as it pertains to mental disorders, the PAR, and functional neural circuit damage.

The predictive adaptive response (PAR)

The PAR is a form of developmental plasticity that equips organisms with the ability to use information from the prenatal and early postnatal environment to alter physiology in order to enhance survival in certain environmental conditions later in life (Bateson et al., 2004). Greater fitness (i.e., chances of survival and reproductive success)

is more likely if these changes *match* with the 'predicted' environmental conditions later in life; however, the organism is at increased risk for adverse outcomes if there is a *mismatch* between changes made in response to perinatal conditions and future environmental conditions (e.g., Nederhof & Schmidt, 2012). Observations of PARs from non-human animal models provide clear examples of this phenomenon. Indeed, freshwater *Daphnia* will develop a spiked helmet-like structure if prenatally exposed to chemical signals that indicate a predator's presence. However, this enhanced structural protection adversely affects the *Daphnia's* health and functioning in predator-free environments (Bateson et al., 2014). PARs can also alter the likelihood of patterns of behaviour and emotion regulatory tendencies. For example, hares (*Lepus timidus*) that are exposed to predator signals prenatally exhibit elevated levels of vigilance and shelter seeking behaviours postnatally (Talge, Neal, & Glover, 2007). While these traits are adaptive when predators are present, in predator-free environments, these behaviours limit adaptive food-seeking behaviours and reproductive success.

When examining outcomes thought to occur as a result of the PAR, we must consider two critical points. First, the changes made in response to perinatal conditions occur in keeping with Darwinian fitness, whereby changes are considered adaptive if they increase the probability that the organism survives to sexual maturity (Burton et al., 2016). Second, PARs evolved during the paleolithic era; therefore, changes that may have increased fitness in these primitive environments may not be adaptive today (Gluckman et al., 2010). For example, in humans, exposure to excessive perinatal stress may indicate an environment that is dangerous and low in resources. As a result, fetal and infant

physiology may be altered to enhance traits associated with increased vigilance, impulsivity, and/or anxiety to maximize chances of survival in these threatening conditions (Glover, 2011). While these traits may have increased chances of survival to sexual maturity in primitive environments, in today's conditions, they could increase the risk for psychopathology. We have further hypothesized that PARs occurring in response to prenatal adversity might even increase the development and stability of long-term personality traits such as neuroticism (see Krzeczkowski & Lieshout, 2018, for narrative review).

Damage to neural circuitry

Although PARs are useful in explaining how adverse environmental conditions might alter neurodevelopment and increase risk for psychopathology, researchers that study the PAR acknowledge that it cannot explain all outcomes resulting from prenatal and early postnatal adverse exposures (Bateson et al., 2004, 2014). Increased risk for mental disorders following exposure to either biological (e.g., maternal and or infant malnutrition, overnutrition, infection) and psychosocial (e.g., parental mental illness or substance abuse, neglect or deprivation) risks may occur as a result of damage to certain neural circuits (often described as 'functional scars within neural networks' or 'biological embedding of adversity'), particularly in those involved in emotion regulation (Bock, Rether, Gröger, Xie, & Braun, 2014; Nelson, 2017).

Decades of studies on institutionalized infants and children have reported on the potential neurophysiological damage occurring as result of adverse exposures (Gee et al., 2013; Nelson, Zeanah, & Fox, 2019). Neurodevelopment, particularly within emotion

regulatory networks, depends on specific environmental inputs provided by caregivers during sensitive periods of development in order to develop optimally; therefore, without these inputs, certain brain areas exhibit poorer development and adverse functioning across the lifespan (Hofer, 2006; Tottenham, 2015). Indeed, institutionalized children exhibit adverse neurodevelopment, including dysregulated emotion and stress response systems, reduced brain volume, adverse development of white matter tracts and poor outcomes across multiple cognitive and socioemotional domains (Nelson et al., 2019). Fortunately, research involving these children has also revealed that interventions appear to be capable of improving neurodevelopment, especially if children receive the intervention before 24 months of age (e.g., Vanderwert, Marshall, Nelson, Zeanah, & Fox, 2010).

Taken together, mechanisms underlying DOHaD are based on exposures that linked to sub-optimal neurodevelopment but are not considered teratogenic. Regardless of whether altered neurodevelopment occurs in keeping with the PAR, or whether perinatal adversity results in damage to particular neural circuits, this evidence strongly supports the negative impact of adverse environmental conditions on neurodevelopment. However, given the plasticity of the brain during the prenatal and early postnatal periods, these developmental windows represent a time of tremendous opportunity during which early interventions might have a lasting, positive impact on neurodevelopment outcomes across the lifespan (Wakschlag et al., 2018, 2019). Since many prenatal and postnatal exposures that adversely affect neurodevelopment are potentially modifiable, interventions targeting

these exposures may potentially to reduce offspring risk or even prevent the development of mental disorders.

The preventative potential of DOHaD research

The DOHaD concept has been integral to the development of many current global health initiatives that strive to reduce the impact of non-communicable diseases (Penkler, Hanson, Biesma, & Müller, 2019). However, despite accumulating evidence supporting the DOHaD hypothesis as it pertains to neurodevelopment and psychopathology, intervention and prevention efforts that target neurodevelopment outcomes have lagged behind those focusing on cardiometabolic and anthropometric outcomes (Penkler et al., 2019). This is likely due in part to a lack of research focused on elucidating the impact of modifiable prenatal and early postnatal risk factors on neurodevelopment, but also since few studies use the DOHaD framework to test the effectiveness of interventions designed to modify these perinatal risk factors (Burton et al., 2016). However, the importance of addressing these evidence gaps is gaining momentum. For example, Part 1 of a series of papers published in *PLoS Medicine* in 2013 highlighted the importance of diagnosing and treating maternal depression to optimize the health of women *and* their offspring (Rahman, Surkan, Cayetano, Rwagatare, & Dickson, 2013). The authors' further state that treating maternal depression is critical to address the United Nations Millennium Developmental Goals, given that almost half of these goals aim to address the health and development of women and their children. More recently in 2016, *The Lancet* published a three-part series on the importance of research, interventions and policy designed to optimize early childhood development to increase human potential and optimize long-

term outcomes (Black et al., 2016; Britto et al., 2016; Richter et al., 2016). This series highlighted the perinatal nutritional environment and maternal depression as two potentially modifiable risk factors that could impact offspring neurodevelopment in the long-term. Finally, the most recent position papers from the international DOHaD society highlight the need to assess outcomes such as emotion regulation in offspring (Hanson, Poston, & Gluckman, 2019). These outcomes are important since they are measurable in infancy and early childhood and indicate an increased risk for multiple physical and mental health outcomes later in life (Calkins, Dollar, & Wideman, 2019; Hanson et al., 2019; Wakschlag et al., 2019).

Moving the DOHaD Hypothesis forward: Critical steps toward elucidating its preventative potential

In his review published in *Neuron,* Nelson (2017) stated:

> If we want children to live up to their developmental potential and to lead meaningful lives, we must afford them that opportunity by limiting their exposure to adversity; we must ensure that their brains receive the types of experiences that foster healthy brain development (and at the right times in development); and we must be mindful of the fact that deviations from the expectable environment during critical periods of development can lead to particularly egregious outcomes.(p. 266).

Elucidating the preventative potential of the DOHaD hypothesis to prevent the development of mental disorders is critical. To address this objective, first, we must utilize data from recent, large cohort studies to elucidate the impact of modifiable risk

factors on the development of mental disorders. Second, we must move toward using the DOHaD framework to test the effectiveness of interventions designed to modify these risk factors. Finally, studies must assess outcome variables such as emotion regulation in offspring. These variables are measurable in infancy and early child and can indicate vulnerability to multiple adverse outcomes later in life. Addressing these objectives would test the preventative potential of the DOHaD hypothesis and enable us to take critical steps forward in our efforts to prevent the development of mental disorders.

Prenatal metabolic complications and offspring neurodevelopment: causal factors or a marker of the causal factors?

One-third of women of child-bearing age are obese, and up to 16% suffer from hyperglycemia (e.g., gestational diabetes mellitus (GDM)) (Ashwal & Hod, 2015; Huda, Brodie, & Sattar, 2010). In keeping with the DOHaD hypothesis, numerous large cohort studies have reported on associations between maternal prenatal metabolic complications and increased risk for numerous problems in offspring linked to poor emotion regulatory capacity, such as internalizing and externalizing problems and cognitive dysfunction (Kong, Chen, Gissler, & Lavebratt, 2020; Perna, Loughan, Le, Tyson, & Hospital, 2015; Rivera et al., 2015; Van Lieshout, 2013; Van Lieshout, Taylor, & Boyle, 2011). Further, studies have also observed adverse neurodevelopment within important emotion regulatory, reward sensitivity and cognitive control regions of the brain (Page et al., 2019) as well as adverse development of white matter tracts and decreased functional connectivity (Li et al., 2016; Ou, Thakali, Shankar, Andres, & Badger, 2015; Salzwedel et al., 2019). Therefore, this evidence suggests that offspring exposed to gestational

metabolic complications appear to be at increased risk for mental disorders. However, despite this evidence, it is well understood that gestational metabolic complications are a multifactorial issue. Therefore, it remains unclear if these metabolic complications causally affect neurodevelopment in offspring or whether these complications are markers of the actual causal perinatal exposure(s) (Kong et al., 2020; Van Lieshout, 2013). These potential exposures (linked to both maternal metabolic complications and offspring neurodevelopment) include: hormone dysregulation, excessive inflammation, nutrient deficiencies (e.g., folate, vitamin D, iron, or even overall prenatal diet quality), elevated perinatal stress or depression or even obstetric complications known to be more common at delivery in obese women (see Van Lieshout et al., 2011 for review). However, to date, large cohort studies have been unable to assess the impact of important potential variables that may be involved in the pathways between maternal metabolic complications and offspring neurodevelopment (Kong et al., 2020). Therefore, we must identify and examine the impact of modifiable risk factors that may play a role in the maternal metabolic complications-to-offspring neurodevelopment pathways (Figure 1).

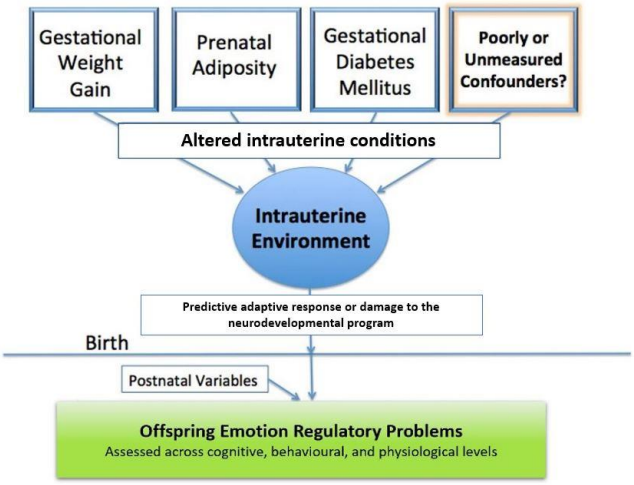

Figure 1: Maternal prenatal metabolic complications may alter intrauterine conditions and increase the risk for adverse offspring outcomes. However, studies that have established these associations have been unable to adjust for important modifiable confounding variables that may play a role in these links.

Addressing the issue of unmeasured/poorly measured confounding variables can be done utilizing data from recently acquired, large cohort studies that include valid and reliable assessments of these potentially modifiable risk factors. Understanding these links is critical to determine the most important variables to target in interventions designed to reduce the risk of mental disorders.

Postpartum depression: Time to intervene

Postpartum depression (PPD) is prevalent, affecting up to 20% of women (Gaynes et al., 2005). While PPD is well understood to negatively affect women's lives, it also has significant adverse effects on the health and development of their infants (Field, 1992; Goodman & Gotlib, 1999; Stein et al., 2014). Decades of evidence have shown that offspring born to women with untreated PPD are at increased risk for a host of adverse outcomes including cognitive and behavioural problems in childhood (Goodman et al.,

2011) and are at increased risk for depression into adulthood (Netsi et al., 2018). Therefore, unlike the links between maternal metabolic complications and offspring psychopathology, there is greater consensus that untreated maternal PPD is on the causal pathway. As a result, it is critical to move beyond describing the adverse effects of untreated PPD on offspring neurodevelopmental outcomes and examine whether treating PPD is capable of reorganizing offspring neurodevelopment, therefore potentially interrupting the intergenerational transmission of psychiatric risk.

Optimal development of emotion regulation in infancy is depends heavily on the quality of interactions with caregivers (Tottenham, 2015). Research in both humans and non-human animal models has shown the importance of sensitive, predictable, maternal caregiving behaviours for the development of the biobehavioural systems underlying offspring emotion regulation (Hofer, 2006; Meaney, 2018). Therefore, adverse development of emotion regulation may be transmitted to offspring through maladaptive mother-infant interaction patterns characteristic of dyads led by mothers with PPD (Murray, Fiori-cowley, & Hooper, 1996; Tronick & Reck, 2009).

PPD exposure has been linked to adverse development within offspring central and peripheral systems key to emotion regulation. These include adverse development of the amygdala and right superior frontal gyrus, as well as greater relative activity within the right anterior cerebral hemisphere and lower vagal tone (Field & Diego, 2008; Field, Pickens, Fox, Nawrocki, & Gonzalez, 1995; Lebel et al., 2016; Lusby, Goodman, Bell, & Newport, 2014). However, given the immense neuroplasticity in the infant brain within the first postpartum year, changes in mothers following PPD treatment (such as cognitive

behavioural therapy (CBT)) may lead to an adaptive reorganization of these emotion regulatory circuits in infants as well as more adaptive mother-infant interactions.

Despite decades of evidence linking exposure to untreated PPD and adverse development of both central and peripheral emotion regulatory systems, no studies have examined whether treating maternal PPD in the first postpartum year is capable of adaptively altering the development of these systems in infants. Further, it is unknown whether PPD treatment can improve the physiological systems that underly mother-infant interaction patterns. Investigating the impact of PPD treatment on these outcomes would not only enable us to test the preventative potential of the DOHaD hypothesis by examining the malleability of systems core to emotion regulation in infants and mother-infant interactions, but also provide important evidence on our capacity to interrupt the intergenerational transmission of psychiatric risk from mother to child (Figure 2).

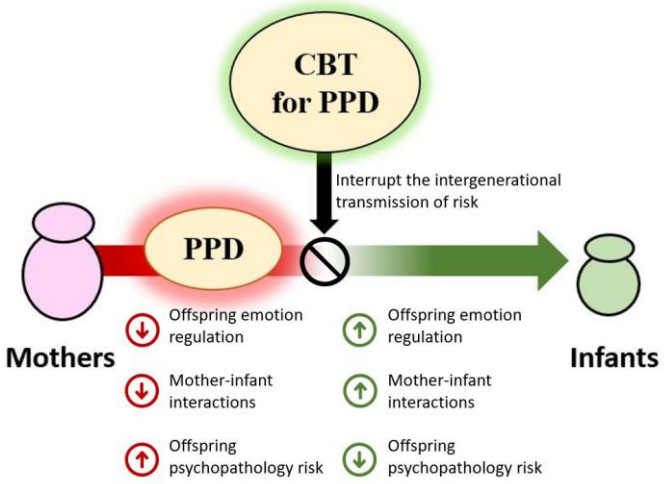

Figure 2: Is treating maternal PPD with cognitive behavioural therapy (CBT) capable of interrupting the intergenerational transmission of risk from mother to offspring? We argue that studies must move beyond describing the impact of exposure to untreated PPD on offspring outcomes and investigate if PPD treatment can improve infant emotion regulation capacity and mother-infant interactions.

Sandwich thesis overview

The five studies presented in this thesis aim to test the preventative potential of the DOHaD hypothesis as it pertains to emotion regulation and risk for mental disorders. This objective was addressed by: i) identifying modifiable perinatal risk factors for offspring emotion regulatory problems and psychopathology using data from large cohort studies, ii) examining whether an early postnatal intervention (maternal cognitive behavioural therapy (CBT) for postpartum depression) can optimize infant emotion regulation and improve mother-infant interaction patterns, and iii) examine outcomes related to offspring emotion regulatory capacity, a key vulnerability factor that is measurable in infancy and early childhood and predicts long-term functioning across emotional, behavioural and cognitive domains.

The studies that comprise this thesis have been previously published, are currently under review, or in preparation. Studies 1 and 2 examine the association between maternal prenatal metabolic complications and offspring cognitive, behavioural and emotional outcomes. We examined associations before and after adjusting for previously unmeasured/poorly measured variables that may play a role in the link between maternal prenatal metabolic complications and adverse offspring outcomes. In both studies, we observed that prenatal maternal diet quality accounted for significant variance in offspring outcomes (similar in magnitude to variables well understood to have an impact on offspring outcomes, including socioeconomic status and postpartum depression). Based on these findings, in Study 3, I examined the association between prenatal maternal

diet quality and the development of the autonomic nervous system, a regulatory system critical to maintaining homeostasis across organ systems. We observed that unhealthy overall prenatal diet quality was associated with lower heart rate variability, suggesting sub-optimal autonomic nervous system development in infants at 6 months of age.

Studies 4 and 5 examined whether treating maternal PPD with 9 weeks of group CBT could optimize offspring emotion regulatory capacity and improve mother-infant interactions. In Study 4 we examined whether treating mothers with CBT could optimize the development of offspring emotion regulatory capacity, assessed across physiological and behavioural levels. Following treatment, we observed significant adaptive changes in infant emotion regulation across all measures. Further, following maternal treatment, infant emotion regulation was indistinguishable from that measured in healthy control infants born to women free of PPD.

Given these findings, I aimed to elucidate further the impact of maternal CBT treatment on offspring emotion regulation. Since mother-infant interaction patterns play a significant role in the development of offspring emotion regulation (Feldman, 2007), I hypothesized that mother-infant physiological synchrony patterns might adaptively change following CBT treatment. Therefore, in Study 5, we used assessed respiratory sinus arrhythmia (RSA) simultaneously in mothers and their infants while the dyad completed the reunion phase of the face to face still-face task. In the healthy dyads at two-time points in infancy, increases in maternal RSA predicted subsequent decreases in infant RSA, and this effect strengthened across the reunion phase. This effect was observed in the PPD dyads, but only following maternal CBT treatment. As a result,

following CBT treatment, results suggested that mothers became more effective at regulating their distressed infant, and infants were more receptive to maternal regulation.

Taken together, the five studies included in this thesis are related. Studies 1,2, and 3 elucidated the impact of modifiable prenatal risk factors on offspring outcomes, and Studies 4 and 5 tested the effects of an intervention designed to address maternal PPD, a known modifiable risk factor for offspring mental disorders. Since Studies 1 and 2 utilized similar methods to address similar questions, there is some duplication in the methods sections of these papers. Additionally, since Studies 2 and 3 used data from the same cohort, the methods in these papers also contain some duplication. Finally, Studies 4 and 5 also used data from the same cohort so they also contain some unavoidable duplication.

References

Ashwal, E., & Hod, M. (2015). Gestational diabetes mellitus: Where are we now? *Clinica Chimica Acta*, *451*(1), 14–20. https://doi.org/10.1016/j.cca.2015.01.021

Barker, D. (1986). infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. *Lancet*, *1*(8489), 1077–1081. https://doi.org/10.1523/JNEUROSCI.2957-12.2012

Barker, D. J. P. (2007). The origins of the developmental origins theory. *Journal of Internal Medicine*, *261*(5), 412–417. https://doi.org/10.1111/j.1365-2796.2007.01809.x

Bateson, P., Barker, D., Clutton-Brock, T., Deb, D., D'Udine, B., Foley, R. A, … Sultan, S. E. (2004). Developmental plasticity and human health. *Nature*, *430*(1), 419–421. https://doi.org/10.1038/nature02725

Bateson, P., Gluckman, P., & Hanson, M. (2014). The biology of developmental plasticity and the Predictive Adaptive Response hypothesis. *The Journal of Physiology*, *592*(11), 2357–2368. https://doi.org/10.1113/jphysiol.2014.271460

Beauchaine, T. P. (2015). Future Directions in Emotion Dysregulation and Youth Psychopathology Future Directions in Emotion Dysregulation and Youth Psychopathology. *Journal of Clinical Child and Adolescent Psychology*, *44*(5), 875–896. https://doi.org/10.1080/15374416.2015.1038827

Black, M. M., Walker, S. P., Fernald, L. C. H., Andersen, C. T., Digirolamo, A. M., Lu, C., … Grantham-Mcgregor, S. (2016). Series Advancing Early Childhood

Development: from Science to Scale 1 Early childhood development coming of age: science through the life course. *Lancet*, *389*(10064), 77–90. https://doi.org/10.1016/S0140-6736(16)31389-7

Bock, J., Rether, K., Gröger, N., Xie, L., & Braun, K. (2014). Perinatal programming of emotional brain circuits: An integrative view from systems to molecules. *Frontiers in Neuroscience*, *8*(8), 1–16. https://doi.org/10.3389/fnins.2014.00011

Britto, P. R., Lye, S. J., Proulx, K., Yousafzai, A. K., Matthews, S. G., Vaivada, T., … Egede, L. (2016). Nurturing care: promoting early childhood development. *Lancet*, *389*(10064), 91–102. https://doi.org/10.1016/S0140-6736(16)31390-3

Brown, A. S., Van Os, J., Driessens, C., Hoek, H. W., & Susser, E. S. (2000). Further evidence of relation between prenatal famine and major affective disorder. *American Journal of Psychiatry*, *157*(2), 190–195. https://doi.org/10.1176/appi.ajp.157.2.190

Burton, G. J., Fowden, A. L., & Thornburg, K. L. (2016). Placental Origins of Chronic Disease. *Physiological Reviews*, *96*(4), 1509–1565. https://doi.org/10.1152/physrev.00029.2015

Calkins, S. D. (1994). Origins and Outcomes of Individual Differences in Emotion Regulation. *Society for Research in Child Develpment*, *59*(2), 53–72.

Calkins, S. D., Dollar, J. M., & Wideman, L. (2019). Temperamental vulnerability to emotion dysregulation and risk for mental and physical health challenges. *Development and Psychopathology*, *31*(3), 957–970. https://doi.org/10.1017/S0954579419000415

Cole, P. M., Martin, S. E., & Dennis, T. A. (2004). Emotion Regulation As A Scientific

Construct-Methodological Challenges And Directions For Child Development Research. *Child Development*, *75*(2), 317–333. https://doi.org/10.1021/cm702990t

Cole, P. M., Michel, M. K., & O'Donnell, T. (1994). The Development of Emotion Regulation and Dysregulation : A Clinical Perspective. *Society for Research in Child Develpment*, *59*(2/3), 73–100.

Cole, P. M., Ram, N., & English, M. S. (2019). Toward a Unifying Model of Self-regulation: A Developmental Approach. *Child Development Perspectives*, *13*(2), 91–96. https://doi.org/10.1111/cdep.12316

Colman, I., Ataullahjan, A., Naicker, K., & Van Lieshout, R. J. (2012). Birth weight, stress, and symptoms of depression in adolescence: Evidence of fetal programming in a National Canadian Cohort. *Canadian Journal of Psychiatry*, *57*(7), 422–428.

Duckworth, R. A. (2015). Neuroendocrine mechanisms underlying behavioral stability: Implications for the evolutionary origin of personality. *Annals of the New York Academy of Sciences, 1360*(1), 54–74. https://doi.org/10.1111/nyas.12797

Feldman, R. (2007). Infant Biological Foundations Synchrony and Developmental Outcomes. *Current Directions in Psychological Science*, *16*(6), 340–345. https://doi.org/10.1111/j.1467-8721.2007.00532.x

Feldman, R. (2015). Sensitive periods in human social development: New insights from research on oxytocin, synchrony, and high-risk parenting. *Development and Psychopathology*, *27*(2), 369–395. https://doi.org/10.1017/S0954579415000048

Field, T. (1992). Infants of depressed mothers. *Development and Psychopathology*, *4*(1), 49–66. https://doi.org/10.1017/S0954579400005551

Field, T., & Diego, M. (2008). Maternal depression effects on infant frontal EEG asymmetry. *The International Journal of Neuroscience*, *118*(8), 1081–1108. https://doi.org/10.1080/00207450701769067

Field, T., Pickens, J., Fox, N. A., Nawrocki, T., & Gonzalez, J. (1995). Vagal tone in infants of depressed mothers. *Development and Psychopathology*, *7*(2), 227–231. https://doi.org/10.1016/j.jad.2014.04.024

Fox, N. (1994). Dynamic Cerebral Processes Underlying Emotion Regulation. *Monographs of the Society for Research in Child Development*, *59*(2/3), 152–166. https://doi.org/10.1007/sl

Fox, N. A. (1998). Temperament and regulation of emotion in the first years of life. *Pediatrics*, *102*(5), 1230–1235.

Freinkel, N. (1980). Banting Lecture 1980 Of Pregnancy and Progeny. *Diabetes*, *29*(12), 1023–1035. https://doi.org/10.2337/diab.29.12.1023

Friedman, J. E. (2015). Obesity and Gestational Diabetes Mellitus Pathways for Programming in Mouse, Monkey, and Man—Where Do We Go Next? The 2014 Norbert Freinkel Award Lecture. *Diabetes Care*, *38*(8), 1402–1411. https://doi.org/10.2337/dc15-0628

Gabard-Durnam, L. J., O'Muircheartaigh, J., Dirks, H., Dean, D. C., Tottenham, N., & Deoni, S. (2018). Human amygdala functional network development: A cross-sectional study from 3 months to 5 years of age. *Developmental Cognitive Neuroscience*, *34*(June), 63–74. https://doi.org/10.1016/j.dcn.2018.06.004

Gage, S. H., Munafò, M. R., & Davey Smith, G. (2016). Causal Inference in

Developmental Origins of Health and Disease (DOHaD) Research. *Annual Review of Psychology*, *67*(1), 576–585. https://doi.org/10.1146/annurev-psych-122414-033352

Gaynes, B. N., Gavin, N., Meltzer-Brody, S., Lohr, K. N., Swinson, T., Gartlehner, G., … Miller, W. C. (2005). Perinatal depression: prevalence, screening accuracy, and screening outcomes. *Evidence Report/Technology Assessment*, (119), 1–8.

Gee, D. G., Gabard-Durnam, L. J., Flannery, J., Goff, B., Humphreys, K. L., Telzer, E. H., … Tottenham, N. (2013). Early developmental emergence of human amygdala-prefrontal connectivity after maternal deprivation. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(39), 15638–15643. https://doi.org/10.1073/pnas.1307893110

Gillman, M. W., Barker, D., Bier, D., Cagampang, F., Challis, J., Fall, C., … Thornburg, K. L. (2007). Meeting Report on the 3rd International Congress on Developmental Origins of Health and Disease (DOHaD). *Pediatric Research*, *61*(5), 625–629. https://doi.org/10.1203/pdr.0b013e3180459fcd

Glover, V. (2011). Annual research review: Prenatal stress and the origins of psychopathology: An evolutionary perspective. *Journal of Child Psychology and Psychiatry and Allied Disciplines*, *52*(4), 356–367. https://doi.org/10.1111/j.1469-7610.2011.02371.x

Gluckman, P. D., Hanson, M. A., & Buklijas, T. (2010). A conceptual framework for the developmental origins of health and disease. *Journal of Developmental Origins of Health and Disease*, *1*(1), 6–18. https://doi.org/10.1017/S2040174409990171

Gluckman, P. D, & Hanson, M. A. (2004a). Living with the past: Evolutino, development and patterns of disease. *Science*, *305*, 1733–1736.

Gluckman, P. D, & Hanson, M. A. (2004b). Living with the past: evolution, development, and patterns of disease. *Science (New York, N.Y.)*, *305*(5691), 1733–1736. https://doi.org/10.1126/science.1095292

Gluckman, P. D, Hanson, M., & Buklijas, T. (2010). A conceptual framework for the developmental origins of health and disease. *Journal of Developmental Origins of Health and Disease*, *1*(1), 6–18. https://doi.org/10.1017/S2040174409990171

Goodman, S. H., & Gotlib, I. H. (1999). Risk for psychopathology in the children of depressed mothers: A developmental model for understanding mechanisms of transmission. *Psychological Review*, *106*(3), 458–490. https://doi.org/10.1037/0033-295X.106.3.458

Goodman, S. H., Rouse, M. H., Connell, A. M., Broth, M. R., Hall, C. M., & Heyward, D. (2011). Maternal Depression and Child Psychopathology: A Meta-Analytic Review. *Clinical Child and Family Psychology Review*, *14*(1), 1–27. https://doi.org/10.1007/s10567-010-0080-1

Hanson, M. A., Poston, L., & Gluckman, P. D. (2019). DOHaD - The challenge of translating the science to policy. *Journal of Developmental Origins of Health and Disease*, *10*(3), 263–267. https://doi.org/10.1017/S2040174419000205

Hofer, M. A. (2006). Psychobiological roots of early attachment. *Current Directions in Psychological Science*, *15*(2), 84–88. https://doi.org/10.1111/j.0963-7214.2006.00412.x

Huda, S. S., Brodie, L. E., & Sattar, N. (2010). Obesity in pregnancy: prevalence and metabolic consequences. *Seminars in Fetal and Neonatal Medicine*, *15*(2), 70–76. https://doi.org/10.1016/j.siny.2009.09.006

Kessler, R. C., Berglund, P., Demler, O., Jin, R., Merikangas, K. R., & Walters, E. E. (2005). Lifetime Prevalence and Age-of-Onset Distributions of DSM-IV Disorders in the National Comorbidity Survey Replication. *Arch Gen Psychiatry*, *62*, 593–602. https://doi.org/10.1001/archpsyc.62.6.593

Kim, D. R., Bale, T. L., & Epperson, C. N. (2015). Prenatal Programming of Mental Illness: Current Understanding of Relationship and Mechanisms. *Current Psychiatry Reports*, *17*(2), 1–9. https://doi.org/10.1007/s11920-014-0546-9

Kong, L., Chen, X., Gissler, M., & Lavebratt, C. (2020). Relationship of prenatal maternal obesity and diabetes to offspring neurodevelopmental and psychiatric disorders: a narrative review. *International Journal of Obesity*. https://doi.org/10.1038/s41366-020-0609-4

Krzeczkowski, J. E., Schmidt, L. A., Savoy, C., Saroj, S., & van Lieshout, R. J. (2018). Frontal EEG asymmetry in extremely low birth weight adult survivors: Links to antenatal corticosteroid exposure and psychopathology. *Clinical Neurophysiology*, *129*(9), 1891–1898. https://doi.org/10.1016/j.biopsych.2018.02.316

Krzeczkowski, J. E., & Van Lieshout, R. J. (2019). Prenatal influences on the development and stability of personality. *New Ideas in Psychology*, *53*(October 2017), 22–31. https://doi.org/10.1016/j.newideapsych.2018.01.003

Lebel, C., Walton, M., Letourneau, N., Giesbrecht, G. F., Kaplan, B. J., & Dewey, D.

(2016). Prepartum and Postpartum Maternal Depressive Symptoms Are Related to Children's Brain Structure in Preschool. *Biological Psychiatry*, *80*(11), 859–868. https://doi.org/10.1016/j.biopsych.2015.12.004

Li, X., Andres, A., Shankar, K., Pivik, R. T., Glasier, C. M., Ramakrishnaiah, R. H., … Ou, X. (2016). Differences in brain functional connectivity at resting-state in neonates born to healthy obese or normal-weight mothers. *International Journal of Obesity*, (August). https://doi.org/10.1038/ijo.2016.166

Lusby, C. M., Goodman, S. H., Bell, M. A., & Newport, D. J. (2014). Electroencephalogram patterns in infants of depressed mothers. *Developmental Psychobiology*, *56*(3), 459–473. https://doi.org/10.1002/dev.21112

Maughan, A., Cicchetti, D., Toth, S. L., & Rogosch, F. A. (2007). Early-occurring maternal depression and maternal negativity in predicting young children's emotion regulation and socioemotional difficulties. *Journal of Abnormal Child Psychology*, *35*(5), 685–703. https://doi.org/10.1007/s10802-007-9129-0

Meaney, M. J. (2018). Perinatal maternal depressive symptoms as an issue for population health. *American Journal of Psychiatry*, *175*(11), 1084–1093. https://doi.org/10.1176/appi.ajp.2018.17091031

Miskovic, V., Schmidt, L. A., Boyle, M., & Saigal, S. (2009). Regional electroencephalogram (EEG) spectral power and hemispheric coherence in young adults born at extremely low birth weight. *Clinical Neurophysiology*, *120*(2), 231–238. https://doi.org/10.1016/j.clinph.2008.11.004

Moffitt, T. E., Arseneault, L., Belsky, D., Dickson, N., Hancox, R. J., Harrington, H., …

Caspi, A. (2011). A gradient of childhood self-control predicts health, wealth, and public safety. *Proceedings of the National Academy of Sciences*, *108*(7), 2693–2698. https://doi.org/10.1073/pnas.1010076108

Murray, L., Fiori-cowley, A., & Hooper, R. (1996). The Impact of Postnatal Depression and Associated Adversity on Early Mother-Infant Interactions and later Infant Outcome. *Child Development*, *67*(5), 2512–2526. https://doi.org/10.1109/TNANO.2009.2031807.Design

Nederhof, E., & Schmidt, M. V. (2012). Mismatch or cumulative stress: Toward an integrated hypothesis of programming effects. *Physiology and Behavior*, *106*(5), 701–706. https://doi.org/10.1016/j.physbeh.2011.12.008

Nelson, C. A. (2017). Hazards to Early Development: The Biological Embedding of Early Life Adversity. *Neuron*, *96*(2), 262–266. https://doi.org/10.1016/j.neuron.2017.09.027

Nelson, C. A., & Gabard-Durnam, L. J. (2020). Early Adversity and Critical Periods: Neurodevelopmental Consequences of Violating the Expectable Environment. *Trends in Neurosciences*, *43*(3), 133–143. https://doi.org/10.1016/j.tins.2020.01.002

Nelson, C. A., Zeanah, C. H., & Fox, N. A. (2019). How early experience shapes human development: The case of psychosocial deprivation. *Neural Plasticity*, *2019*. https://doi.org/10.1155/2019/1676285

Netsi, E., Pearson, R. M., Murray, L., Cooper, P., Craske, M. G., & Stein, A. (2018). Association of persistent and severe postnatal depression with child outcomes. *JAMA Psychiatry*, *75*(3), 247–253.

https://doi.org/10.1001/jamapsychiatry.2017.4363

Nyaradi, A., Li, J., Hickling, S., Foster, J., & Oddy, W. H. (2013). The role of nutrition in children's neurocognitive development, from pregnancy through childhood. *Frontiers in Human Neuroscience*, *7*(March), 97. https://doi.org/10.3389/fnhum.2013.00097

O'Donnell, K. J., & Meaney, M. J. (2016). Fetal Origins of Mental Health: The Developmental Origins of Health and Disease Hypothesis. *American Journal of Psychiatry*, appi.ajp.2016.1. https://doi.org/10.1176/appi.ajp.2016.16020138

Ou, X., Thakali, K. M., Shankar, K., Andres, A., & Badger, T. M. (2015). Maternal adiposity negatively influences infant brain white matter development. *Obesity*, *23*(5), 1047–1054. https://doi.org/10.1002/oby.21055

Page, K. A., Luo, S., Wang, X., Chow, T., Alves, J., Buchanan, T. A., & Xiang, A. H. (2019). Children exposed to maternal obesity or gestational diabetes mellitus during early fetal development have hypothalamic alterations that predict future weight gain. *Diabetes Care*, *42*(8), 1473–1480. https://doi.org/10.2337/dc18-2581

Pappas, A., Adams-Chapman, I., Shankaran, S., McDonald, S. A., Stoll, B. J., Laptook, A. R., … Higgins, R. D. (2017). Neurodevelopmental and Behavioral Outcomes in Extremely Premature Neonates With Ventriculomegaly in the Absence of Periventricular-Intraventricular Hemorrhage. *JAMA Pediatrics*, *172*(1), 32–42. https://doi.org/10.1001/jamapediatrics.2017.3545

Penkler, M., Hanson, M., Biesma, R., & Müller, R. (2019). DOHaD in science and society: Emergent opportunities and novel responsibilities. *Journal of*

Developmental Origins of Health and Disease, *10*(3), 268–273. https://doi.org/10.1017/S2040174418000892

Perna, R., Loughan, A. R., Le, J., Tyson, K., & Hospital, W. R. (2015). Gestational Diabetes : Long-Term Central Nervous System Developmental and Cognitive Sequelae. *HAPC*, *4*(3), 217–220. https://doi.org/10.1080/21622965.2013.874951

Rahman, A., Surkan, P. J., Cayetano, C. E., Rwagatare, P., & Dickson, K. E. (2013). Grand Challenges: Integrating Maternal Mental Health into Maternal and Child Health Programmes. *PLoS Medicine*, *10*(5). https://doi.org/10.1371/journal.pmed.1001442

Räikkönen, K., Pesonen, A. K., Roseboom, T. J., & Eriksson, J. G. (2012). Early determinants of mental health. *Best Practice and Research: Clinical Endocrinology and Metabolism*, *26*(5), 599–611. https://doi.org/10.1016/j.beem.2012.03.001

Richter, L. M., Daelmans, B., Lombardi, J., Heymann, J., Boo, F. L., Behrman, J. R., … Darmstadt, G. L. (2016). Investing in the foundation of sustainable development: pathways to scale up for early childhood development. *The Lancet*, *6736*(16). https://doi.org/10.1016/S0140-6736(16)31698-1

Rivera, H. M., Christiansen, K. J., & Sullivan, E. L. (2015). The role of maternal obesity in the risk of neuropsychiatric disorders. *Frontiers in Neuroscience*, *9*, 1–16. https://doi.org/10.3389/fnins.2015.00194

Saigal, S., Szatmari, P., Rosenbaum, P., Campbell, D., & King, S. (1991). Cognitive abilities and school performance of extremely low birth weight children and matched term control children at age 8 years: A regional study. *The Journal of Pediatrics*,

118(5), 751–760. https://doi.org/10.1016/S0022-3476(05)80043-5

Salzwedel, A. P., Gao, W., Andres, A., Badger, T. M., Glasier, C. M., Ramakrishnaiah, R. H., … Ou, X. (2019). Maternal Adiposity Influences Neonatal Brain Functional Connectivity. *Frontiers in Human Neuroscience*, *12*(January), 1–12. https://doi.org/10.3389/fnhum.2018.00514

Schlotz, W., & Phillips, D. I. W. (2009). Fetal origins of mental health: Evidence and mechanisms. *Brain, Behavior, and Immunity*, *23*(7), 905–916. https://doi.org/10.1016/j.bbi.2009.02.001

Smetain, P., Stiff, D., Briante, C., Adair, C. E., Ahmad, S., & Khad, M. (2011). *The human face of mental health and mental illness in Canada* (Mental Health Commission of Canada).

Stein, A., Pearson, R. M., Goodman, S. H., Rapa, E., Rahman, A., McCallum, M., … Pariante, C. M. (2014). Effects of perinatal mental disorders on the fetus and child. *The Lancet*, *384*(9956), 1800–1819. https://doi.org/10.1016/S0140-6736(14)61277-0

Susser, E., & St Clair, D. (2013). Prenatal famine and adult mental illness: Interpreting concordant and discordant results from the Dutch and Chinese Famines. *Social Science and Medicine*, *97*, 325–330. https://doi.org/10.1016/j.socscimed.2013.02.049

Talge, N. M., Neal, C., & Glover, V. (2007). Antenatal maternal stress and long-term effects on child neurodevelopment: How and why? *Journal of Child Psychology and Psychiatry and Allied Disciplines*, *48*(3–4), 245–261. https://doi.org/10.1111/j.1469-7610.2006.01714.x

Thompson, R. A. (1994). Emotion Regulation: A Theme in Search of Definition. *Monographs of the Society for Research in Child Development*, *59*(2), 25–52.

Tottenham, N. (2015). Social scaffolding of human amygdala-mPFCcircuit development. *Social Neuroscience*, *10*(5), 489–499. https://doi.org/10.1080/17470919.2015.1087424

Tottenham, N. (2019). Early Adversity and the Neotenous Human Brain. *Biological Psychiatry*, *16*, 1–10. https://doi.org/10.1016/j.biopsych.2019.06.018

Tronick, E., & Reck, C. (2009). Infants of Depressed Mothers. *Harvard Review of Psychiatry*, *17*(2), 147–156. https://doi.org/10.1080/10673220902899714

Van Den Bergh, B. R. H. (2011). Developmental programming of early brain and behaviour development and mental health: a conceptual framework. *Developmental Medicine and Child Neurology*, *53*, 19–23. https://doi.org/10.1111/j.1469-8749.2011.04057.x

van den Bergh, B. R. H., van den Heuvel, M. I., Lahti, M., Braeken, M., de Rooij, S. R., Entringer, S., … Schwab, M. (2017). Prenatal developmental origins of behavior and mental health: the influence of maternal stress in pregnancy. *Neuroscience & Biobehavioral Reviews*, (November 2016). https://doi.org/https://doi.org/10.1016/j.neubiorev.2017.07.003

Van Lieshout, R. J. (2013). Role of maternal adiposity prior to and during pregnancy in cognitive and psychiatric problems in offspring. *Nutrition Reviews*, *71*(SUPPL1), 95–101. https://doi.org/10.1111/nure.12059

Van Lieshout, R. J., Taylor, V. H., & Boyle, M. H. (2011). Pre-pregnancy and pregnancy

obesity and neurodevelopmental outcomes in offspring: A systematic review. *Obesity Reviews*, *12*(501), 548–559. https://doi.org/10.1111/j.1467-789X.2010.00850.x

Vanderwert, R. E., Marshall, P. J., Nelson, C. A., Zeanah, C. H., & Fox, N. A. (2010). Timing of intervention affects brain electrical activity in children exposed to severe psychosocial neglect. *PLoS ONE*, *5*(7), 3–7. https://doi.org/10.1371/journal.pone.0011415

Vargas, A. O. (2009). Did Paul Kammerer discover epigenetic inheritance? A modern look at the controversial midwife toad experiments. *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution*, *312*(7), 667–678. https://doi.org/10.1002/jez.b.21319

Wadhwa, P. D., Buss, C., Entringer, S., & Swanson, J. M. (2009). Developmental origins of health and disease: Brief history of the approach and current focus on epigenetic mechanisms. *Seminars in Reproductive Medicine*, *27*(5), 358–368. https://doi.org/10.1055/s-0029-1237424

Wakschlag, L. S., Perlman, S. B., Blair, R. J., Leibenluft, E., Briggs-Gowan, M. J., & Pine, D. S. (2018). The neurodevelopmental basis of early childhood disruptive behavior: Irritable and callous phenotypes as exemplars. *American Journal of Psychiatry*, *175*(2), 114–130. https://doi.org/10.1176/appi.ajp.2017.17010045

Wakschlag, L. S., Roberts, M. Y., Flynn, R. M., Smith, J. D., Krogh-Jespersen, S., Kaat, A. J., … Davis, M. M. (2019). Future Directions for Early Childhood Prevention of Mental Disorders: A Road Map to Mental Health, Earlier. *Journal of Clinical Child*

and Adolescent Psychology, *48*(3), 539–554.

https://doi.org/10.1080/15374416.2018.1561296

Chapter 2: Maternal Metabolic Complications in Pregnancy and Offspring Behavior Problems at 2 Years of Age (Study 1)

Study 1 Overview

Title: Maternal Metabolic Complications in Pregnancy and Offspring Behavior Problems at 2 Years of Age

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Context and Implications of this study: The first study in this sandwich thesis investigates the link between gestational metabolic complications and offspring behavior problems before and after adjusting for previously unmeasured confounding variables, including prenatal diet quality. As outlined in the background, the impact of unmeasured, potentially modifiable confounding variables is an important factor in DOHaD research, and is potentially limiting our ability to unlock the preventative potential of the DOHaD hypothesis.

Children exposed to maternal metabolic complications while in utero (e.g., maternal obesity, gestational diabetes mellitus) appear to be at an increased risk for emotional and behavioral problems later in life. However, no studies have examined whether maternal gestational diet confounds these associations. We found that gestational diet plays an important role in links between metabolic complications during pregnancy

and offspring behavioral problems. Further, the effect of gestational diet was similar in magnitude to socioeconomic disadvantage and postpartum depression, two well-known risk factors for offspring psychopathology

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Abstract

Objectives: Prenatal maternal metabolic problems such as pre-pregnancy adiposity, excess gestational weight gain, and gestational diabetes mellitus (GDM) are associated with an increased risk of psychopathology in offspring. We examined whether these exposures were linked to symptoms of emotional and behavioral problems in offspring at two years of age, or if associations were due to confounding variables.

Methods: Data from 815 mother-child pairs enrolled at the Edmonton site of the Canadian Healthy Infant Longitudinal Development cohort were used to examine associations between gestational metabolic complications and scores on the externalizing and internalizing scales of the Child Behavior Checklist (CBCL-1½ to 5) at age two. Associations between maternal metabolic complications and offspring psychopathology were assessed before and after adjustment for gestational diet, socioeconomic status (SES), postpartum depression (PPD), prenatal smoking and breastfeeding.

Results: Pre-pregnancy body mass index and GDM, but not gestational weight gain, predicted more offspring externalizing and internalizing problems. However, after adjustment for confounding variables, these associations were no longer statistically significant. *Post-hoc* analyses revealed that gestational diet accounted for unique variance in both externalizing (semi-partial r_{diet}=-0.20, p<0.001) and internalizing (semi-partial $r_{diet=}$-0.16, p=0.01) problems. PPD and SES also accounted for a similar amount of variance for both externalizing (semi-partial r_{PPD}=0.17, p<0.001; r_{ses}=-0.11, p=0.03) and internalizing problems (semi-partial r_{PPD}=0.21, p<0.001; r_{ses}=-0.14, p=0.004).

Conclusions: Since the confounding effect of gestational diet persisted after adjustment for, and was similar in magnitude to, SES and PPD, future research should consider the impact of unhealthy prenatal diets on offspring neurodevelopment.

Introduction

Emotional and behavioral problems affect up to 20% of children under two years of age (Skovgaard et al., 2007; von Klitzing, Döhnert, Kroll, & Grube, 2015) and predict an increased risk for psychopathology from pre-school age through adulthood (Masi, Pisano, Milone, & Muratori, 2015; Moffitt et al., 2011). The identification of modifiable risk factors causally related to early behavioral problems is therefore of critical importance. The Developmental Origins of Health and Disease (DOHaD) hypothesis posits that prenatal and early postnatal adversity can increase disease susceptibility across the lifespan (Gluckman, Hanson, Cooper, & Thornburg, 2008). Since major neural networks mediating emotion and behavior develop prenatally, fetal exposure to sub-optimal intrauterine conditions, such as those associated with excessive maternal adiposity and gestational diabetes mellitus (GDM), may increase the risk of psychiatric disorders later in life (Bale et al., 2010; Tau & Peterson, 2010). Indeed, numerous observational studies have linked prenatal exposure to pre-pregnancy adiposity, excessive gestational weight gain (GWG), and GDM to emotional, behavioral, and cognitive problems in offspring, problems that may emerge as early as 2 years of age (Nomura et al., 2012; Rivera, Christiansen, & Sullivan, 2015; Van Lieshout, Schmidt, Robinson, Niccols, & Boyle, 2012). Additionally, the effects of both elevated maternal pre-pregnancy body mass index (BMI) together with excessive GWG may further increase risk for neurodevelopmental problems in offspring relative to either exposure alone (Huang et al., 2014).

Despite this evidence, our knowledge of the clinical applicability of the DOHaD hypothesis to the prevention and amelioration of mental disorders in children remains limited. A major barrier to this is a widespread inability to adjust for known modifiable confounding variables that may impact the link between prenatal metabolic complications and offspring behavior problems, such as maternal diet and postpartum depression. Investigating the potential confounding effect of these variables would provide a more complete understanding of the mechanisms underlying the observed associations between metabolic complications and offspring behavioral problems and could provide potential targets for intervention.

Given this background, we used data from the Edmonton site of the Canadian Healthy Infant Longitudinal Development (CHILD) Cohort study to determine if 1) maternal pre-pregnancy adiposity, GWG, their interaction, and/or GDM were associated with offspring behavioral problems at 2 years of age, and 2) if these associations persisted after accounting for key confounding variables including gestational diet and postpartum depression. This study extends previous research in this area by examining the confounding effect of maternal diet quality during pregnancy, a previously unstudied confounder of associations between maternal metabolic complications and offspring emotional and behavioral problems.

Methods

Sample

The CHILD study is a longitudinal birth cohort study, recruiting pregnant women in their second and third trimesters from the general population in four centres across

Canada (see Subbarao et al., 2015 for study details). Mother-child dyads (n=815, of which 586 (72%) had complete outcome data on child behaviour at two years, (Figure 1) enrolled in the Edmonton (Alberta, Canada) sub-cohort of the CHILD study were examined.

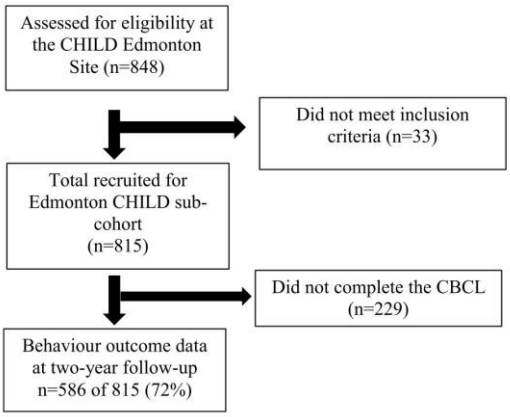

Figure 1: Flow diagram of the sample of individuals with CBCL outcome data at two years of age

Edmonton was the only CHILD study site that examined behavioural outcomes in children. Each woman had only one child in this study. Enrollment for this study was carried out between 2008 and 2012. Women provided informed consent prior to inclusion in the study and at each study visit. Women participating in the study provided data at enrollment and at follow up visits occurring at 36 weeks gestation and at 3, 12, 24 and 36 months postpartum. At each visit, women completed questionnaires about family and child characteristics (see supplemental table e1). In the current study, we used data collected during pregnancy and from the 12, 24 and 36-month visits. Women were excluded if they were younger than 18 years old, could not speak or read English, if they delivered a multiple birth, had conceived via in-vitro fertilization, their infant had a major

congenital abnormality or was born preterm (before 35.5 weeks gestation; preterm infants were excluded since the original CHILD study investigated the development of allergy and asthma and infants born prior to 35.5 weeks can have respiratory problems making the diagnosis of asthma difficult. See supplemental e2 and Takaro et al., 2015 for details). CHILD study ethics approval was obtained from the University of Alberta Health Ethics Research Office (Pro00002099).

Predictors

Pre-pregnancy Body Mass Index (BMI)

In the province of Alberta, perinatal clinical data is captured and recorded in the Alberta Perinatal Health Program (APHP)(Kaul et al., 2015) database, which collects clinical information from all hospital and registered midwife deliveries within the province (Donovan, Savu, Edwards, Johnson, & Kaul, 2015). A total of 727 (89%) of women had BMI data. Maternal pre-pregnancy BMI (weight in kg/ height m^2) was calculated using pre-pregnancy weight extracted from medical charts (n=350), retrospectively reported when the child was 3 years of age (if the prenatal record was not available (n=117)) or estimated by weight obtained at 1 year after birth (n=260) if neither were available. Maternal height was obtained from medical charts (n=332) or obtained when the child was 1 year of age (n=395). Azad et al. (2016) also used this method to obtain BMI. No significant differences between women with BMI calculated from prenatal chart weight (M_{charts}=25.8, SD=5.28) vs. those that retrospectively self-reported their weight ($M_{self\text{-}report}$=25.2, SD=4.95; p=0.29) were observed. No differences were found between women with BMI calculated from chart weight and the women with BMI

data estimated using a weight variable obtained at 1 year after birth ($M_{1\text{-}year}$=26.5, *SD*=6.6) (*p*=0.06). BMI was examined as both a continuous and a dichotomous variable using World Health Organization BMI cut-offs defined as normal; BMI=18.5-24.9 kg/m^2 vs. overweight/obese; BMI≥25 kg/m^2 (WHO, 2009). We decided 'a priori' to exclude women who were underweight (n=18) because complications in these women and their offspring differ from those manifesting the adverse effects of overnurition (Buss et al., 2012; Van Lieshout et al., 2013).

Gestational Weight Gain (GWG)

Maternal GWG was determined by subtracting pre-pregnancy weight from the last weight recorded on the prenatal chart before delivery (at an average gestational age of 37.1 weeks, *SD*=2.93). GWG was retrospectively self-reported when the child was 3 years of age if GWG was unavailable from the prenatal chart (n=15). Those that were retrospectively self-reported (*M*=13.5, *SD*=4.30) were not different from those with prenatal chart data (*M*=15.1, *SD*=6.50) (*p*=0.36). GWG was examined as a dichotomous variable in keeping with the Institute of Medicine guidelines (recommended vs. excessive). A GWG of >15 kg was considered excessive for individuals with a normal BMI, while excessive GWG for the overweight/obese category comprised overweight women (BMI=25-29.99) with a GWG >11kg, and obese women (BMI>30) with GWG >9kg (Rasmussen & Yaktine, 2009).

Gestational Diabetes Mellitus (GDM)

GDM is screened for between 24 and 28 weeks gestation and is captured and recorded in the APHP (Kaul et al., 2015) database. The woman's diagnosis of GDM was

obtained from the pregnancy record completed by their prenatal health care provider. A total of 805 women (98%) had GDM data. Plasma glucose levels were determined using a 1-hour 50g glucose challenge test (DynaLife Testing, 2014). One-hour oral glucose tolerance test plasma glucose levels above 7.8 mmol/L defined GDM status.

Primary Outcome

Emotional and Behavioral Problems

Symptoms of emotion and behavioral problems in offspring were reported by mothers at the 24 month follow-up visit using the 1½-5 year-old version of the Child Behavior Checklist [CBCL 1 ½-5] (On average, mothers completed the CBCL when their children were 2.02 years of age (*SD*=0.32)). The CBCL 1 ½ -5 is a validated, 99-item measure of child behavior that can be aggregated into internalizing and externalizing scales (Achenbach & Reschorla, 2000). The internalizing scale (36-items) is comprised of emotionally reactive, anxious/depressed, somatic complaints and withdrawn subscales (Cronbach α=0.89). The 24 item externalizing scale (Cronbach α=0.92) consists of aggressive behavior and attention problems subscales. Internalizing and externalizing raw scores were examined as continuous outcomes to fully capture behavioral variability and maximize statistical power. Higher scores on each of the CBCL scales indicate more behavioural problems.

Confounding variables

Confounding variables were chosen on an *a priori* basis if the literature suggested consistent associations with both our predictors (pre-pregnancy BMI, GWG, GDM) and child emotional and behavioral problems.

Maternal gestational dietary quality

A food frequency questionnaire (FFQ) developed by the Fred Hutchinson Cancer Research Center was modified to reflect Canadian multi-ethnic food choices. This 175-item self-administered FFQ was completed by women (n=699/815, 86%) at study enrolment (in the second and third trimester). Women reported the average frequency and portion sizes of food consumed since becoming pregnant.

From this FFQ, the overall quality of maternal diet in pregnancy was determined using the Healthy Eating Index-2010 (HEI-2010), a dietary quality index designed to capture dietary habits in accordance with accepted Dietary Guidelines for adults (Guenther et al., 2014). The HEI-2010 has been validated in numerous populations including pregnant women (Guenther et al., 2013). The HEI-2010 is comprised of 12 components: total fruit, whole fruit, total vegetables, greens+beans, whole grains, dairy, total protein foods, seafood+plant proteins, fatty acids, refined grains, sodium and empty calories which includes consumption of solid fats, alcohol, added sugar. The final 3 components are foods to be consumed in moderation and are therefore reverse scored. Hence, higher scores on all subscales indicate better quality of diet. All 12 components are summed to yield an HEI total score variable with a maximum of 100, the highest quality diet measurable. The HEI total score was used in all analyses. In acknowledgement of the potential causal association between an unhealthy diet, higher BMI and gestational weight gain, we considered gestational diet as a confounding variable in our adjusted models. Since the pre-pregnancy BMI and GWG predictor variables consider weight prior to pregnancy; we argue that our measure of diet quality

assessed during gestation could not be causally associated to these pre-pregnancy predictor variables. However, a higher BMI and greater GWG may be associated with poorer diet during gestation.

Socioeconomic Status

Socioeconomic status (SES) was assessed using the McArthur scale of Subjective Social Status at the 12 month visit (Adler, Epel, Castellazzo, & Ickovics, 2000). This instrument uses a visual analogue scale that consists of two ladders with 10 rungs each. Women are instructed to place an 'x' on the rung of the ladder indicative of their social standing relative to other citizens nationally, and then compared to individuals within their community. This scale has shown adequate reliability and has convergent validity with objective SES indicators. This scale also predicts health outcomes over and above objective measures of SES (Cundiff, Smith, Uchino, & Berg, 2013).

Maternal Postpartum Depression*:*

The Center for Epidemiological Studies Depression scale (CES-D) is a self-reported 20-item scale that is a reliable and valid measure of depression during the postpartum period and beyond (Navarro et al., 2007). The total score was used to assess maternal depression at 12 months postpartum.

Maternal Smoking During Pregnancy

At enrollment (second and third trimester), women were asked if they smoked cigarettes (yes/no) at any time during pregnancy.

Breastfeeding Duration

Mothers were asked to report the age (in months) at which their child stopped any breastfeeding at the 24-month visit.

Statistical Analyses

Associations between our predictors and outcomes were examined using two separate statistical regression models. The first contained each predictor and each outcome separately (unadjusted models). We also investigated the interaction between BMI and GWG using the interaction term of a 2x2, between-subjects ANOVA in the unadjusted model. Our second, fully adjusted statistical models contained the variables in each of the first models plus all confounding variables. Pre-pregnancy BMI was also added as a confounder in the adjusted analysis for GDM. We conducted a post-hoc analysis to investigate the unique contribution of each variable in the fully adjusted models using semi-partial correlations (Johnson & Wichern, 2014). All statistical tests were 2-tailed and statistical significance was set at $\alpha=0.05$. Data were analyzed using IBM SPSS version 23. All continuous data passed the statistical assumptions for regression models (e.g., normality tests). All associations are displayed as unstandardized beta (B) values that indicate for every one-unit change in the predictor, an *x* unit change is observed in the outcome (e.g., a one point increase in BMI would be associated with an *x* increase in behavioral problems).

To account for missing data, we examined both complete case and imputed data. Missing data for all variables were imputed using the fully conditional specification multiple imputation in SPSS 23. Since no significant differences between completed case and imputed data were observed, the results reported used complete data only.

Missing pre-pregnancy body mass index (BMI) data was associated shorter breastfeeding duration [*M*=6.0(5.6) vs. 8.7(5.6), p<0.01], single or separated relationship status (OR=0.39, 95% CI=0.20-0.77), Caucasian ethnicity (OR=0.59, 95% CI=037-0.96) and younger age [*M*=29.6(5.2) vs. 31.5(4.3), *p*<0.01]. Missing GWG data also associated with single or separated relationship status (OR=0.4, 95% CI= 0.25-0.77). The 9 women missing GDM data did not differ from those for whom data were present. No significant differences were observed between women with and without any of our metabolic predictors and offspring internalizing and externalizing problems. Finally, women that did not complete the CBCL on their children were younger [*M*=29.6(5.0) vs. 31.5(4.4), *p*<0.01] of lower SES [*M*=6.2(1.8) vs. 6.6(1.63), *p*<0.01] and had fewer years of education [*M*=15.5(2.9) vs. 16.2(2.6), *p*<0.01].

Results

The mean pre-pregnancy BMI of the sample was 26.1 (SD=5.7) and mean age was 31.5 years (SD=4.52). Women with a BMI in the overweight/obese range had fewer years of education, a lower HEI-2010 score, shorter breastfeeding duration, were of lower SES, and a greater proportion had excessive GWG relative to women within the normal BMI category. Those diagnosed with GDM were older, had infants with a younger gestational age, were more likely to be Caucasian, and reported lower SES compared to those without GDM (Table 1). The offspring average raw externalizing score was 9.8 (SD=6.8) and scores ranged from 0-39. The average internalizing score was 5.1 (SD=4.9) and the range was 0-34.

Table 1: Characteristics of the total study sample (n=815)[a]

	Body Mass Index[b]			Presence of Gestational Diabetes Mellitus		
Demographic Variables	Normal (n=367)	Overweight/ Obese (n=342)	*p*	GDM (n=60)	No-GDM (n=746)	*p*
Maternal Age in years (M, SD)	31.5 (4.4)	31.5 (4.3)	0.98	32.7(4.9)	31.2(4.4)	0.01
Marital Status (n, %)						
Married/Common-law	333 (95.1)	298 (92.5)	0.16	53(93.0)	658(93.2)	0.95
Divorced/ Separated	10 (4.9)	12 (7.5)		4(7.0)	48(6.8)	
Maternal education in years (M, SD)	16.5 (2.7)	15.7 (2.5)	<0.01	15.9(2.8)	16.1(2.6)	0.72
Any smoking during pregnancy (n, %)	14 (4.0)	13 (3.9)	0.94	3(5.3)	31(4.4)	0.76
Healthy eating index total score (M, SD)[c]	74.2 (7.7)	71.9 (8.1)	<0.01	73.6(6.9)	73.0(8.3)	0.62
MacArthur Scale of Subject Social Status score (M, SD)	6.7 (1.5)	6.4 (1.8)	0.03	5.83(1.9)	6.56(1.6)	<0.01
Birth order First (n, %)	168 (45.8)	140 (40.9)	0.36	27(45.0)	325(43.6)	0.83
Gestational age in weeks (M, SD)	39.5 (1.3)	39.4 (1.4)	0.36	38.7(1.4)	39.5(1.3)	<0.01
Breastfeeding duration (M, SD)	9.5 (5.7)	7.9 (5.6)	<0.01	8.07(6.2)	8.57(5.7)	0.56
Child's Sex (Male-n, %)	187(51.0)	168 (49.1)	0.63	35(58.3)	377(50.5)	0.25
Child's Ethnicity (n, %)						
Caucasian	245 (69.0)	233 (69.3)	0.93	31(54.4)	491(68.8)	0.03
Non-Caucasian	110 (31.0)	103 (30.7)		26(45.6)	223(31.2)	
Child Behavior (M, SD)[d]						
Internalizing	4.5 (4.3)	5.7 (5.4)	<0.01	7.2 (5.6)	4.9 (4.8)	<0.01
Externalizing	9.0 (6.5)	10.6 (7.2)	<0.01	12.2 (6.2)	9.7 (9.9)	0.02
Presence of GDM	23 (6.3)	33 (9.7)	0.10			
Gestational weight gain (n, %)[e]	Normal (n=249)	Overweight/obese (n=218)		GDM	No-GDM	

Adequate	69(35)	34(17.8)	<0.01	6(19.4)	97(27.2)	
Excessive	128(65)	157(82.2)		25(80.6)	260(72.8)	0.34

[a] n=709 women with BMI data (excluding the 18 underweight women) and 806 had GDM data. Discrepancies in the demographic variable frequencies are due to missing data (e.g., 24 women with normal BMI were missing data on marital status).
[b] BMI (kg): Normal =18.5-25, Overweight/obese≥ 25
[c] Higher scores indicate better diet quality (mean scores fell within 51-79, the "needs improvement/fair" category)
[d] Behavior scores were consistent with those reported in other studies (e.g., Rescorla et al., 2011)
[e] GWG was obtained for only women with pre-pregnancy BMI and calculated separately for normal, overweight and obese categories as per IOM guidelines: Adequate =11-15 kg for normal BMI, <6 kg for overweight, <4 kg for obese; Excessive≥15 kg for normal, >11 kg for overweight, >9 kg for obese.

For our unadjusted models, statistically significant associations were observed between pre-pregnancy BMI examined as both a continuous and dichotomous (normal vs. overweight obese) predictor and offspring externalizing symptoms ($BMI_{continuous}$: B=0.15, 95% CI [0.05;0.26]; $BMI_{dichotomous}$: B=1.60, 95% CI [0.45;2.74]) and internalizing symptoms ($BMI_{continuous}$: B=0.09, 95% CI [0.02;0.16]; $BMI_{dichotomous}$: B=1.2, 95% CI [0.35;2.0]) at 2 years of age. Neither excessive GWG nor the interaction between BMI and GWG were associated with offspring outcomes. GDM was associated with higher levels of externalizing (B=2.56, 95% CI [0.41;4.71]) and internalizing (B=2.28, 95% CI [0.75;3.81]) symptoms (Table 2) Statistically significant associations were no longer observed between BMI, GDM, and internalizing and externalizing behaviors following adjustment for confounders (Table 3).

Table 2: Unadjusted associations between maternal metabolic complications during pregnancy and CBCL 1½ -5 externalizing and internalizing problems (N=586 with behavioural follow-up data when offspring were two years of age)

Predictors (B, 95-CI)	Externalizing	Internalizing
Body Mass Index[a]	0.15 (0.05; 0.26)**	0.09 (0.02; 0.16)*
Body Mass Index (Categorical)[b] Normal-Overweight/Obese	1.6 (0.45; 2.74)**	1.2 (0.35; 2.0)**
Gestational weight gain		

Adequate vs. Excess[c]	0.79 (-0.78; 2.37)	0.33(-0.75; 1.40)
Body mass index x Gestational Weight Gain[d]	1.91(NS)	0.10 (NS)
GDM[e]	2.56 (0.41; 4.71)*	2.28 (0.75; 3.81)**

Unstandardized betas (B) indicate for every one-unit increase in the predictor, there is an *x*-unit increase in the outcome

[a] Continuous BMI (Of women that completed the CBCL, 569/586 (94%) also had BMI data)

[b] Normal vs. overweight (BMI 25-29.9) Obese (BMI >30)

[d] BMI (normal vs. overweight/obese) by GWG (adequate vs. excessive), *F* statistics shown from 2x2 between subjects ANOVA

[e] For the GDM model, of the women that completed the CBCL, 582/586 (99%) had GDM data)

* p<0.05

** p<0.01

Table 3: Associations between maternal metabolic complications during pregnancy, each confounding variable and offspring CBCL 1½ -5 externalizing and internalizing problems measured at two years of age

Predictors (B, 95-CI)	Externalizing	Internalizing
Adjusted Model 1		
Body mass index[a]	0.07 (-0.05; 0.19)	0.03 (-0.06; 0.12)
Smoking	1.30 (-5.1; 2.3)	3.25 (-5.82; -0.7)*
Maternal Diet	-0.18 (-0.27; -0.09)**	-0.10 (-0.16; -0.05)**
SES	-0.45 (-0.84;-0.05)*	-0.41 (-0.68;-0.13)**
Depression	0.17 (0.08;0.27)**	0.15 (0.08;0.22)**
Breastfeeding duration	-0.10 (-0.21 0.01)	-0.08 (-0.16; 0.01)
Adjusted Model 2		
BMI (Categorical)		
Normal- Overweight/Obese[b]	0.59 (-0.68; 1.87)	0.68 (-0.21; 1.56)
Smoking	1.52 (-5.2; 2.2)	3.3 (-5.8; -0.72)*
Maternal Diet	-0.18 (-0.27;-0.09)**	-0.10 (-0.16; -0.04)**
SES	-0.46 (-0.85;-0.06)*	-0.40 (-0.68;-0.13)**
Depression	0.18 (0.08;0.27)**	0.15 (0.08; 0.21)**
Breastfeeding duration	-0.11 (-0.22; 0.005)	-0.08 (-0.16; 0.01)
Adjusted Model 3		
GDM[c]	1.7 (-0.77; 4.24)	1.25 (-0.46; 2.96)
BMI	0.07 (-0.05;0.19)	0.02 (-0.06; 0.11)
Smoking	1.4 (-5.20; 2.25)	3.27 (5.84; -0.70)*
Maternal Diet	-0.18 (-0.27; -0.10)**	-0.11 (-0.16; -0.05)**
SES	-0.39 (-0.79; 0.009)	-0.38 (-0.7; -0.10)**
Depression	0.16 (0.07;0.26)**	0.14 (0.08; 0.2)**
Breastfeeding duration	-0.10 (-0.21; 0.01)	-0.08 (-0.16;0.02)

Adjusted for SES, maternal smoking, prenatal maternal diet (measured by HEI total score), maternal depression at 12 months postpartum and breastfeeding duration

[a] For the adjusted model BMI model, n=391 had complete data on every variable included in the model

[b] BMI normal (18.5-24.9) vs. BMI >25

[c] GDM: Gestational diabetes mellitus, n=392 had complete data for every variable

* p<0.05

A *post-hoc* examination of semi-partial correlations between all variables in the fully adjusted models revealed that total scores on the HEI-2010, PPD at 12 months, and SES each independently accounted for statistically significant associations between our predictors and internalizing and externalizing problems. Semi-partial correlations display the unique variance of an individual variable in the model in the outcome while removing the influence that all other variables have on this outcome. Total diet accounted for the most unique variance in externalizing problems ($r_{part,diet}$ =-0.20, p<0.01). Additionally, PPD and SES also accounted for a significant amount of variance in externalizing problems ($r_{part,\ PPD}$=0.17, p<0.01, $r_{part,\ ses}$= -0.11, p<0.05). These variables, along with smoking in pregnancy accounted for internalizing problems ($r_{part,\ PPD}$= 0.21, p<0.01, $r_{part,diet}$ =-0.16, p<0.01, $r_{part,\ ses}$= -0.14, p<0.01, $r_{part,\ smoke}$=0.12, p<0.05) (semi-partial correlation coefficients between HEI-2010 total, postpartum depressions scores, SES and offspring behavior problems within the adjusted BMI model are shown, See table 4 for both BMI and GDM models).

Table 4: *Post-hoc* analyses of semi-partial correlations accounting for the associations between BMI, GDM and offspring behaviour problems at two years of age[a].

Model		Semi-partial correlation coefficients (r_s, p-value)	
BMI (continuous)		Externalizing	Internalizing
	Maternal Diet	-0.20(<0.01)	-0.16 (<0.01)
	Empty calories[b]	-0.18 (<0.01)	-0.12 (<0.05)
	Depression	0.17 (<0.01)	0.21 (<0.01)
	SES	-0.11 (<0.05)	-0.14 (<0.01)
	Smoking	0.04 (N.S.)	0.12 (<0.05)
	Breastfeeding	-0.08 (N.S.)	-0.09 (N.S.)
	BMI	0.06 (N.S.)	0.03 (N.S.)
GDM			
	Maternal Diet	-0.20 (<0.01)	-0.17 (<0.01)
	Empty calories[b]	-0.17 (<0.01)	-0.12 (<0.05)
	Depression	0.16 (<0.01)	0.20 (<0.01)
	SES	-0.09 (0.055)	-0.13 (<0.01)

	Smoking	0.04 (N.S.)	0.12 (<0.05)
	Breastfeeding	-0.08 (N.S.)	-0.09 (N.S.)
	BMI	0.05 (N.S.)	0.03 (N.S.)
	GDM	0.07 (N.S)	0.07 (N.S)

[a] Semi-partial correlations are used to display the unique variance that each individual variable in the fully adjusted model accounts for in the outcome while removing the influence that all other variables have on this outcome. (e.g., the unique variance that maternal diet accounts for in offspring behavioural problems while accounting for all other variables in the model).
[b]The HEI-2010 (maternal diet) component that remained significant when added to the fully adjusted model *in place* of HEI-total.

Component subscale scores of the HEI-2010 were then substituted into fully adjusted models *in place* of total diet quality (e.g., the empty calories subscale (calories from solid fats and added sugars) was substituted into the adjusted model in place of HEI-2010 total). The empty calories component retained statistically significant associations with externalizing ($r_{\text{part, empty cal}}$= -0.18, p<0.01) and internalizing ($r_{\text{part, empty cal}}$= -0.12, p<0.05) in both the BMI and GDM fully adjusted models. A sensitivity analysis that utilized a predictor variable of BMI calculated using only recorded pre-pregnancy weights (n=467 participants) confirmed these findings (Supplementary Tables e3, e4 and e5).

Discussion

Prior to adjustment for confounding variables, statistically significant associations were observed between maternal pre-pregnancy BMI, GDM, and offspring externalizing and internalizing symptoms at 2 years of age. However, these were no longer significant after adjustment for known confounding variables. Indeed, associations between BMI and offspring emotional and behavioral problems were accounted for by each of maternal diet, postpartum depression, and SES. To our knowledge, this is the first study to show that the effect of pre-pregnancy adiposity and GDM on offspring emotional and

behavioral problems may be accounted for by poor maternal pregnancy diet, postpartum depression, and SES.

Previously published studies have reported associations between maternal metabolic complications of pregnancy (GDM, pre-pregnancy BMI and GWG) and offspring behavior problems in young children (Nomura et al., 2012; Robinson et al., 2012; Van Lieshout et al., 2012). Most of this prior work has linked metabolic complications in pregnancy to delayed cognitive development (Pugh et al., 2015), autism spectrum disorders (Li, Fallin, Riley, Landa, & Walker, 2016) and externalizing problems including hyperactivity and inattention despite adjustment for confounders (Ornoy, Reece, Pavlinkova, Kappen, & Miller, 2015; Rodriguez, 2010). However, to our knowledge, none contained data on maternal diet quality during pregnancy and few have assessed postpartum depression using a validated scale.

Post-hoc analyses revealed that maternal diet quality, postpartum depression, and SES each accounted for a significant amount of unique variance in offspring behavioral problems in fully adjusted models. However, overall maternal diet and particularly empty calories accounted for a significant amount of variance in both internalizing and externalizing problems. This supports previous findings that diet is linked to better offspring neurodevelopment outcomes (Bolduc et al., 2016; Nyaradi, Li, Hickling, Foster, & Oddy, 2013) and is similar to the results of large cohort studies that examined associations between diet in pregnancy and offspring externalizing problems at age 5 and 6 years (Jacka et al., 2013; Steenweg-de Graaff et al., 2014). However, these studies did

not examine the role that gestational diet played in the associations between maternal BMI or GDM and offspring behavior.

Poor gestational diet, accounted for a significant amount of variance in offspring behaviour problems, even in the presence of well-known risk factors for offspring psychopathology such as SES and postpartum depression. Moreover, the magnitude of the effect of poor maternal diet was similar to these established contributors to behavioral problems in early childhood. Therefore, future studies investigating the mental health and neurodevelopment of offspring exposed to maternal metabolic complications in-utero should adjust for maternal diet quality.

Further, we investigated the subscales of the HEI-2010 to determine if the effect of overall diet was driven by any dietary components in particular. Our finding that much of the effect of overall diet was driven by the empty calories scale of the HEI-2010 (e.g., calories from solid fats, added sugar), is supported by studies in rodent models which have shown that high-fat diets can contribute to altered neurotransmitter signaling, neuroendocrine dysgregulation and inflammation (Golan, Lev, Hallak, Sorokin, & Huleihel, 2005; Sullivan, Riper, Lockard, & Valleau, 2015). These effects appear to be mediated by epigenetic alterations in offspring mesocorticolimbic dopamine systems (Vucetic, Kimmel, Totoki, Hollenbeck, & Reyes, 2010). Indeed, chronically low dopamine levels developing in response to hypomethylation of the promoter region of the dopamine transporter gene could produce an increased propensity of reward seeking behaviors in order to restore homeostatic dopamine balance (Vucetic et al., 2010).

Epigenetic alterations occurring in response to maternal high fat diets could therefore potentially be involved in the development of behaviour problems in humans.

The results of this study should be interpreted with the following limitations in mind. First, the observational nature of this study limits causal inference. Second, whether our measurement of diet quality specifically measures nutrition during pregnancy or longer-term dietary habits (e.g., before pregnancy, or across a woman's whole life) that may influence offspring neurodevelopment is unclear. Third, these associations may be due to confounders of links between dietary quality and our outcomes. Fourth, although we adjusted for breastfeeding duration, we cannot rule out the possibility that child postnatal diet outside of breastfeeding contributes to these findings, because we did not have access to these data. However, Jacka et al. (2012) did adjust for child diet and found that maternal prenatal diet retained significant associations with offspring externalizing problems. We also were unable to adjusted for exclusive breastfeeding. Fifth, we only had reports on offspring from a single informant (the child's mother). Sixth, a subset of our maternal weight values were not obtained from prenatal charts, therefore, although these values were not different from prenatal chart weights, they may be subject to response bias. Finally, we did not have the capacity to control for genetic factors potentially influencing maternal behaviors including dietary choices. However, the CHILD study is in the process of obtaining and analysing genetic data, providing opportunities to consider these factors in future research.

In order to further advance the field, future observational studies should attempt to examine other potentially modifiable confounders of associations between metabolic

complications during pregnancy and offspring neurodevelopmental outcomes including maternal physical inactivity. These studies could also examine the interactions between metabolic complications and behavioral lifestyle factors (e.g., nutrition, physical inactivity) to further elucidate the impact of these exposures on offspring neurodevelopment. Future studies should also investigate these associations using larger more diverse sample sizes. Studies should also examine more specific components of offspring behavior problems (e.g., impulsivity, reward sensitivity). Ongoing randomized controlled trials of maternal lifestyle interventions could also be utilized to investigate the impact of maternal lifestyle on offspring brain and behavior. If improving diet prenatally is shown to benefit offspring neurodevelopment, these interventions could have immense public health potential (Van Lieshout & Krzeczkowski, 2016).

Our data suggest that associations between maternal metabolic complications during pregnancy and offspring behavior problems are accounted for by SES, postpartum depression and maternal diet. Since numerous studies have observed moderate to strong continuity between behavior and emotion problems in early childhood and adult psychopathology, the examination of modifiable risk factors for emotional and behavioral problems is integral to the prevention and/or amelioration of mental disorders across the lifespan. Given the significant preventive promise of the DOHaD hypothesis, future studies should attempt to determine if optimizing maternal diet during pregnancy could be utilized to improve offspring neurodevelopment, in addition to its other well-known benefits for women and children.

References

Achenbach, T. M., & Reschorla, L. A. (2000). *Manual for the ASEBA Preschool Forms & Profiles*. Burlington VT: University of Vermont, Research Centre for Children, Youth, & Families.

Adler, N. E., Epel, E. S., Castellazzo, G., & Ickovics, J. R. (2000). Relationship of subjective and objective social status with psychological and physiological functioning: preliminary data in healthy white women. *Health Psychology*, 19, 586–592.

Azad, M. B., Sharma, A. K., de Souza, R. J., Dolinsky, V. W., Becker, A. B., Mandhane, P. J., … Sears, M. R. (2016). Association Between Artificially Sweetened Beverage Consumption During Pregnancy and Infant Body Mass Index. *JAMA Pediatrics*, 170, 662–670.

Bale, T. L., Baram, T. Z., Brown, A. S., Goldstein, J. M., Insel, T. R., McCarthy, M. M., … Nestler, E. J. (2010). Early life programming and neurodevelopmental disorders. *Biological Psychiatry*, 68, 314–319.

Buss, C., Entringer, S., Davis, E. P., Hobel, C. J., Swanson, J. M., Wadhwa, P. D., & Sandman, C. A. (2012). Impaired executive function mediates the association between maternal pre-pregnancy body mass index and child ADHD symptoms. *PLoS ONE*, 7, e37758.

Cundiff, J. M., Smith, T. W., Uchino, B. N., & Berg, C. A. (2013). Subjective Social Status: Construct Validity and Associations with Psychosocial Vulnerability and Self-Rated Health. *International Journal of Behavioral Medicine*, 20, 148–158.

Donovan, L. E., Savu, A., Edwards, A. L., Johnson, J. A., & Kaul, P. (2015). Prevalence and Timing of Screening and Diagnostic Testing for Gestational Diabetes: A Population-Based Study in Alberta, Canada. *Diabetes Care*, 39. 55–60.

DynaLife Diagnostic Testing. (2014). Gestational Diabetes Mellitus Screen. https://www.dynalifedx.com.

Gluckman, P. D., Hanson, M. A, Cooper, C., & Thornburg, K. L. (2008). Effect of In Utero and Early-Life Conditions on Adult Health and Disease. *New England Journal of Medicine*, 359, 61–73.

Golan, H. M., Lev, V., Hallak, M., Sorokin, Y., & Huleihel, M. (2005). Specific neurodevelopmental damage in mice offspring following maternal inflammation during pregnancy. *Neuropharmacology*, 48, 903–917.

Guenther, P. M., Casavale, K. O., Reedy, J., Kirkpatrick, S. I., Hiza, H. a B., Kuczynski, K. J., … Krebs-Smith, S. M. (2013). Update of the Healthy Eating Index: HEI-2010. *Journal of the Academy of Nutrition and Dietetics*, 113, 569–580.

Guenther, P. M., Kirkpatrick, S. I., Reedy, J., Krebs-Smith, S. M., Buckman, D. W., Dodd, K. W., … Carroll, R. J. (2014). The Healthy Eating Index-2010 is a valid and reliable measure of diet quality according to the 2010 Dietary Guidelines for Americans. *The Journal of Nutrition*, 144, 399–407.

Huang, L., Yu, X., Keim, S., Li, L., Zhang, L., & Zhang, J. (2014). Maternal prepregnancy obesity and child neurodevelopment in the Collaborative Perinatal Project. *International Journal of Epidemiology*, 43, 783–92.

Jacka, F. N., Ystrom, E., Brantsaeter, A. L., Karevold, E., Roth, C., Haugen, M., … Berk,

M. (2013). Maternal and Early Postnatal Nutrition and Mental Health of Offspring by Age 5 Years: A Prospective Cohort Study. *Journal of the American Academy of Child & Adolescent Psychiatry*, 52, 1038–1047.

Johnson, R. A., & Wichern, D. W. (2014). *Applied multivariate statistical analysis* (Vol. 4). New Jersey: Prentice-Hall.

Kaul, P., Savu, A., Nerenberg, K. A., Donovan, L. E., Chik, C. L., Ryan, E. A., & Johnson, J. A. (2015). Impact of gestational diabetes mellitus and high maternal weight on the development of diabetes, hypertension and cardiovascular disease: A population-level analysis. *Diabetic Medicine*, 32, 164–173.

Li, M., Fallin, M. D., Riley, A., Landa, R., & Walker, S. O. (2016). The Association of Maternal Obesity and Diabetes With Autism and Other Developmental Disabilities. *Pediatrics*, 137, e20152206.

Masi, G., Pisano, S., Milone, A., & Muratori, P. (2015). Child behavior checklist dysregulation profile in children with disruptive behavior disorders: A longitudinal study. *Journal of Affective Disorders*, 186, 249–253.

Moffitt, T. E., Arseneault, L., Belsky, D., Dickson, N., Hancox, R. J., Harrington, H., … Caspi, A. (2011). A gradient of childhood self-control predicts health, wealth, and public safety. *Proceedings of the National Academy of Sciences*, 108, 2693–2698.

Navarro, P., Ascaso, C., Garcia-Esteve, L., Aguado, J., Torres, A., & Martan-Santos, R. (2007). Postnatal psychiatric morbidity: a validation study of the GHQ-12 and the EPDS as screening tools. *General Hospital Psychiatry*, 29, 1–7.

Nomura, Y., Marks, D. J., Grossman, B., Yoon, M., Loudon, H., Stone, J., & Halperin, J.

M. (2012). Exposure to Gestational Diabetes Mellitus and Low Socioeconomic Status. *Archives of Pediatric Adolescent Medicine*, 166, 337–343.

Ornoy, A., Reece, E. A., Pavlinkova, G., Kappen, C., & Miller, R. K. (2015). Effect of maternal diabetes on the embryo, fetus, and children: congenital anomalies, genetic and epigenetic changes and developmental outcomes. *Birth Defects Research. Part C, Embryo Today : Reviews*, 105, 53–72.

Rasmussen K.M. & Yaktine A.K. (2009). *Weight Gain During Pregnancy: Reexamining the Guidelines.* National Academy Press: Washington DC.

Rescorla, L. A., Achenbach, T. M., Ivanova, M. Y., Harder, V. S., Otten, L., Bilenberg, N., … Verhulst, F. C. (2011). International comparisons of behavioral and emotional problems in preschool children: Parents' reports from 24 societies. *Journal of Clinical Child and Adolescent Psychology*, *40*(3), 456–467.

Rijlaarsdam, J., Cecil, C. A. M., Walton, E., Mesirow, M. S. C., Relton, C. L., Gaunt, T. R., … Barker, E. D. (2016). Prenatal unhealthy diet, insulin-like growth factor 2 gene (IGF2) methylation, and attention deficit hyperactivity disorder symptoms in youth with early-onset conduct problems. *Journal of Child Psychology and Psychiatry and Allied Disciplines*, 1, 19–27.

Rivera, H. M., Christiansen, K. J., & Sullivan, E. L. (2015). The role of maternal obesity in the risk of neuropsychiatric disorders. *Frontiers in Neuroscience*, *9*, 1–16.

Robinson, M., Zubrick, S. R., Pennell, C. E., Van Lieshout, R. J., Jacoby, P., Beilin, L. J., … Oddy, W. H. (2012). Pre-pregnancy maternal overweight and obesity increase the risk for affective disorders in offspring. *Journal of Developmental Origins of Health*

and Disease, *4*, 1–7.

Rodriguez, A. (2010). Maternal pre-pregnancy obesity and risk for inattention and negative emotionality in children. *Journal of Child Psychology and Psychiatry and Allied Disciplines*, 51, 134–143.

Skovgaard, A. M., Houmann, T., Christiansen, E., Landorph, S., Jørgensen, T., Olsen, E. M., … Lichtenberg, A. (2007). The prevalence of mental health problems in children 1(1/2) years of age - the Copenhagen Child Cohort 2000. *Journal of Child Psychology and Psychiatry, and Allied Disciplines*, 48, 62–70.

Steenweg-de Graaff, J., Tiemeier, H., Steegers-Theunissen, R. P. M., Hofman, A., Jaddoe, V. W. V, Verhulst, F. C., & Roza, S. J. (2014). Maternal dietary patterns during pregnancy and child internalising and externalising problems. The Generation R Study. *Clinical Nutrition*, 33, 115–121.

Subbarao, P., Anand, S. S., Becker, A. B., Befus, A. D., Brauer, M., Brook, J. R., … Sears, M. R. (2015). The Canadian Healthy Infant Longitudinal Development (CHILD) Study: examining developmental origins of allergy and asthma. *Thorax*, 70, 998–1000.

Sullivan, E. L., Riper, K. M., Lockard, R., & Valleau, J. C. (2015). Maternal high-fat diet programming of the neuroendocrine system and behavior. *Hormones and Behavior*, 76, 153–161.

Takaro, T. K., Scott, J. A, Allen, R. W., Anand, S. S., Becker, A. B., Befus, A. D., … Brook, J. R. (2015). The Canadian Healthy Infant Longitudinal Development (CHILD) birth cohort study: assessment of environmental exposures. *Journal of*

Exposure Science and Environmental Epidemiology, 25, 1–13.

Tau, G. Z., & Peterson, B. S. (2010). Normal development of brain circuits. *Neuropsychopharmacology,* 35, 147–168.

Van Lieshout, R. J., & Krzeczkowski, J. E. (2016). Just DO(HaD) It! Testing the clinical potential of the DOHaD hypothesis to prevent mental disorders using experimental study designs. *Journal of Developmental Origins of Health and Disease*, 1–9.

Van Lieshout, R. J., Schmidt, L. A., Robinson, M., Niccols, A., & Boyle, M. H. (2012). Maternal Pre-pregnancy Body Mass Index and Offspring Temperament and Behavior at 1 and 2 Years of Age. *Child Psychiatry & Human Development*, 44, 382–390.

von Klitzing, K., Döhnert, M., Kroll, M., & Grube, M. (2015). Mental Disorders in Early Childhood. *Deutsches Ärzteblatt International*, 112, 375–386.

Vucetic, Z., Kimmel, J., Totoki, K., Hollenbeck, E., & Reyes, T. M. (2010). Maternal high-fat diet alters methylation and gene expression of dopamine and opioid-related genes. *Endocrinology*, 151, 4756–4764.

World Health Organization. *Global Database on Body Mass Index.* WHO: Geneva, 2009.

Chapter 3: Neurodevelopment in 3–4 year old children exposed to maternal hyperglycemia or adiposity in utero (Study 2)

Study 2 Overview

Title: Neurodevelopment in 3–4 year old children exposed to maternal hyperglycemia or adiposity in utero

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Context and Implications: This study aimed to replicate and extend upon our results from Study 1 using data from a second large Canadian birth cohort, and by examining another offspring outcome that depends critically on optimal emotion regulatory capacity, that of cognitive function. In study 2, we observed that associations between maternal metabolic complications and offspring cognition at 3-4 years of age are due to confounders (particularly gestational diet quality). Indeed, prenatal maternal diet confounded associations between metabolic complications in pregnancy and offspring neurodevelopment, even after the effects of traditional risk factors (maternal depression, education and quality of the home environment) were accounted for, providing further support that it plays an important role in the neurodevelopment of offspring born to mothers with metabolic complications during pregnancy. Taken together, evidence from Studies 1 and 2 suggest that prenatal diet quality may be an important modifiable risk factor that could be targeted in future interventions designed to optimize the development of offspring emotion regulation

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Abstract

Background: Prenatal exposure to maternal metabolic complications has been linked to offspring neurodevelopmental problems. However, no studies investigating these links have adjusted for maternal prenatal diet.

Aims: To determine if prenatal exposure to maternal adiposity or hyperglycemia is associated with neurodevelopmental problems in 3-4 year old children, and if links persist following adjustment for confounding variables, including prenatal diet.

Method: 808 mother-child pairs from the Maternal-Infant Research on Environmental Chemicals-Child Development Plus cohort were used to examine associations between pre-pregnancy body mass index (BMI), hyperglycemia and offspring verbal, performance and full-scale IQ scores, as well as internalizing and externalizing problems. Associations were examined before and after adjustment for prenatal diet along with home environment, maternal depression, education and prenatal smoking. Semi-partial correlations were examined post-hoc to assess the impact of each confounder in the adjusted models.

Results: In the unadjusted models, BMI and hyperglycemia predicted lower verbal and full-scale IQ. BMI was also linked to externalizing problems. However, associations were not significant after adjustment. In adjusted models, post-hoc analysis revealed that prenatal diet and home environment accounted for significant variance in verbal and full-scale IQ. The home environment and maternal depression accounted for significant variance in externalizing problems.

Conclusion: In the adjusted models, maternal metabolic complications were not

associated with offspring neurodevelopment. Even while adjusting for well-known risk factors for adverse offspring cognition (home environment, maternal depression), we show for the first time that maternal prenatal diet is an important confounder of the links between maternal metabolic complications and offspring cognition.

Introduction

Nearly 40% of women are overweight or obese during pregnancy, and up to 16% of pregnancies are complicated by maternal hyperglycemia (i.e., impaired glucose tolerance or diabetes mellitus)[1–3]. Given the rapid rate of prenatal neurodevelopment, the fetal brain is particularly vulnerable to the pathological effects of prenatal adiposity and/or hyperglycemia[4,5]. Indeed, both pre-pregnancy adiposity and hyperglycemia have been linked to abnormalities of central nervous system development including defects of the neural tube[6–8]. As a result, prenatal metabolic complications may have the potential to affect more complex neural systems underlying the development of higher mental functions such as cognition and emotion regulation.

Indeed, in keeping with the developmental origins of health and disease (DOHaD) hypothesis, prenatal exposure to excess maternal adiposity has been linked to poorer cognitive and language skills in children [9–11]. Higher rates of attention deficit hyperactivity disorder and externalizing problems are also seen in the children of overweight/obese women[12,13]. Similar outcomes have been noted in children born to those with hyperglycemia during gestation including a doubling of the risk of both cognitive and behavioral problems[14,15].

Despite this accumulating evidence, the impact of potential unmeasured confounding variables on the associations between prenatal metabolic complications and offspring neurodevelopment remains a significant issue in the field. In an attempt to address these limitations, researchers have utilized twin and sibling studies, as well as paternal obesity as a negative control in order to strengthen the case for causal links

between prenatal metabolic complications and offspring neurodevelopmental problems. However, while maternal adiposity had a stronger association with offspring cognitive and behavioral problems relative to paternal obesity in four studies [16–19], in three others it did not [20–22]. Prenatal metabolic complications were also linked to poorer neurodevelopment in one twin [23] and one sibling study [9], but two others using sibling designs reported null results [24,25].

Despite the advantages of sibling and twin studies in adjusting for genetic and other familial factors, current studies lack the ability to adjust for important *environmental* confounding variables, particularly for those that may be modifiable. Indeed, no studies have adjusted for overall prenatal diet quality when examining the associations between maternal metabolic complications and offspring neurodevelopment. Controlling for overall prenatal quality in pregnancy would significantly advance our understanding of these links and could help us move closer to identifying more specific targets for intervention.

Given high rates of excess maternal adiposity and dysglycemia, and the importance of early cognition and behavior to health and success in life, understanding potential mechanisms underlying these links is of significant importance. Therefore, we utilized data from the pan-Canadian Maternal-Infant Research on Environmental Chemicals-Child Development Plus (MIREC-CD+) cohort to: a) determine if prenatal exposure to maternal overweight/obesity or hyperglycemia is associated with cognitive and behavioral problems in 3-4 year old offspring, and b) if these links persist following

adjustment for confounding variables including prenatal diet quality, a previously unmeasured confounding variable of these links.

Methods

Subjects

MIREC is a longitudinal pregnancy cohort that recruited women from ten Canadian cities during their first trimester (<14 weeks gestation) between 2008 and 2011[26]. Eligibility criteria included fluency in English or French, maternal age >18 years, plans to deliver locally, and an agreement to provide a cord blood sample. Women were excluded if there were abnormalities or malformations of the fetus in the current pregnancy, a history of medical complications such as heart disease, or a history of illicit drug use. Women provided sociodemographic information over three prenatal visits (one per trimester), and clinical information was obtained from medical charts following delivery.

The current study utilized data from the MIREC-CD plus cohort (a sub-study of the original MIREC cohort) designed to assess cognitive and behavioral outcomes in 800 3-4 year old children (n=808, m_{age}=40.7 months, SD=3.73). Parents provided written consent prior to participation and study procedures were approved by research ethics boards at Health Canada and all recruitment sites.

Predictors

We examined the independent impact of pre-pregnancy adiposity and hyperglycemia separately on each of our outcomes since not all overweight women develop hyperglycemia, and not all women with hyperglycemia are overweight.

Pre-Pregnancy Adiposity-Body Mass Index (BMI): Maternal pre-pregnancy

BMI was calculated at the first trimester visit by dividing self-reported pre-pregnancy weight (kg) by height (m^2), obtained by research staff. BMI was considered both a continuous and a dichotomous variable (Normal: 18.5 < BMI < 25 vs. Overweight/obese: BMI>25)[27]. Underweight cases (BMI< 18.5, n=20) were excluded since complications in pregnancy associated with being underweight are different from complications due to maternal overweight/obesity [28,29].

Hyperglycemia (GDM/IGT): Of the women who participated in MIREC-CD Plus, 594 were assessed for hyperglycemia based on medical chart review. A dichotomous variable for hyperglycemia (gestational diabetes mellitus (GDM) or impaired glucose tolerance (IGT)) was created where GDM or IGT status =1 and no GDM or IGT status = 0. GDM/IGT was determined based on the results of a glucose challenge test (GCT), or a 50g or 100g oral glucose tolerance test (OGTT). GDM was diagnosed if 1) fasting glucose levels after a 1 hour 50g GCT result exceeded 10.3 mmol/L, or 2) if two or more of the following cut-off values were exceeded following either a 50g or 100g OGTT: [50g OGTT criteria: a) 1 hour post >10.6 mmol/L, b) 2 hour >8.9 mmol/L, or c) fasting plasma glucose level >5.3mmol/L; 100g OGTT criteria: a) 1 hour post >10.6 mmol/L, b) 2 hour >9.2 mmol/L, c) 3 hours>8.0 mmol/L, or d) fasting plasma glucose level>5.8 mmol/L]. IGT was diagnosed if 1 of these OGTT levels was exceeded. GDM and IGT were combined because adverse health outcomes in offspring have been observed following exposure to maternal glycemic levels below diagnostic criteria for gestational diabetes mellitus [30].

Outcomes

Cognition: Wechsler Preschool and Primary Scale of Intelligence-III (WPPSI-III): Qualified research staff assessed child cognitive functioning using the WPPSI-III, a gold standard measure of intellectual function in children aged 2 ½ to 7 ½ years. Age-corrected verbal IQ, performance IQ and full-scale IQ scores served as our cognitive outcomes. The verbal IQ scale measures acquired knowledge, verbal comprehension and reasoning. The performance scale measures spatial abilities including visual and motor skills. Full-scale IQ score measures general intelligence. Software provided by the test publishers were used to calculate these three scales. Composite scales have a mean of 100, a standard deviation of 15, and a maximum possible score of 160.

Behavior: The Behavioral Assessment System for Children-Second Edition (BASC-II): Mothers completed the 134-item BASC-II that measures emotional and behavioral problems in 2 to 5 year old children. Individual items are scored on a 4-point Likert scale (0=never to 3=almost always) with higher scores indicating more problems. T-scores on the internalizing and externalizing composite scales were utilized. Each t-score has a mean of 50 and a standard deviation of 10. The externalizing scale is comprised of 22 items from the aggression and hyperactivity subscales (α=0.93), and the internalizing problems scale consists of 37 items drawn from the anxiety, depression, and somatization subscales (α=0.90).

Confounding variables

Confounding variables were selected based on previous long-standing evidence of associations between the confounder and both our predictors and outcomes.

Prenatal Diet Quality: Prenatal diet was assessed using a 46-item food frequency questionnaire (FFQ) to assess the frequency and serving sizes of foods consumed in the past month. The FFQ was administered to mothers between 16 and 21 weeks of pregnancy and assessed intake of foods in 8 subgroups (vegetables, fruits, meat, poultry, fish and alternatives, dairy products, grain produces and other foods)[31]. The Healthy Eating Index-2010 (HEI) was used to convert FFQ data into a measure of total prenatal diet quality (see reference [32] for a step by step guide on the methods used to convert FFQ data to HEI scores). The HEI assesses diet quality according to national dietary guidelines and has been validated in pregnant women [33]. It is comprised of 8 "adequacy" components including total fruit, whole fruit, total vegetables, greens and beans, whole grains, dairy, total protein foods, seafood and plant proteins, and fatty acids. Higher scores indicate better diet quality. The HEI also contains 3 "moderation" components including refined grains, sodium, and empty calories (from fats and added sugars). The moderation components are reverse-scored, therefore higher scores indicate better diet quality. The sum of the adequacy and moderation components yields an HEI total score with a maximum score out of 100. The HEI total score was used in our statistical analyses. HEI total scores in our sample were consistent with scores in other samples of pregnant women [34,35].

The Home Environment: Trained research staff assessed the quality of the child's home environment using The Home Observation for Measurement of the Environment (HOME) scale [36]. The HOME scale is comprised of both an observation of the family's home environment and a semi-structured interview with the mother.

Research staff used a binary (yes/no) scale to indicate the *presence* of stimulating factors in the home environment and adaptive maternal parenting behaviors, as well as an *absence* of harmful factors. Stimulating factors include the presence and variety of educational toys, games and books (e.g., child has: 3 or more puzzles, toys that help them learn animal names, toy or real musical instruments) and adaptive parenting behaviors (e.g., parent converses with child, reads stories, sets routines) [37]. The absence of harmful factors is also measured (e.g., house and surrounding environment are free of hazards, parent does not yell or scold child during the visit) [37]. This assessment was carried out when children were 3-4 years of age and in the home in which the child spends the most time. Administration of this scale takes approximately 1 hour. Total HOME scale scores were calculated and higher scores (maximum of 55 points) indicate more adaptive parenting behaviors and a more stimulating home environment [37].

Maternal Depression: Symptoms of maternal depression were assessed when offspring were 3 years of age using the 10-item Center for Epidemiological Studies Depression (CES-D) scale. Mothers rated depressive symptoms experienced during the previous week using a four-point scale (1= “rarely” to 4=“all of the time”) with higher scores representing more depression symptoms [38].

Education: Maternal education (high school or less vs. post-secondary education or greater) was used to as a measure of maternal cognitive function since education is considered the most efficient substitute of maternal IQ [39].

Prenatal Smoking: Self-reported maternal smoking behavior was captured at the third prenatal visit categorically: never smoked, former smoker, smoke currently/quit in pregnancy.

Statistical Analysis

Descriptive statistics: Between-group comparisons (normal vs. overweight/obese BMI and normal glucose vs. hyperglycemia) were examined using independent samples t-tests and chi-squared tests for continuous and categorical variables respectively.

Unadjusted associations: A series of linear regression models were used to examine the links between each of our predictors (Overweight/Obesity and hyperglycemia) and outcome variables (Verbal IQ, Performance IQ, Full-Scale IQ, Internalizing Behavior, Externalizing Behavior).

Adjusted associations: Multiple linear regression models were used to examine the same associations observed in our unadjusted models, but while adjusting for each of our confounding variables (BMI was also added as a confounder in the hyperglycemia model). The variance inflation factors in the adjusted models were below 10, therefore no evidence for multi-collinearly was detected.

Adjusted associations--post-hoc assessment of confounding variables: Finally, a post-hoc analysis of the semi-partial correlations in the adjusted models was completed to determine which variables accounted for the most unique variance in our outcomes.

For all results, unstandardized regression coefficients (B) values and 95% confidence intervals (CI) are presented, given that these B values display the change in the outcome per one unit change in the predictor. Statistical tests were 2-tailed with

significance set at $\alpha=0.05$ and carried out in SPSS v23.

Sensitivity Analysis

The interaction between BMI and hyperglycemia was assessed using linear regression in order to determine whether having both hyperglycemia and overweight BMI was linked to greater offspring problems.

Missing Data

Both complete case and imputed data for all variables were examined using the fully conditional specification multiple imputation method in SPSS 23. We report only the results for subjects with complete predictor and outcomes data since there were no significant differences between complete and imputed data. A comparison of characteristics between women with and without BMI and hyperglycemia data is presented in Supplemental Table S1 (online).

Results

Descriptive statistics: Mothers were on average 32.80 (*SD*=4.81) years of age at enrollment and had an average pre-pregnancy BMI of 25.10 (*SD*=5.63) (Table 1). At enrollment, 37% of women were overweight, and 8% had hyperglycemia. Of the women that had data for both BMI and hyperglycemia (n=531), 26 (12.5%) of the overweight women, and 13 (4%) of the normal weight women were hyperglycemic. Fifteen (2.80%) women of the 531 women were diagnosed with GDM and 32 (6.03%) had IGT. The average child full-scale IQ in the sample was 106.90 (*SD*=13.48). The mothers of children that participated in the MIREC-CD plus sub-study were not different from mothers that participated only in the original MIREC cohort on each of our predictors and

covariates (however, MIREC-CD plus mothers were more likely to have pursued education beyond high school).

Table 1: Demographic information of the MIREC-CD Plus Sample

	Body Mass Index			Hyperglycemia		
Demographic Variables[a]	Normal (n=464)	Overweight/ Obese (n=273)	*p*	Normal Glucose (n=547)	GDM/IGT (n=47)	*p*
Maternal education (n,%)						
High School or less	37 (8.0)	35 (12.8)	0.03	60 (11)	3 (6.4)	0.33
College Educated/ University Degree	425 (92.0)	238 (87.2)		486 (89.0)	44 (93.6)	
Marital Status (n,%)						
Married/Common law	448 (96.5)	260 (95.2)	0.75	527 (96.4)	46 (97.8)	0.97
Divorced/Separated	3 (0.6)	1 (0.4)		4 (0.7)	0	
Single	13 (2.8)	12 (4.4)		16 (2.9)	1 (2.2)	
Maternal age (M. SD)	32.9 (4.8)	32.9 (4.8)	0.96	32.7 (4.8)	33.7 (4.5)	0.17
Household Income (n,%)						
Less than 50 000	51 (11)	46 (16.8)	<0.01	78 (14.3)	10 (21.3)	0.12
50 001-100 000	183 (39.4)	133 (48.7)		226 (41.3)	23(48.9)	
Greater than 100 000	230 (49.6)	94 (34.4)		243 (44.4)	14 (29.8)	
Smoking (n,%)						
Never	293 (63.1)	185(67.8)	0.44	349 (63.8)	31 (66.0)	0.79
Former	131 (28.2)	68 (24.9)		147 (26.9)	13 (27.7)	
Current/Quit in pregnancy	40 (8.6)	20 (7.3)		51 (9.3)	3 (6.4)	
Birth Country (n,%)						
Canada	370 (79.7)	237 (86.8)	0.02	445 (81.4)	37 (78.7)	0.66
Elsewhere	94 (20.3)	36 (13.2)		102 (18.6)	10 (21.3)	
HOME total (M,SD)	48.0 (3.7)	46.7 (4.9)	0.001	47.4(4.3)	46.0 (4.42)	0.09
Maternal depression (M,SD)	5.0 (3.9)	6.2 (4.5)	0.001	5.4 (4.2)	7.9(5.7)	0.01
Maternal diet quality (M,SD)[b]	73.5 (7.4)	70.4(8.4)	<0.01	71.9 (8.0)	69.7 (7.5)	0.07
Breastfeeding (M, SD)	5.6 (2.0)	5.3 (2.1)	0.14	5.4 (2.2)	4.9 (2.9)	0.27
Presence of hyperglycemia (n,%)						
GDM	4 (1.2)	17 (6.2)	<0.01		15 (32)	
IGT	9 (1.9)	9 (3.3)			32 (68)	
Infant Sex (n,%)						
Male	220 (47.4)	147 (53.8)	0.09	279 (51)	24 (51.1)	0.99

Female	244 (52.6)	126 (46.2)		268 (49)	23 (48.9)	
Gestational Age (M,SD)	39.1 (1.53)	38.7 (1.84)	<0.01	38.9(1.7)	38.2 (1.7)	<0.01
Birth Weight (g) (M,SD)	3419.2 (489.2)	3486.8 (579.4)	0.11	3438.9 (531.4)	3355.9 (608.1)	0.31

[a] Any discrepancies in n for demographic variables are due to missing data (e.g., two women in the normal BMI category did not have information on education)
[b]Diet quality scores between 60 and 79.99 are considered 'average' in pregnant women [34]
M=Mean
SD=Standard deviation

Unadjusted associations: In unadjusted statistical models containing BMI as a predictor, a per unit increase in maternal pre-pregnancy BMI was associated with significantly lower verbal (BMI_{cont} B=-0.30, 95% CI [-0.48;-0.11]) and full-scale IQ scores (BMI_{cont} B=-0.24, 95% CI [-0.44;-0.05]) (Table 2). Maternal BMI in the overweight/obese category was associated with significantly lower verbal BMI_{Di} B=-3.41, 95% CI [-5.69;-1.12]) and full-scale IQ scores BMI_{Di} B=-3.21, 95% CI [-5.21;-0.88]) compared to women with a normal pregnancy BMI. Maternal BMI was also a risk factor of increased levels of externalizing problems (BMI_{cont} B=0.10, 95% CI [0.001;0.21], BMI_{Di} B=1.87, 95% CI [0.72;1.72]), but not performance IQ or internalizing difficulties (Table 3).

Table 2: Unadjusted and fully adjusted associations between maternal metabolic risk factors and cognitive outcomes (WPPSI-III) in children at 3-4 years of age

Predictor β, (95% CI)	Verbal IQ		Performance IQ		Full-Scale IQ	
	Unadjusted[1]	Adjusted[2]	Unadjusted	Adjusted	Unadjusted	Adjusted
BMI $_{continuous}$	-0.30** (-0.48;-0.11)	-0.16 (-0.35;0.03)	-0.11 (-0.32; 0.10)	-0.13 (-0.23;0.21)	-0.24* (-0.44;-0.05)	-0.12 (-0.31;0.07)
BMI $_{dichotomous}$[3]	-3.41** (-5.69;-1.12)	-1.70 (-4.01;0.61)	-1.79 (-4.4; 0.78)	-0.43 (-3.11;2.24)	-3.21** (-5.21;-0.88)	-1.48 (-3.81;0.85)
Hyperglycemia [4]	-5.17** (-8.67;-1.67)	-2.09 (-6.15;1.96)	-0.70 (-4.47; 3.14)	2.29 (-1.82;7.20)	-3.48* (-7.0; 0.00)	-0.34 (-3.63;4.32)

[1] Unadjusted associations
[2] Adjusted for gestational diet quality, home environment, maternal depression, prenatal smoking and maternal education, (and BMI in the hyperglycemia models)

[3] Normal (18.5-24.9 kg/m^2) vs. Overweight or obese (≥ 25kg/m^2)
[4] GDM and IGT vs. No-GDM
* p<0.05
** p<0.01

In the unadjusted hyperglycemia models, GDM/IGT was associated with reduced verbal (B=-5.17, 95% CI [-8.67;-1.67]) and full-scale IQ scores (B=-3.48, 95% CI [-7.00; -0.01]), but not performance IQ, or behavior problems (Tables 2 and 3). A sensitivity analysis examining the interaction between maternal BMI and hyperglycemia was tested but was not statistically significant. Finally, there were no significant differences in our results when stratifying by offspring sex.

Table 3: Unadjusted and fully adjusted associations between maternal metabolic risk factors and behavioral outcomes (BASC-II) in children at 3-4 years of age

Predictor B, 95% CI	Externalizing Unadjusted[1]	Adjusted[2]	Internalizing Unadjusted	Adjusted
BMI $_{continuous}$	0.10*	0.01	-0.02	-0.04
	(0.001; 0.21)	(-0.11; 0.13)	(-0.12; 0.09)	(-0.16; 0.08)
BMI $_{dichotomous}$[3]	1.87**	0.54	0.43	0.08
	(0.72; 3.02)	(-0.90; 1.98)	(-0.79; 1.66)	(-1.40; 1.56)
Hyperglycemia[4]	-0.09	-1.03	0.27	-0.19
	(-1.90; 1.72)	(-3.56; 1.50)	(-1.67; 2.21)	(-2.73;2.30)

[1] Unadjusted associations
[2] Adjusted for gestational diet quality, home environment, maternal depression, maternal education, prenatal smoking (and BMI in the hyperglycemia models)
[3] Normal (18.5-24.9 kg/m^2) vs. Overweight or obese (≥ 25kg/m^2)
[4] GDM and IGT vs No-GDM
* p<0.05
** p<0.01

Adjusted associations: Following adjustment for confounding variables, no significant associations persisted between our predictors and outcomes.

Adjusted associations- post-hoc assessment of confounding variables: Semi-partial correlations (r_{part}) were used to examine the variables that remained significant in the fully adjusted models. Significant variance in verbal IQ was accounted for by the home environment (r_{part}=0.28, p<0.001) and prenatal diet (r_{part}=0.12, p<0.01). A

significant amount of variance in full-scale IQ was also accounted for by the home environment (r_{part}=0.31, p<0.01) and prenatal diet (r_{part}=0.08, p<0.05). The home environment (r_{part}=-0.20, p<0.01) and symptoms of maternal depression (r_{part}=0.18, p<0.01) accounted for variance in externalizing problems (Table 4).

Table 4: Confounding effect of variables in the fully adjusted BMI model

BMI Model	WPPSI-II				BASC-II	
	Verbal IQ B (95% CI)	r_{part}[a]	Full-Scale IQ B (95% CI)	r_{part}	Externalizing B (95% CI)	r_{part}
HOME[b]	0.91** (0.65;1.18)	0.28	1.02** (0.75;1.29)	0.31	-0.40** (-0.57;-0.24)	-0.20
Depression symptoms	0.06 (-0.21;0.33)	0.02	0.14 (-0.13;0.41)	0.04	0.37** (1.98;0.56)	0.18
Education	2.43 (-1.54;6.40)	0.05	5.04** (1.02;9.06)	0.10	0.77 (-1.73;3.28)	0.03
Smoking	-0.70 (-2.46;1.06)	-0.03	-0.49 (-2.27;1.29)	-0.02	0.69 (-0.39;1.79)	0.05
Maternal Diet Quality[c]	0.20** (0.06;0.35)	0.12	0.14* (0.01;0.28)	0.08	0.05 (-0.04;0.14)	0.05

[a] Semi-Partial Correlation: The amount of unique variance each variable accounts for in the fully adjusted models
[b] Home Observation for Measurement of the Environment Scale
[c] Healthy Eating Index 2010
* p<0.05
** p<0.01

In the fully adjusted hyperglycemia model, the home environment accounted for significant variance in verbal IQ (r_{part}=0.34, p<0.01) and full-scale IQ (r_{part}=0.38 p<0.01). Maternal prenatal diet also accounted for variance in verbal (r_{part}=0.17, p<0.01) and full-scale IQ (r_{part}=0.11, p<0.05) (Table 5).

Table 5: Confounding effect of variables in the fully adjusted hyperglycemia model

Hyperglycemia Model	WPPSI-III				BASC-II	
	Verbal IQ B (95% CI)	r_{part}	Full-Scale IQ B (95% CI)	r_{part}	Externalizing B (95% CI)	r_{part}
BMI[a]	-0.001	-0.001	-0.004	-0.002	0.05	0.03

	(-0.22;0.22)		(-0.22;0.21)		(-0.91;0.18)	
HOME[b]	1.10**	0.34	1.2**	0.38	-0.46**	-0.23
	(0.79;1.4)		(0.92;1.54)		(-0.65;-0.26)	
Depression	0.09	0.03	0.19	0.06	0.35**	0.17
symptoms	(-0.23;0.4)		(-0.12;0.49)		(0.15;0.55)	
Education	0.33	0.01	4.07	0.08	0.79	0.03
	(-4.37;5.02)		(-0.53;8.68)		(-2.20;3.77)	
Smoking	-0.96	-0.05	0.44	-0.02	0.73	0.06
	(-3.0;1.08)		(-2.4;1.55)		(-0.53;1.99)	
Maternal Diet	0.28**	0.17	0.18*	0.11	0.05	0.05
Quality[c]	(0.12;0.44)		(0.03;0.34)		(-0.05;0.15)	

[a] Continuous body mass index
[b] Home Observation for Measurement of the Environment Scale
[c] Healthy Eating Index 2010
* $p<0.05$

Although not selected a-priori as confounding variables, maternal income, birth country and infant gestational age differed significantly between BMI groups (Table 1). Therefore, we ran additional analyses with these variables added as confounders to our adjusted models and results were unchanged (diet and home environment still accounted for the most unique variance in verbal and full scale IQ and home environment and maternal depression accounted for unique variance in offspring externalizing problems).

Discussion

In a large sample of children, prenatal exposure to maternal adiposity and hyperglycemia was associated with lower verbal and full-scale IQ scores at 3-4 years of age. Exposure to maternal adiposity was also associated with more externalizing problems in offspring. However, after adjustment for confounding variables, these associations were no longer statistically significant. Associations between prenatal maternal metabolic problems and offspring cognitive outcomes appeared to be due to confounding variables, particularly the home environment, maternal depression symptoms and overall maternal diet quality reported during pregnancy.

Our primary objective was to examine the associations between maternal metabolic complications and offspring neurodevelopment, and our unadjusted findings are consistent with the majority of studies that have observed these links. Numerous studies have observed poorer cognitive performance in children born to obese women [9–11,16,40–45], and in children exposed to prenatal hyperglycemia [14,46–48]. These problems are generally in the language domain [9,14,16,41,46]. Elevated levels of behavior problems, particularly externalizing problems, have also been observed in offspring as young as two [49–52]. However, since most of these studies were not designed to specifically examine links between maternal metabolic complications and offspring neurodevelopment, many fail to contain data on key confounding variables. Indeed, to our knowledge, no studies have considered the confounding effect of prenatal diet quality and just 5 have objectively assessed stimulation of the home environment [11,41–43,53].

A novel finding of this study is that total maternal diet quality during pregnancy remained significant in both our adjusted adiposity and hyperglycemia models for verbal and full-scale IQ, suggesting that overall prenatal diet quality is an important confounding variable in associations between excess adiposity/hyperglycemia in mothers and offspring cognitive problems.

Maternal diet is a potentially modifiable factor known to play a significant role in fetal brain development [54,55]. Indeed, prenatal exposure to a nutrient-dense diet (high in fruits, vegetables, and seafood) has been linked to higher offspring IQ [56] and may be particularly important for the development of verbal IQ in children [57]. Conversely,

consumption of a low nutrient diet, high in processed, energy-dense foods has been linked to reduced cognitive function in 8 year old offspring [57], as well as a 3-point decrease in IQ relative to children whose mothers consumed a healthier diet [56]. Experimental studies using non-human animal models have noted that prenatal exposure to high-fat, high-sugar diets results in worse performance on tasks assessing learning and memory, reduced levels of brain derived neurotropic factor in the hippocampus and altered expression of genes involved in hippocampal synaptic plasticity [58].

Observational studies have also noted associations between maternal diet and behavioral problems in offspring [59,60]; however, in the current study, diet was not significant in our models predicting child behavior. This could be due to the age at which behavior problems were assessed, maternal reporting bias, or a true lack of effect.

Women of lower socioeconomic status (SES) are more likely to consume an unhealthy diet [e.g., 61]. Therefore, child cognitive development may be more of a reflection of the family SES rather than prenatal diet quality. However, by including both prenatal diet quality and the assessment of the child's home environment together in each adjusted model, we observed that both variables accounted for significant unique variance in offspring cognitive function. Additionally, we did not find any evidence of multicollinearity between these two variables. Therefore, despite the greater amount of variance accounted for by the home environment relative to prenatal diet, our results indicate that these are disparate constructs and that prenatal diet also has an impact on offspring cognitive function.

In each of the adjusted models, the home environment accounted for the most

variance in offspring cognitive function and offspring behavior, and maternal depression accounted for significant unique variance in offspring behavior. It is well documented that the home environment[62,63] and maternal depression[64] play a significant role in child development, therefore, it is not surprising that these variables accounted for a significant amount of variance in our models.

Despite the potential importance of these findings, this study must be examined in the context of the following limitations. First, since some MIREC centers only screened participants at high risk for GDM, of the 808 subjects participating in MIREC-CD Plus, 214 were missing hyperglycemia data which may partially explain why our prevalence of hyperglycemia lower than other studies. Second, women in our sample had higher levels of education and income relative to the Canadian population [65], which needs to be taken into account when generalizing to other populations. Additionally, a greater proportion of women of low income were missing information on BMI (Supplementary Table 1). Since it is well understood that higher BMI in pregnancy is linked to lower SES, this may have contributed to the slightly lower prevalence of overweight women observed in our sample relative to that seen in other studies. Third, we were unable to adjust for maternal IQ; however, we did measure maternal education and evidence suggests that HOME scale scores at least partially mediate associations between maternal and offspring IQ [62]. Fourth, while the FFQ we used covered a wide range of foods commonly consumed by Canadians, it was comprised of only 46 items. This may have limited the scope of our diet assessment; however, other studies calculated the HEI using an FFQ with a similar number of items[66] and previous research suggests that short

FFQs are valid for assessing overall diet quality [67]. Future studies could extend upon these findings by using other diet quality indices (e.g., alternative health eating index for pregnancy). We also did not consider intake of multivitamins or other dietary supplements. Fifth, reports of maternal BMI and child behavior problems were made only by mothers and therefore could be affected by response bias. Sixth, despite only occurring in a small percentage of the population, potential prenatal confounding factors that lead to neonatal hospitalizations, such as failure to thrive, could be adjusted for in future studies. Additionally, we excluded women with a fetus with any known abnormalities or malformations and future studies could also consider these factors. Finally, we were unable to adjust for child diet; however, the home environment has been associated with access to healthy food and healthy eating behaviors in children [68].

Future studies should aim to extend follow-ups of offspring born to women that participated in prenatal healthy lifestyle randomized controlled trials (e.g., the Maternal Obesity Management Trial[69]) to elucidate potential causal associations between prenatal maternal diet and offspring cognition[70,71]. This is important since factors that optimize cognitive function may have a significant impact at the population level. Since a 1-point increase in the national average IQ is associated with a 0.11% increase in gross domestic product per person annually [72], optimizing cognitive development represents a tremendous opportunity to improve the health and success of the population.

Numerous studies have reported on associations between prenatal maternal metabolic complications and offspring neurodevelopmental problems. However, none of these studies have examined the role prenatal maternal diet quality might play in these

links. We show for the first time that prenatal diet quality is a significant confounder of these associations, even in models that also adjusted for traditional risk factors for adverse cognitive development (e.g., maternal depression and the home environment). Due to the preventative potential of early life interventions, assessing the mechanisms that underlie links between maternal prenatal metabolic complications and offspring neurodevelopment is of particular interest; therefore, future studies must adjust for prenatal diet quality in order to fully elucidate the mechanisms underlying these links.

References

[1] I. Guelinckx, R. Devlieger, K. Beckers, G. Vansant, Maternal obesity: Pregnancy complications, gestational weight gain and nutrition, Obes. Rev. 9 (2008) 140–150.

[2] E. Ashwal, M. Hod, Gestational diabetes mellitus: Where are we now?, Clin. Chim. Acta. 451 (2015) 14–20. doi:10.1016/j.cca.2015.01.021.

[3] IDF Diabetes Atlas, 7th Edition. 2015. doi:10.1289/image.ehp.v119.i03.

[4] W. Gao, W. Lin, K. Grewen, J.H. Gilmore, Functional Connectivity of the Infant Human Brain: Plastic and Modifiable., Neuroscientist. (2016) doi:10.1177/1073858416635986.

[5] G.Z. Tau, B.S. Peterson, Normal development of brain circuits., Neuropsychopharmacology. 35 (2010) 147–168. doi:10.1038/npp.2009.115.

[6] A. Bharadwaz, G. Madhab, Review Article Burden of Maternal Obesity on Congenital Anomalies : Implications and Future Trend, Ann. Women Child Heal. 2 (2016) 1–8.

[7] A. Ornoy, E.A. Reece, G. Pavlinkova, C. Kappen, R.K. Miller, Effect of maternal diabetes on the embryo, fetus, and children: congenital anomalies, genetic and epigenetic changes and developmental outcomes., Birth Defects Res. C. Embryo Today. 105 (2015) 53–72. doi:10.1002/bdrc.21090.

[8] E. Villamor, K. Tedroff, M. Peterson, S. Johansson, M. Neovius, G. Petersson, S. Cnattingius, Association Between Maternal Body Mass Index in Early Pregnancy and Incidence of Cerebral Palsy., Jama. 317 (2017) 925–936.

doi:10.1001/jama.2017.0945.

[9] L. Huang, X. Yu, S. Keim, L. Li, L. Zhang, J. Zhang, Maternal prepregnancy obesity and child neurodevelopment in the Collaborative Perinatal Project., Int. J. Epidemiol. 43 (2014) 783–92. doi:10.1093/ije/dyu030.

[10] E. Basatemur, J. Gardiner, C. Williams, E. Melhuish, J. Barnes, A. Sutcliffe, Maternal prepregnancy BMI and child cognition: A longitudinal cohort study, Pediatrics. 131 (2013) 56–63. doi:10.1542/peds.2012-0788.

[11] W.Y. Craig, G.E. Palomaki, L.M. Neveux, Maternal body mass index during pregnancy and offspring neurocognitive development, Obstet. Med. (2013) 20–25.

[12] A. Rodriguez, J. Miettunen, T.B. Henriksen, J. Olsen, C. Obel, a Taanila, H. Ebeling, K.M. Linnet, I. Moilanen, M.-R. Järvelin, Maternal adiposity prior to pregnancy is associated with ADHD symptoms in offspring: evidence from three prospective pregnancy cohorts., Int. J. Obes. (Lond). 32 (2008) 550–7. doi:10.1038/sj.ijo.0803741.

[13] A. Rodriguez, Maternal pre-pregnancy obesity and risk for inattention and negative emotionality in children, J. Child Psychol. Psychiatry Allied Discip. 51 (2010) 134–143. doi:10.1111/j.1469-7610.2009.02133.x.

[14] G. Dionne, M. Boivin, J.R. Séguin, D. Pérusse, R.E. Tremblay, Gestational diabetes hinders language development in offspring., Pediatrics. 122 (2008) e1073-9. doi:10.1542/peds.2007-3028.

[15] R. Perna, A.R. Loughan, J. Le, K. Tyson, W.R. Hospital, Gestational Diabetes :

Long-Term Central Nervous System Developmental and Cognitive Sequelae, HAPC. 4 (2015) 217–220. doi:10.1080/21622965.2013.874951.

[16] M. Casas, L. Chatzi, A.E. Carsin, P. Amiano, M. Guxens, M. Kogevinas, K. Koutra, N. Lertxundi, M. Murcia, M. Rebagliato, I. Riaño, C.L. Rodríguez-Bernal, T. Roumeliotaki, J. Sunyer, M. Mendez, M. Vrijheid, Maternal pre-pregnancy overweight and obesity, and child neuropsychological development: Two southern european birth cohort studies, Int. J. Epidemiol. 42 (2013) 506–517. doi:10.1093/ije/dyt002.

[17] M. Robinson, S.R. Zubrick, C.E. Pennell, R.J. Van Lieshout, P. Jacoby, L.J. Beilin, T. a. Mori, F.J. Stanley, J.P. Newnham, W.H. Oddy, Pre-pregnancy maternal overweight and obesity increase the risk for affective disorders in offspring, J. Dev. Orig. Health Dis. 4 (2012) 1–7. doi:10.1017/S2040174412000578.

[18] V. Daraki, T. Roumeliotaki, K. Koutra, V. Georgiou, M. Kampouri, A. Kyriklaki, M. Vafeiadi, S. Papavasiliou, Effect of parental obesity and gestational diabetes on child neuropsychological and behavioral development at 4 years of age : the Rhea mother – child cohort , Crete , Greece, Eur. Child Adolesc. Psychiatry. (2017). doi:10.1007/s00787-016-0934-2.

[19] S.H. Mikkelsen, L. Hohwü, J. Olsen, B.H. Bech, Z. Liew, Original Contribution Parental Body Mass Index and Behavioral Problems in Their Offspring : A Danish National Birth Cohort Study, (2017) 1–10. doi:10.1093/aje/kwx063.

[20] M. Brion, M. Zeegers, V. Jaddoe, F. Verhulst, D.A. Lawlor, G.D. Smith, Maternal

Pre-pregnancy Overweight and Child Cognition and Behavior: Exploring Intrauterine Effects in Two Pregnancy Cohorts, Pediatrics. 127 (2011) 1–18. doi:10.1542/peds.2010-0651.Maternal.

[21] M. Bliddal, J. Olsen, H. Støvring, H.L.F. Eriksen, U.S. Kesmodel, T.I.A. Sørensen, E.A. Nøhr, Maternal pre-pregnancy BMI and intelligence quotient (IQ) in 5-year-old children: A cohort based study, PLoS One. 9 (2014) 3–9. doi:10.1371/journal.pone.0094498.

[22] P. Surén, N. Gunnes, C. Roth, M. Bresnahan, M. Hornig, D. Hirtz, K.K. Lie, W.I. Lipkin, P. Magnus, T. Reichborn-Kjennerud, S. Schjølberg, E. Susser, A.-S. Oyen, G.D. Smith, C. Stoltenberg, Parental obesity and risk of autism spectrum disorder., Pediatrics. 133 (2014) e1128-38. doi:10.1542/peds.2013-3664.

[23] E.E. Antoniou, T. Fowler, K. Reed, T.R. Southwood, J.P. McCleery, M.P. Zeegers, Maternal pre-pregnancy weight and externalising behaviour problems in preschool children: A UK-based twin study, BMJ Open. 4 (2014) no pagination. doi:10.1136/bmjopen-2014-005974.

[24] A. Fraser, C. Almqvist, H. Larsson, N. Långström, D.A. Lawlor, Maternal diabetes in pregnancy and offspring cognitive ability: Sibling study with 723,775 men from 579,857 families, Diabetologia. 57 (2014) 102–109. doi:10.1007/s00125-013-3065-z.

[25] Q. Chen, A. Sjolander, N. Langstrom, A. Rodriguez, E. Serlachius, B.M. D'Onofrio, P. Lichtenstein, H. Larsson, Maternal pre-pregnancy body mass index

and offspring attention deficit hyperactivity disorder: A population-based cohort study using a sibling-comparison design, Int. J. Epidemiol. 43 (2014) 83–90. doi:10.1093/ije/dyt152.

[26] T.E. Arbuckle, W.D. Fraser, M. Fisher, K. Davis, C.L. Liang, N. Lupien, S… , E. Ouellet, Cohort profile: The maternal-infant research on environmental chemicals research platform, Paediatr. Perinat. Epidemiol. 27 (2013) 415–425. doi:10.1111/ppe.12061.

[27] World Health Organization, WHO: Global Database on Body Mass Index, World Heal. Organ. (2012). doi:papers3://publication/uuid/DF45B6E6-94B7-4B8A-9775-3A1313BA45EC.

[28] R.J. Van Lieshout, M. Robinson, M.H. Boyle, Maternal pre-pregnancy body mass index and internalizing and externalizing problems in offspring, Can. J. Psychiatry. 58 (2013) 151–159.

[29] C. Buss, S. Entringer, E.P. Davis, C.J. Hobel, J.M. Swanson, P.D. Wadhwa, C.A. Sandman, Impaired executive function mediates the association between maternal pre-pregnancy body mass index and child ADHD symptoms, PLoS One. 7 (2012). doi:10.1371/journal.pone.0037758.

[30] B. Metzger, L. Lowe, A. Dyer, E. Trimble, U. Chaovarindr, D. Coustan, Hyperglycemia and Adverse Pregnancy Outcomes, N. Engl. J. Med. 358 (2008) 1991–2002.

[31] A. Morisset, H.A. Weiler, L. Dubois, J. Ashley-martin, G.D. Shapiro, L. Dodds, I.

Massarelli, M. Vigneault, T.E. Arbuckle, W.D. Fraser, Rankings of iron, vitamin D , and calcium intakes in relation to maternal characteristics of pregnant Canadian women, Appl. Physiol. Nutr. Metab. 757 (2016) 749–757.

[32] Overview & Background of The Healthy Eating Index. NIH National Cancer Institute. Division of Cancer Control and Population Sciences, 2015 (accessed 10 January 2018) https://epi.grants.cancer.gov/hei/

[33] P.M. Guenther, K.O. Casavale, J. Reedy, S.I. Kirkpatrick, H. A. B. Hiza, K.J. Kuczynski, L.L. Kahle, S.M. Krebs-Smith, Update of the Healthy Eating Index: HEI-2010, J. Acad. Nutr. Diet. 113 (2013) 569–580. doi:10.1016/j.jand.2012.12.016.

[34] M. Tsigga, V. Filis, K. Hatzopoulou, C. Kotzamanidis, M.G. Grammatikopoulou, Healthy Eating Index during pregnancy according to pre-gravid and gravid weight status., Public Health Nutr. 14 (2011) 290–6. doi:10.1017/S1368980010001989.

[35] M.E. Pick, M. Edwards, D. Moreau, E.A. Ryan, Assessment of diet quality in pregnant women using the Healthy Eating Index, J. Am. Diet. Assoc. 105 (2005) 240–246. doi:10.1016/j.jada.2004.11.028.

[36] B.M. Caldwell R.H. Bradley, HOME inventory and administration manual, 2001 University of Arkansas for Medical Sciences and University of Arkansas at Little Rock.

[37] V. Totsika, K. Sylva, The Home Observation for Measurement of the Environment

revisited., Child Adolesc. Ment. Health. 9 (2009) 25–35. doi:10.1046/j.1475-357X.2003.00073.x.

[38] K.L. Smarr, A.L. Keefer, Measures of depression and depressive symptoms: Beck Depression Inventory-II (BDI-II), Center for Epidemiologic Studies Depression Scale (CES-D), Geriatric Depression Scale (GDS), Hospital Anxiety and Depression Scale (HADS), and Patient Health Questionna, Arthritis Care Res. 63 (2011) 454–466. doi:10.1002/acr.20556.

[39] J.D. Matarazzo, D.O. Herman, Relationship of education and IQ in the WAIS—R standardization sample., J. Consult. Clin. Psychol. 52 (1984) 631–634. doi:http://dx.doi.org/10.1037/0022-006X.52.4.631.

[40] U. Heikura, A. Taanila, A.L. Hartikainen, P. Olsen, S.L. Linna, L. Von Wendt, M.R. Jorvelin, Variations in prenatal sociodemographic factors associated with intellectual disability: A study of the 20-year interval between two birth cohorts in Northern Finland, Am. J. Epidemiol. 167 (2008) 169–177. doi:10.1093/aje/kwm291.

[41] R. Tanda, P.J. Salsberry, P.B. Reagan, M. Fang, The impact of prepregnancy obesity on children's cognitive test scores, Matern. Child Heal. J. 17 (2013) 222–229. doi:10.1038/nature13314.A.

[42] S.J. Pugh, G.A. Richardson, J.A. Hutcheon, K.P. Himes, M.M. Brooks, N.L. Day, L.M. Bodnar, Maternal Obesity and Excessive Gestational Weight Gain Are Associated with Components of Child Cognition, J. Nutr. (2015) 1–8.

doi:10.3945/jn.115.215525.

[43] Y.H. Neggers, R.L. Goldenberg, S.L. Ramey, S.P. Cliver, Maternal prepregnancy body mass index and psychomotor development in children, Acta Obstet. Gynecol. Scand. 82 (2003) 235–240. doi:10.1034/j.1600-0412.2003.00090.x.

[44] K.O. Duffany, K.H. McVeigh, T.S. Kershaw, H.S. Lipkind, J.R. Ickovics, Maternal Obesity: Risks for Developmental Delays in Early Childhood, Matern. Child Health J. 20 (2016) 219–230. doi:10.1007/s10995-015-1821-z.

[45] S.N. Hinkle, L.A. Schieve, A.D. Stein, D.W. Swan, U. Ramakrishnan, A.J. Sharma, Associations between maternal prepregnancy body mass index and child neurodevelopment at 2 years of age, Int. J. Obes. 36 (2012) 1312–1319. doi:10.1038/ijo.2012.143.

[46] A. Fraser, S.M. Nelson, C. MacDonald-Wallis, D.A. Lawlor, Associations of existing diabetes, gestational diabetes, and glycosuria with offspring iq and educational attainment: The avon longitudinal study of parents and children, Exp. Diabetes Res. 2012 (2012) 1–7. doi:10.1155/2012/963735.

[47] A. Ornoy, A. Wolf, N. Ratzon, C. Greenbaum, M. Dulitzky, Neurodevelopmental outcome at early school age of children born to mothers with gestational diabetes, Arch Dis Child Fetal Neonatal Ed. 81 (1999) F10-4. doi:10.1136/fn.81.1.F10.

[48] M.C. Robles, C. Campoy, L.G. Fernandez, J.M. Lopez-Pedrosa, R. Rueda, M.J. Martin, Maternal diabetes and cognitive performance in the offspring: A systematic review and meta-analysis, PLoS One. 10 (2015) 1–16.

doi:10.1371/journal.pone.0142583.

[49] R.J. Van Lieshout, Role of maternal adiposity prior to and during pregnancy in cognitive and psychiatric problems in offspring, Nutr. Rev. 71 (2013) 95–101. doi:10.1111/nure.12059.

[50] P. Krakowiak, C.K. Walker, a. a. Bremer, a. S. Baker, S. Ozonoff, R.L. Hansen, I. Hertz-Picciotto, Maternal Metabolic Conditions and Risk for Autism and Other Neurodevelopmental Disorders, Pediatrics. 129 (2012) e1121–e1128. doi:10.1542/peds.2011-2583.

[51] M. Li, M.D. Fallin, A. Riley, R. Landa, S.O. Walker, The Association of Maternal Obesity and Diabetes With Autism and Other Developmental Disabilities, Pediatrics. 137 (2016) e20152206.

[52] K.D. Getz, M.T. Anderka, M.M. Werler, S.S. Jick, Maternal Pre-pregnancy Body Mass Index and Autism Spectrum Disorder among Offspring: A Population-Based Case-Control Study, Paediatr. Perinat. Epidemiol. (2016). doi:10.1111/ppe.12306.

[53] S. Pugh, J. Hutcheon, G. Richardson, M. Brooks, K. Himes, N. Day, L. Bodnar, Gestational weight gain, prepregnancy body mass index and offspring attention-deficit hyperactivity disorder symptoms and behaviour at age 10, Bjog. (2016) 1–10. doi:10.1111/1471-0528.13909.

[54] B.M.Y. Leung, K.P. Wiens, B.J. Kaplan, Does prenatal micronutrient supplementation improve children's mental development? A systematic review., BMC Pregnancy Childbirth. 11 (2011) 12. doi:10.1186/1471-2393-11-12.

[55] A. Nyaradi, J. Li, S. Hickling, J. Foster, W.H. Oddy, The role of nutrition in children's neurocognitive development, from pregnancy through childhood., Front. Hum. Neurosci. 7 (2013) 97. doi:10.3389/fnhum.2013.00097.

[56] A.A. Freitas-Vilela, R.M. Pearson, P. Emmett, J. Heron, A.D.A.C. Smith, A. Emond, J.R. Hibbeln, M.B.T. Castro, G. Kac, Maternal dietary patterns during pregnancy and intelligence quotients in the offspring at 8 years of age: Findings from the ALSPAC cohort, Matern. Child Nutr. (2017) 1–11. doi:10.1111/mcn.12431.

[57] E.D. Barker, N. Kirkham, J. Ng, S.K.G. Jensen, Prenatal maternal depression symptoms and nutrition, and child cognitive function, Br. J. Psychiatry. 203 (2013) 417–421. doi:10.1192/bjp.bp.113.129486.

[58] K.C. Page, E.K. Jones, E.K. Anday, Maternal and postweaning high-fat diets disturb hippocampal gene expression, learning, and memory function., Am. J. Physiol. Regul. Integr. Comp. Physiol. 306 (2014) R527–R37. doi:10.1152/ajpregu.00319.2013.

[59] F.N. Jacka, E. Ystrom, A.L. Brantsaeter, E. Karevold, C. Roth, M. Haugen, H.M. Meltzer, S. Schjolberg, M. Berk, Maternal and Early Postnatal Nutrition and Mental Health of Offspring by Age 5 Years: A Prospective Cohort Study, J. Am. Acad. Child Adolesc. Psychiatry. 52 (2013) 1038–1047. doi:10.1016/j.jaac.2013.07.002.

[60] J. Steenweg-de Graaff, H. Tiemeier, R.P.M. Steegers-Theunissen, A. Hofman,

V.W. V Jaddoe, F.C. Verhulst, S.J. Roza, Maternal dietary patterns during pregnancy and child internalising and externalising problems. The Generation R Study, Clin. Nutr. 33 (2014) 115–121. doi:10.1016/j.clnu.2013.03.002.

[61] S.K. Ng, C.M. Cameron, A.P. Hills, R.J. McClure, P.A. Scuffham, Socioeconomic disparities in prepregnancy BMI and impact on maternal and neonatal outcomes and postpartum weight retention: The EFHL longitudinal birth cohort study, BMC Pregnancy Childbirth. 14 (2014) 1–15. doi:10.1186/1471-2393-14-314.

[62] L. Ronfami, L. Vecci-Brumatti, M. Mariuz, V. Tognin, M. Bin, V. Ferluga, A. Knowles, M. Montico, F. Barbone, The Complex Interaction between Home Environment , Socioeconomic Status , Maternal IQ and Early Child Neurocognitive Development : A Multivariate Analysis of Data Collected in a Newborn Cohort Study, PLoS One. (2015) 1–13. doi:10.1371/journal.pone.0127052.

[63] S. Tong, P. Baghurst, G. Vimpani, A. McMichael, Socioeconomic Position, Maternal IQ, Home Environment, and Cognitive Development, J. Pediatr. 151 (2007). doi:10.1016/j.jpeds.2007.03.020.

[64] S.H. Goodman, M.H. Rouse, A.M. Connell, M.R. Broth, C.M. Hall, D. Heyward, Maternal Depression and Child Psychopathology: A Meta-Analytic Review, Clin. Child Fam. Psychol. Rev. 14 (2011) 1–27. doi:10.1007/s10567-010-0080-1.

[65] Statistics Canada. Education and Labour (2011) https://www12.statcan.gc.ca/nhs-enm/2011/as-sa/99-014-x/99-014-x2011003_2-eng.cfm accessed on July 18th 2018.

[66] P. Clarys, T. Deliens, I. Huybrechts, P. Deriemaeker, B. Vanaelst, W. De Keyzer, M. Hebbelinck, P. Mullie, Comparison of nutritional quality of the vegan, vegetarian, semi-vegetarian, pesco-vegetarian and omnivorous diet, Nutrients. 6 (2014) 1318–1332. doi:10.3390/nu6031318.

[67] C.L. Cleghorn, R.A. Harrison, J.K. Ransley, S. Wilkinson, J. Thomas, J.E. Cade, Can a dietary quality score derived from a short-form FFQ assess dietary quality in UK adult population surveys?, Public Health Nutr. 19 (2016) 2915–2923. doi:10.1017/S1368980016001099.

[68] R.R. Rosenkranz, D.A. Dzewaltowski, Model of the home food environment pertaining to childhood obesity, 66 (2008) 123–140. doi:10.1111/j.1753-4887.2008.00017.x.

[69] K.B. Adamo, Z.M. Ferraro, G. Goldfield, E. Keely, D. Stacey, S. Hadjiyannakis, S. Jean-Philippe, M. Walker, N.J. Barrowman, The Maternal Obesity Management (MOM) Trial Protocol: A lifestyle intervention during pregnancy to minimize downstream obesity, Contemp. Clin. Trials. 35 (2013) 87–96. doi:10.1016/j.cct.2013.02.010.

[70] R.J. Van Lieshout, J.E. Krzeczkowski, Just DO(HaD) It! Testing the clinical potential of the DOHaD hypothesis to prevent mental disorders using experimental study designs, J. Dev. Orig. Health Dis. (2016) 1–9. doi:10.1017/S2040174416000441.

[71] R.C. Richmond, A. Al-Amin, G. Davey Smith, C.L. Relton, Approaches for

drawing causal inferences from epidemiological birth cohorts: A review, Early Hum. Dev. 90 (2014) 769–780. doi:10.1016/j.earlhumdev.2014.08.023.

[72] G. Jones, W.J. Schneider, Intelligence, human capital, and economic growth: a bayesian averaging of classical estimates (Bace) approach, J. Econ. Growth. (2006).

Chapter 4: Maternal Pregnancy Diet Quality Is Directly Associated with Autonomic Nervous System Function in 6-Month-Old Offspring (Study 3)

Study 3 Overview

Title: Maternal Pregnancy Diet Quality Is Directly Associated with Autonomic Nervous System Function in 6-Month-Old Offspring

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Context and implications of this study: Studies 1 and 2 of this thesis used data from two large Canadian cohort studies to show that maternal prenatal diet quality appears to play an important role in the link between gestational metabolic complications and offspring outcomes related to poor emotion regulation capacity, including early cognitive and behavioural problems. Therefore, I hypothesized that maternal prenatal diet might be involved in programming the development of neurophysiological regulatory systems known to index emotion regulatory capacity. To test this hypothesis, in Study 3, I used data from the MIREC cohort to examine the association between prenatal diet quality and offspring heart rate variability - an index of autonomic nervous system activity and a commonly assessed biomarker of regulatory capacity and later psychopathology. This study was the first to report on an association between poorer prenatal diet quality and lower heart rate variability. Therefore, exposure to a poor prenatal diet may alter

development of the autonomic nervous system and could increase risk for a host of mental disorders later in life.

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Abstract

Background: Many pregnant women are consuming diets of poor overall quality. While many studies have linked poor prenatal diet quality to an increased risk of specific diseases in offspring, it is not known if exposure to poor prenatal diet affects core neurophysiological regulatory systems in offspring known to lie upstream of multiple diseases.

Objective: To examine associations between prenatal diet quality and autonomic nervous system (ANS) function in infants at 6 months of age.

Methods: Data from 400 women (>18 years, with uncomplicated pregnancies) and their infants participating in the Maternal-Infant Research on Environmental Chemicals-Infant Development (MIREC-ID) cohort were used to investigate links between prenatal diet quality and infant ANS function at 6 months of age. Prenatal diet quality was assessed using the Healthy Eating Index (2010), calculated from a validated food frequency questionnaire completed by women during the first trimester. Infant ANS function was measured using two assessments of heart rate variability (HRV) including root mean square of successive differences [RMSSD] and standard deviation of N-N intervals [SDNN]. Associations were analyzed before and after adjustment for socioeconomic status, maternal depression symptoms, maternal cardiometabolic dysfunction and prenatal smoking.

Results: Poorer prenatal diet quality was associated with lower infant HRV assessed using RMSSD (B=0.07, 95% CI (0.01, 0.13), R^2=0.013) and SDNN (B=0.18 95% CI

(0.02, 0.35), R^2=0.011). These associations remained significant after adjustment for confounding variables (RMSSD: B=0.09, 95% CI (0.003; 0.18), semi-partial (sp^2) correlation=0.14, SDNN B=0.24 (0.0, 0.49), sp^2=0.13).

Conclusions: In a large cohort study, poorer prenatal diet quality was associated with lower offspring heart rate variability, a marker of decreased capacity of the ANS to respond adaptively to challenge. Therefore, poor prenatal diet may play a significant role in the programming of multiple organ systems and could increase general susceptibility to disease in offspring.

Introduction

Approximately half of all adults consume diets of poor overall quality (such as a Western-style diet, low in nutrients, and high in fats and sugars(1–3)) which can contribute to an increased risk for a range of non-communicable diseases (1,2). Women of childbearing age are no exception, because the majority enter pregnancy consuming a diet of suboptimal overall quality (4–6). Since organ systems rapidly develop prenatally, fetal exposure to poor prenatal diet has the potential to alter the development of these systems and increase offspring disease susceptibility(7,8).

The developmental origins of health and disease (DOHaD) hypothesis posits that prenatal and early postnatal environments affect health and disease risk throughout life (9,10). Studies in humans have consistently reported links between poor prenatal diet and increased risk of adverse offspring outcomes including obesity (11), cardiometabolic problems (12–14), and psychopathology (15,16) in children up to 6 years of age. Factors associated with a poor prenatal diet including maternal obesity, have also been linked to a host of offspring health problems in adulthood (14,17,18).

Studies in non-human animal models suggest that exposure to a diet low in nutrients and high in fats and sugars during pregnancy has detrimental effects on offspring cardiac morphology (19,20), metabolic functioning (21,22), and neurodevelopment (23–25). This research has also noted alterations in set-points within systems that coordinate hormonal, and inflammatory responses to stressors, which can affect the functioning of multiple organ systems and increase the risk of a variety of chronic diseases (23,26–28).

Despite these findings, studies in humans have focused mainly on links between exposure to poor prenatal diet quality and risk for pathology in individual organ systems or single diseases. This work has generally overlooked the co-morbid nature of many chronic diseases (e.g., cardiovascular disease and depression) as well as the impact of prenatal diet on the development of the more basic neurophysiological regulatory systems that lie upstream of *multiple* chronic diseases in offspring.

Investigating how prenatal diet quality impacts the development of physiological regulatory systems can provide a more complete understanding of the role of prenatal diet in offspring disease propensity in general and move the field closer to an understanding of the mechanisms through which poor prenatal diet may affect offspring development in humans.

The autonomic nervous system (ANS) is a core regulatory system that functions to maintain homeostasis across organ systems by coordinating adaptive responses to external environmental and internal physiological demands (29). ANS function can be assessed by measuring the variability between inter-beat intervals of the heart, or heart rate variability (HRV) (30,31). Greater HRV reflects a more flexible ANS capable of coordinating adaptive responses to a range of environmental challenges (29,32). Conversely, low HRV is suggestive of a more rigid system with a limited physiological capacity to respond to stressors (33,34).

Given its central role to homeostatic maintenance, autonomic dysfunction is linked to a host of diseases and comorbid conditions across the lifespan (35). Indeed, low HRV is

linked to poorer regulatory capacity in infants and toddlers (36,37), cardiovascular and mental health problems in children (38,39), precedes future cardiovascular events in adults (40), is directly related to disease severity (41), and is a key risk factor for all-cause mortality (35).

The development of the ANS begins early in the first trimester and autonomic innervation of organ systems progresses rapidly throughout gestation (36,42). While ANS development appears to be sensitive to intrauterine conditions associated with maternal stress [e.g., 43 for review], no studies have examined associations between overall prenatal diet quality and offspring ANS development. The few studies that have examined dietary influences and offspring HRV have examined individual nutrients in isolation (B12 (44), zinc (45), omega-3 fatty acids (46)), and have small or highly-selected samples (43,44).

Given this background, investigating whether a modifiable exposure like prenatal diet is linked to an objective measure of ANS activity in offspring is important to our understanding of the mechanisms underlying links between adverse prenatal exposures and disease risk. We set out to examine the link between overall prenatal diet quality, and HRV an important predictor of disease risk and a well-validated, objective biomarker of ANS function in a population-based cohort of Canadian mothers and their infants.

Methods

Sample: The Maternal-Infant Research on Environmental Chemicals (MIREC) study is a population-based longitudinal pregnancy cohort that recruited pregnant women

from prenatal clinics (mean gestational age at recruitment 11.99 weeks, SD=1.5) between 2008 and 2011 from across Canada (Vancouver, Edmonton, Winnipeg, Sudbury, Toronto, Hamilton, Kingston, Ottawa, Montreal and Halifax). Women were eligible to participate if they were fluent in English or French, >18 years of age, planned to deliver locally and agreed to provide a cord blood sample. Women were excluded if they were carrying a fetus with a known abnormality, had any medical complications, or reported any history of recreational drug use (47).

The current study used data from the MIREC-Infant Development (MIREC-ID) sub-study (an in-depth clinical assessment of n=400 infants) designed to follow-up and assess development in the 6-month-old infants of women that participated in the original MIREC study. MIREC infants were eligible to participate in the MIREC-ID sub-study if they were a singleton and free of congenital birth defects or neurological disorders. (for demographic comparison of MIREC vs. MIREC-ID infants, see Supplementary Table 1). Women provided written consent prior to participation and study procedures were approved by research ethics boards at Health Canada and all testing sites.

Predictor: Prenatal Diet Quality

The assessment of overall prenatal diet quality involved two steps. First, a food frequency questionnaire (FFQ) (48) asked women to report on serving sizes and frequency of foods consumed in the past month. Second, the Healthy Eating Index-2010 (HEI) (49) used information obtained from the FFQ to calculate an overall diet quality score.

i) Food Frequency Questionnaire (FFQ): A validated semi-quantitative short FFQ was used by women to report on the intake of 46 food items across 6 sub-groups (vegetables; fruits; meat, poultry, fish and alternatives; dairy products; grain products and other foods)(48). The FFQ was administered between 16 and 21 weeks gestation. Previous studies have shown that short-form FFQs are valid in assessing overall diet quality (50–52) and FFQs with a similar number of items have previously been used by the HEI to calculate total diet quality (53).

ii) Healthy Eating Index-2010 (HEI). Based on the dietary intake information obtained from the FFQ, the HEI was used to estimate total diet quality (54) (see (55) for the methods used to convert FFQ data to HEI scores). We used the HEI scoring algorithm method since it is the recommended method to use when the goal is to examine the association between HEI diet scores based on dietary data from an FFQ and 'another health outcome variable'. The HEI assesses diet quality according to national dietary guidelines and has been validated in pregnant women (54). The HEI calculates 8 "adequacy" sub-components (total fruit, whole fruit, total vegetables, greens and beans, whole grains, dairy, total protein foods, seafood and plant proteins, and fatty acids). The HEI also contains 3 sub-components comprised of foods that should be consumed in moderation (refined grains, sodium, and empty calories [from saturated fats and added sugars]). These items are reverse-scored and so higher scores indicate less consumption of these 'moderation' components. The sum of all sub-components yields an HEI total score (maximum score=100). Higher overall scores indicate greater consumption of

adequacy components and less consumption of the moderation components. The HEI total score was used in our analyses. Our HEI total scores were consistent with those from other samples of pregnant women (56,57).

Outcome: Autonomic Nervous System Function: Time Domain Heart Rate Variability

Heart rate variability (HRV) is a measure of the variability between adjacent normal R waves (NN intervals) acquired from an electrocardiogram (ECG). Seven electrodes were placed on the infant's chest at the following locations: i) right clavicle, lateral to the sternum, ii) left clavicle, lateral to the sternum, iii) left clavicle at the mid-clavicular line, iv) lower right chest wall, v) fourth intercostal space at the right sternal edge, vi) 6^{th} rib, at the left mid-clavicular line, vii) 5th intercostal space at the left axillary line. HRV was obtained during a short anthropometric assessment and throughout two validated tasks (tilt procedure (58) and arm restraint task (59), see Supplementary Table 2 for protocols) that are commonly used to invoke fluctuations in HRV. HRV was averaged across tasks to obtain a global measure of HRV reactivity. Therefore, lower HRV throughout challenges reflects maladaptive, diminished autonomic flexibility (33,60,61). Recordings were obtained using the MARS ambulatory ECG system and the ECG recording length was m=43 minutes (SD=21.3).

The two most utilized time series measures of HRV-the standard deviation of all N-N intervals (SDNN) and the square root mean of the sum of squares of differences between adjacent NN intervals (RMSSD) (62)-were used in analyses. SDNN assesses reactions to environmental stimulation and is considered a gold standard index of the

cardiovascular response to changing demands (63). RMSSD reflects the beat-to-beat variance in heart rate and is the primary time-domain measure of parasympathetically mediated vagal tone (60). All ECGs were examined for artifacts by a cardiologist. Data were excluded if >2% of the recordings had noise (n=10 excluded- No significant differences were observed on any of our demographic variables between the 390 dyads analyzed and the 10 that were excluded).

Confounding variables

Potential confounding variables were included in our adjusted model if previous empirical evidence exists linking this variable to both our predictor and our outcome.

Maternal cardiometabolic dysfunction: To assess prenatal exposure to maternal metabolic dysfunction we used pre-pregnancy body mass index (BMI) (64). Maternal pre-pregnancy BMI was calculated at the first-trimester visit by dividing self-reported pre-pregnancy weight (kg) by height (m^2) obtained from medical charts. BMI was examined categorically (Underweight: BMI =<18.5, Normal: 18.5 < BMI =< 25, Overweight: 25< BMI=<29; Obese BMI>30)(65).

Maternal Depression Symptoms: Depressive symptoms were assessed when offspring were 6 months of age using the 10-item Center for Epidemiological Studies Depression (CES-D) scale. Women rated depressive symptoms experienced during the previous week using a four-point scale (0= “rarely” to 3=“all of the time”) with higher scores representing more symptoms of depression (66).

Household income: Women self-reported their total household income before tax on a 9-point scale- 'less than $10,000' and increases in $10,000 increments to greater than $100,000 (Canadian dollars).

Prenatal Smoking: Self-reported smoking was captured at the third prenatal visit and was defined categorically: never smoked, former smoker, or smoke currently/quit in pregnancy.

Sex: Offspring sex was included as a confounder since programming effects of prenatal exposure to poor diet may differ depending on sex (67). Sex was also examined as a moderator of these links.

Recording Duration: Due to the variability in recording length, this was also adjusted for as a confounding variable.

Statistical Analyses

Differences between women participating in MIREC vs. the MIREC-ID sub-study were examined using independent sample t-tests and chi-squared tests. Two linear regression models were used to examine associations between the predictor (overall prenatal diet quality) and each outcome variable (RMSSD, SDNN). We also tested an offspring sex by prenatal diet quality interaction. Multivariable linear regression models were used to examine associations assessed in our unadjusted models with the addition of each confounding variable. Variance inflation factors (VIF) were examined in the adjusted models to check for multicollinearity. VIF values were below 10, therefore no multi-collinearly was observed between our variables. To assess the impact of a one-point

change in diet on HRV, we present unstandardized beta (B) values. Hypotheses were tested using two-tailed significance tests ($p < 0.05$). All analyses were conducted using SPSS v23.

Results

Sample Characteristics

The characteristics of the MIREC-ID study sample are presented in Table 1 (Table 1 includes information for all dyads with infant HRV data n=400, however, 10 dyads had to be excluded from further analyses due to excessive noise in the ECG trace, therefore analyses are conducted for n=390 dyads). The average age of mothers was 32 ±SD=4.7 years and 41% had an overweight/obese pre-pregnancy BMI. Average maternal prenatal diet quality (HEI) score was 72.1 ±SD=8.1, min=42.9 max=89.0; and 75.1% of women in our sample had scores in the 'average' prenatal diet quality range for pregnant women [60.0-79.9], scores >80 are considered 'adequate' (57)). The majority of women in this sample were married (n=382, 95.2%) and were college/university educated (n=384, 87.4%). Infants were born at 39.2 ±SD=1.4 weeks gestation and tested at 6.7 ±SD=0.83 months. Demographic characteristics of women participating in MIREC-ID were also similar to women participating in the nationally representative population-based Canadian Health Measures Survey (Cycle 1 2007-09) (47).

Table 1: Characteristics of MIREC-ID Sample (n=400)

Demographic Variables	Maternal/Family Characteristics		Infant RMSSD (m,SD)	Infant SDNN (m,SD)
Diet quality [m, SD][a]	72.1	(8.1)		
Age (years) [m, SD]	32	(4.7)		
Marital Status [n, (%)]				

Married/Common law	382	(95.2)	15.2 (4.8)	38.0 (15.5)
Divorced/Separated	3	(0.8)	14.5 (4.9)	35.0 (15.1)
Single	15	(3.8)	15.6 (3.7)	37.9 (9.01)
Household Income [n, (%)]				
Less than $50 000	71	(17.8)	15.6 (5.3)	40.4 (17.1)
$50 001-$100 000	186	(46.5)	15.1 (5.1)	38.8 (14.2)
Greater than $100 000	143	(35.8)	15.4 (4.3)	38.7 (14.8)
Education [n, (%)]				
High School or less	50	(12.6)	15.9 (4.9)	38.0 (13.1)
College Educated/ University Degree	348	(87.4)	15.1 (4.9)	39.2 (15.3)
Birth Country [n, (%)]				
Canada	349	(87.3)	15.2 (4.8)	38.5 (14.9)
Elsewhere	51	(12.7)	15.9 (5.4)	42.6 (14.8)
Breastfeeding [n, (%)]				
Yes	380	(96.0)	15.3 (3.7)	39.2 (15.1)
Never or only once	16	(4.0)	14.3 (4.9)	35.6 (11.9)
Smoking [n, %]				
Never	265	(66.3)	15.3 (4.9)	39.1 (15.5)
Former	102	(25.5)	15.5 (4.8)	39.4 (13.3)
Current/Quit in pregnancy	33	(8.3)	14.3 (4.2)	37.2 (15.8)
Pre-pregnancy BMI (kg/m^2) [n, (%)]				
Underweight	7	(1.9)	15.4 (5.9)	52.4 (27.5)
Normal	210	(56.9)	15.2 (4.5)	38.3 (13.5)
Overweight	86	(23.3)	15.3 (4.9)	39.1 (17.4)
Obese	66	(17.9)	15.6 (5.3)	38.9 (14.5)
Depressive symptoms (CES-D10)[b]	6.0	(4.0)		
	Infant Characteristics			
Age (months) [m, SD]	6.7	(0.83)		
Heart rate [m, SD]	140.6	(10.4)		
RMSSD [m, SD]	15.3	(4.9)		
SDNN [m, SD]	39.0	(15.0)		
Birth weight (g) [m, SD]	3497.7	(497.4)		
Gestational age (weeks) [m,SD]	39.2	(1.4)		
Infant Sex [n, (%)]				
Male	205	(51.2)		

m=Mean
SD=Standard deviation
[a]Diet quality scores between 60 and 79.99 are of 'average' quality in pregnant women, in our sample 75.1% consumed a diet of average quality
[b] Maximum possible score on the CES-D10 is 30 points. 89% of the sample had scores <=10, and scores above 10 are considered a cut-off for depression risk
Infant RMSSD or SDNN did not differ between any of our demographic variables

Analyses

In unadjusted associations, a significant positive association between poor overall prenatal diet quality and lower HRV assessed using both RMSSD and SDNN measures (Figure 1 and Table 2) was observed.

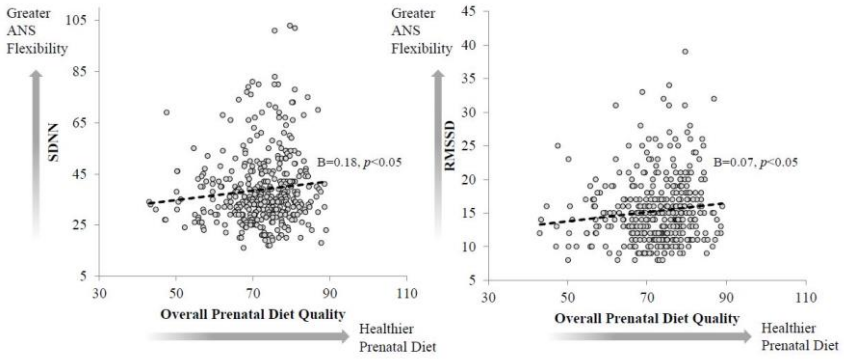

Figure 1: Unadjusted associations between overall prenatal diet quality and offspring HRV

Table 2: Unadjusted and adjusted associations between overall prenatal diet quality and offspring heart rate variability

	RMSSD		SDNN	
	B	95% CI	B	95% CI
Prenatal diet quality	0.07	(0.01; 0.13)	0.18	(0.02; 0.35)
R^2	0.013		0.011	

Associations remained significant following adjustment for confounders (Table 3). Variance accounted for by prenatal diet did not change in adjusted models. The potential presence of heteroscedasticity was tested for using the Breusch-Pagan test and White's test. Both tests were not significant (p=0.21, p=0.38), therefore there did not appear to be any violations of the homoscedasticity assumption. Finally, offspring sex did not modify these relationships.

Table 3: Adjusted associations between overall prenatal diet quality and heart rate variability at 6 months of age

	RMSSD			SDNN		
	B	95% CI	sp^2	B	95% CI	sp^2
Prenatal diet quality	0.09	[0.03; 0.18]	0.14	0.24	[0.00; 0.49]	0.13
Maternal body mass index	0.35	[-0.29; 0.98]	0.06	-0.05	[-2.07; 1.90]	-0.003
Infant age	-0.06	[-0.65; 0.53]	-0.01	0.95	[-0.88; 2.78]	0.06
Maternal Smoking	-0.30	[-0.83; 0.23]	-0.06	-1.02	[-2.6; 0.57]	-0.07
Total household income	-0.07	[-0.28; 0.15]	-0.03	-0.26	[-0.90; 0.38]	-0.06
Maternal depressive symptoms	0.14	[0.008; 0.27]	0.11	0.25	[-0.15; 0.65]	0.06
Recording duration	0.08	[0.06; 0.11]	0.34	0.31	[0.24; 0.39]	0.42
Sex	-0.13	[-1.11; 0.90]	-0.14	-0.77	[-3.73; 2.20]	-0.04
Adjusted R^2	0.14	$p<0.001$		0.18	$p<0.001$	

Sp^2: Squared semi-partial correlation- indicates the amount of unique variance accounted for by each individual IV to the total variance of the DV (rmssd and sdnn) [1]

Discussion

In this large sample of Canadian mothers and their infants, maternal prenatal diet quality was associated with lower offspring HRV even after adjustment for known confounders. To our knowledge, this study is the first to observe a link between overall prenatal diet, a common and modifiable exposure, and HRV, a well-validated objective marker of ANS function and disease risk. These findings suggest that prenatal diet may play a role in the programming of multiple organ systems in offspring.

The high rates of consumption of diets of poor quality in women of childbearing age (~60% (5)) highlight the importance of investigating its link to core physiological regulatory systems in offspring like the ANS. Despite the potentially modifiable nature of this exposure, only four studies have investigated associations between prenatal nutrition and diet exposures and offspring ANS function. Lower HRV measured during gestation was observed in the fetuses of adolescent mothers deficient in zinc in the 3rd trimester (45). This was also noted in the 5 year old children born to women low in vitamin B-12

during pregnancy from urban south India (44). Conversely, offspring of women that consumed healthier carbohydrates prenatally exhibited a trend toward higher HRV ($p=0.06$) (69), and greater HRV was noted in the infants of women supplemented prenatally with omega-3 fatty acids (46). However, the utilization of small and highly selected samples or restriction to single macro or micronutrient components limits the generalizability and clinical utility of these findings. Additionally, since nutrients have synergistic effects on development, focusing on overall prenatal diet quality could reflect the effect of multiple nutrient imbalances on offspring ANS function (70).

ANS development appears to be sensitive to intrauterine conditions. Studies examining proxies of intrauterine adversity (e.g., survivors of very low birth weight (<1500g)) have noted poorer autonomic functioning in offspring (71). Maternal prenatal mental disorders (72–77) and substance abuse (78–83) may also impact offspring HRV. However, the proportion of women with mood/anxiety disorders (affecting 12-15% of women prenatally (84,85)), substance use problems (affecting 1-14% of women prenatally (86)), and who have VLBW infants (<1% of births (87)) is much smaller than those consuming diets of poor overall quality. Additionally, the vast majority of these findings have been reported in neonates (72–75,78,80–83), and since ANS development continues throughout infancy (36) it has been unclear whether these associations persist beyond the neonatal period.

While the mechanisms by which prenatal overall diet affects offspring autonomic function are not known, they could occur through fetal alterations in the development of

central regulators of ANS activity. In non-human animal models, the periventricular nucleus of the hypothalamus, the mesocorticolimbic dopamine system (88,89), and the prefrontal cortex (90), all of which mediate autonomic cardiovascular control(32), are adversely affected in offspring exposed to high fat diets prenatally (21,90,91). Other studies have observed prenatal nutritional influences on epigenetic alterations in genes integral to ANS development. Indeed, Mash1 mRNA, was significantly reduced in the offspring of dams fed a high fat diet prenatally (25). Alterations in genes laying the foundation for ANS development and adverse development of important brain areas known to modulate cardiovascular control could reflect a reduced capacity to adaptively modulate physiological resources in response to challenges. This is consistent with work that observed associations between prenatal adversity and ANS function when measured during tasks designed to induce adaptive changes in HRV. Prenatal adversity may program differences in regulatory physiology that are more likely to be detected when the system is challenged, relative to quiet rest (76,91). Indeed, exposure to early life adversity has been linked to a blunted stress response (92) and dysregulated stress reactivity predicts a host of health problems later in life (93). As a result, measuring ANS activity during stressors (as done in the current study) may be important as this could reflect a diminished capacity of the system to respond effectively to challenges.

While the goal of the current study was to determine if a link exists between overall diet quality and offspring ANS function, specific nutrients that comprise healthier overall diets may act synergistically to promote optimal ANS development in offspring.

Zinc, iron, choline and long-chain polyunsaturated fatty acids play a significant role in DNA synthesis, energy metabolism, neurotransmitter synthesis, myelination and synaptogenesis and therefore are important components of fetal nervous system development (94). Pregnant women do not consume enough of these nutrients and physiological requirements of these nutrients increase during pregnancy (95). Consumption of an optimal healthy overall diet may decrease the likelihood of deficiencies in these important nutrients, therefore, reducing the risk of adverse ANS development.

The following limitations of this study must also be considered. First, it should be acknowledged that some participants with high diet quality had infants with low HRV and some with poor diet quality had infants with high HRV. Since it is well known that HRV can change depending on state (e.g., strong, rapid increases in negative affectivity, increased drowsiness) HRV obtained from these few participants may have been affected by state related changes prior to, or during ECG acquisition. Unfortunately, we did not obtain information on infant state prior to the ECG recordings. Additionally, unmeasured variables, such as shared genetics or prenatal exercise, may also have affected our findings for these dyads. Second, replicating this study using samples of pregnant women at elevated risk for poor diet consumption (such as those of lower SES) and their offspring would be important to determine if a positive association between diet quality and offspring HRV can also be found in these populations. Indeed, MIREC only recruited healthy pregnant women and since diets of Canadian women are generally healthier than

American women (75.1% of our sample consumed a diet of average quality) (56), this should be considered when generalizing findings to other populations. However, given the strong programming effects of prenatal diet on offspring development reported on in both experimental animal model literature and epidemiological studies in humans (e.g., 15,16,24) we hypothesize that associations between diet and offspring HRV could be even stronger in samples that include more women at risk for poor diet consumption. Third, we are unable to examine whether low offspring HRV persists beyond 6 months of age and if HRV in the infants in this study predicts later disease. Fourth, it is unclear whether offspring ANS functioning could be affected by diet specifically in pregnancy or by the woman's diet over the course of her life leading up to pregnancy. Since women are born with their lifetime complement of ova, a woman's diet across the lifespan may also play a role in offspring ANS development. Fifth, although short FFQs are valid in assessing overall diet quality (50–52), future studies could use more comprehensive measures to further investigate dietary patterns and offspring ANS functioning. Further, it should be acknowledged that FFQ-reported intakes themselves can be biased - which could have affected our HEI total scores. Finally, while our associations between prenatal diet and offspring HRV were significant, we acknowledge that they were small in magnitude. However, given that a 1% increase in heart rate variability was linked to a 1% decrease in risk for cardiovascular disease (41), we believe that our findings could have significant public health implications.

While data in humans suggest that poor prenatal diet quality is associated with an increased risk for chronic diseases and their comorbidity in offspring, these studies have tended to examine risks for individual diseases in isolation and have not investigated mechanisms that might underlie broad chronic disease risk. The data from this large cohort study suggest that a common modifiable exposure, prenatal diet quality, may adversely affect offspring ANS function. These findings suggest that for some dyads, prenatal diet could play a role in altering this core physiological regulatory system known to underlie risk for multiple chronic conditions across the lifespan. As a result, our findings could have significant public health implications and could one day inform the development of interventions aimed at improving health for both women and their infants.

References

1. Rehm CD, Peñalvo JL, Afshin A, Mozaffarian D. Dietary intake among US adults, 1999–2012. JAMA 2016;315:2542–53
2. Wang DD, Leung CW, Li Y, Ding EL, Chiuve SE, Hu FB, Willett WC. Trends in dietary quality among adults in the United States, 1999 through 2010. JAMA Intern Med 2014;174:1587–95.
3. Krebs-Smith SM, Guenther PM, Subar AF, Kirkpatrick SI, Dodd KW. Americans do not meet federal dietary recommendations. J Nutr 2010;140:1832–8.1
4. Martin JC, Zhou SJ, Flynn AC, Malek L, Greco R, Moran L. The assessment of diet quality and its effects on health outcomes pre- pregnancy and during pregnancy. Semin Reprod Med 2016;34:83–92.
5. Bodnar LM, Simhan HN, Parker CB, Meier H, Mercer BM, Grobman WA, Haas DM, Wing DA, Hoffman MK, Parry S, et al. Racial or ethnic and socioeconomic inequalities in adherence to national dietary guidance in a large cohort of US pregnant women. J Acad Nutr Diet 2017;117:867–77.
6. RasmussenMA, Maslova E,Halldorsson TI, Olsen SF. Characterization of dietary patterns in the Danish National Birth Cohort in relation to preterm birth. PLoS One 2014;9(4):e93644.
7. Hanson MA, Gluckman PD, Godfrey KM. Developmental epigenetics and risks of later non-communicable disease. In: Seckl JR, Christen Y, editors. Hormones, intrauterine health and programming.Research and perspectives in endocrine

interactions, vol. 12. Berlin: Springer; 2014. pp. 175–83.

8. Emmett PM, Jones LR, Golding J. Pregnancy diet and associated outcomes in the Avon Longitudinal Study of Parents and Children. Nutr Rev 2015;73:154–74.
9. Gluckman PD,HansonMA,Cooper C,ThornburgKL. Effect of in utero and early-life conditions on adult health and disease. N Engl J Med 2008;359:61–73.
10. Gluckman PD, Hanson MA, Buklijas T. A conceptual framework for the developmental origins of health and disease. J Dev Orig Health Dis 2010;1:6–18.
11. Shapiro ALB, Kaar JL, Crume TL, Starling AP, Siega-Riz AM, Ringham BM, Glueck DH, Norris JM, Barbour LA, Friedman JE, et al. Maternal diet quality in pregnancy and neonatal adiposity: the Healthy Start Study. Int J Obes 2016;40(7):1056–62.
12. Murrin C, Shrivastava A, Kelleher CC. Maternal macronutrient intake during pregnancy and 5 years postpartum and associations with child weight status aged five. Eur J Clin Nutr 2013;67:670–9.
13. Salter AM, Tarling EJ, Langley-Evans SC. Influence of maternal nutrition on the metabolic syndrome and cardiovascular risk in the offspring. Clin Lipidol 2009;4:145–58.
14. Gaillard R. Maternal obesity during pregnancy and cardiovascular development and disease in the offspring. Eur J Epidemiol 2015; 30:1141–52
15. : Jacka FN, Ystrom E, Brantsaeter AL, Karevold E, Roth C, Haugen M, Meltzer HM, Schjolberg S, Berk M. Maternal and early postnatal nutrition and mental

health of offspring by age 5 years: a prospective cohort study. J Am Acad Child Adolesc Psychiatry 2013;52: 1038–47

16. Steenweg-de Graaff J, Tiemeier H, Steegers-Theunissen RPM, Hofman A, Jaddoe VWV, Verhulst FC, Roza SJ. Maternal dietary patterns during pregnancy and child internalising and externalising problems. The Generation R Study. Clin Nutr 2014;33:115–21.
17. Van Lieshout RJ, Robinson M, Boyle MH. Maternal pre-pregnancy body mass index and internalizing and externalizing problems in offspring. Can J Psychiatry 2013;58:151–9
18. Godfrey KM, Reynolds RM, Prescott SL, Nyirenda M, Jaddoe VWV, Eriksson JG, Broekman BFP. The influence of maternal obesity on the long-term health of the offspring. Lancet Diabetes Endocrinol 2017;5:53–64
19. Loche E, BlackmoreHL, Carpenter AA,Beeson JH,Pinnock A,Ashmore TJ, Aiken CE, De Almeida-Faria J, Schoonejans JM, Giussani DA, et al. Maternal diet-induced obesity programmes cardiac dysfunction in male mice independently of post-weaning diet. Cardiovasc Res 2018;114(10):1372–84.
20. Armitage JA, Lakasing L, Taylor PD, Balachandran AA, Jensen RI, Dekou V, Ashton N, Nyengaard JR, Poston L. Developmental programming of aortic and renal structure in offspring of rats fed fat- rich diets in pregnancy. J Physiol 2005;565:171–84.
21. Gali Ramamoorthy T, Allen TJ, Davies A, Harno E, Sefton C, Murgatroyd C,

White A. Maternal overnutrition programs epigenetic changes in the regulatory regions of hypothalamic Pomc in the offspring of rats. Int J Obes 2018;42(8):1431–44.

22. De Jong KA, Barrand S, Wood-Bradley RJ, de Almeida DL, Czeczor JK, Lopaschuk GD, Armitage JA, McGee SL. Maternal high fat diet induces early cardiac hypertrophy and alters cardiac metabolism in Sprague Dawley rat offspring. Nutr Metab Cardiovasc Dis 2018;28: 600–9.
23. Grissom NM, George R, Reyes TM. Suboptimal nutrition in early life affects the inflammatory gene expression profile and behavioral responses to stressors. Brain Behav Immun 2017;63:115–26.
24. Vucetic Z,Kimmel J, Totoki K, Hollenbeck E, Reyes TM.Maternal high- fat diet alters methylation and gene expression of dopamine and opioid- related genes. Endocrinology 2010;151:4756–64.
25. Lemes SF, de Souza ACP, Payolla TB, Versutti MD, de Fátima da Silva Ramalho A, Mendes-da-Silva C, Souza CM,Milanski M, Torsoni AS, Torsoni MA. Maternal consumption of high-fat diet in mice alters hypothalamic notch pathway, NPY cell population and food intake in offspring. Neuroscience 2018;371:1–15
26. Bellisario V, Panetta P, Balsevich G, Baumann V, Noble J, Raggi C, Nathan O, Berry A, Seckl J, SchmidtM, et al. Maternal high-fat diet acts as a stressor increasing maternal glucocorticoids' signaling to the fetus and disrupting maternal behavior and brain activation in C57BL/6J mice. Psychoneuroendocrinology

2015;60:138–50.

27. Wu T, Deng S, Li WG, Yu Y, Li F, Mao M. Maternal obesity caused by overnutrition exposure leads to reversal learning deficits and striatal disturbance in rats. PLoS One 2013;8(11):e78876.

28. Naef L, Gratton A, Walker C-D. Exposure to high fat during early development impairs adaptations in dopamine and neuroendocrine responses to repeated stress. Stress 2013;16:540–8.

29. Berntson GG, Cacioppo JT, Quigley KS. Respiratory sinus arrhythmia: autonomic origins, physiological mechanisms, and psychophysiological implications. Psychophysiology 1993;30:183–96.

30. Berntson GG. Heart rate variability: origins, methods, and interpretive caveats. Psychophysiology 1997;34(6):623–48.

31. Stein PK, Bosner MS, Kleiger RE, Conger BM. Heart rate variability: a measure of cardiac autonomic tone. Am Heart J 1994;127:1376–81.

32. Thayer JF, Lane RD. A model of neurovisceral integration in emotional regulation and dysregulation. J Affect Disord 2000;61:201–16..

33. Thayer JF, Sternberg E. Beyond heart rate variability: Vagal regulation of allostatic systems. Ann N Y Acad Sci. 2006;1088:361–72.

34. Goto M, Nagashima M, Baba R, Nagano Y, Yokota M, Nishibata K, Tsuji A. Analysis of heart rate variability demonstrates effects of development on vagal modulation of heart rate in healthy children. J Pediatr 1997;130:725–9.

35. Thayer JF, Yamamoto SS, Brosschot JF. The relationship of autonomic imbalance, heart rate variability and cardiovascular disease risk factors. Int J Cardiol 2010;141(2):122–31

36. Porges SW, Furman SA. The early development of the autonomic nervous system provides a neural platform for social behaviour: a polyvagal perspective. Infant Child Dev 2011;20:106–18..

37. Calkins SD, Keane SP. Cardiac vagal regulation across the preschool period: stability, continuity, and implications for childhood adjustment. Dev Psychobiol 2004;45:101–12..

38. Zhou Y, Xie G,Wang J, Yang S. Cardiovascular risk factors significantly correlate with autonomic nervous system activity in children. Can J Cardiol 2012;28:477–82.

39. Michels N, Sioen I, Clays E, De Buyzere M, Ahrens W, Huybrechts I, Vanaelst B, De Henauw S. Children's heart rate variability as stress indicator: association with reported stress and cortisol. Biol Psychol 2013;94:433–40

40. Wulsin LR,Horn PS, Perry JL,Massaro JM, Agostino RBD. Autonomic imbalance as a predictor of metabolic risks, cardiovascular disease, diabetes, and mortality. J Clin Endocrinol Metab 2016;100:2443–8..

41. Hillebrand S, Gast KB, De Mutsert R, Swenne CA, Jukema JW, Middeldorp S, Rosendaal FR, Dekkers OM. Heart rate variability and first cardiovascular event in populations without known cardiovascular disease: meta-analysis and dose-

response meta-regression. Europace 2013;15:742–9

42. Francis NJ, Landis SC. Cellular and molecular determinants of sympathetic neuron development.Annu Rev Neurosci 1999;22:541–66

43. Propper CB, Holochwost SJ. The influence of proximal risk on the early development of the autonomic nervous system. Dev Rev 2013;33: 151–67.

44. Sucharita S, Dwarkanath P, Thomas T, Srinivasan K, Kurpad AV, Vaz M. Low maternal vitamin B12 status during pregnancy is associated with reduced heart rate variability indices in young children. Matern Child Nutr 2014;10:226–33.

45. Spann MN, Smerling J, Gustafsson H, Foss S, Altemus M, Monk C. Deficient maternal zinc intake—but not folate—is associated with lower fetal heart rate variability. Early Hum Dev 2015;91:169–72.

46. Gustafson KM, Carlson SE, Colombo J, Yeh HW, Shaddy DJ, Li S, Kerling EH. Effects of docosahexaenoic acid supplementation during pregnancy on fetal heart rate and variability: a randomized clinical trial. Prostaglandins Leukot Essent Fatty Acids 2013;88:331–8

47. Arbuckle TE, Fraser WD, Fisher M, Davis K, Liang CL, Lupien N, Bastien S, Velez MP, Von Dadelszen P, Hemmings DG, et al. Cohort profile: the Maternal-Infant Research on Environmental Chemicals research platform. Paediatr Perinat Epidemiol 2013;27:415–25.

48. Morisset A, Weiler HA, Dubois L, Ashley-Martin J, Shapiro GD, Dodds L, Massarelli I, VigneaultM, Arbuckle TE, Fraser WD.Rankings of iron, vitamin D,

and calcium intakes in relation to maternal characteristics of pregnant Canadian women. Appl Physiol Nutr Metab 2016;757: 749–57..

49. Guenther PM, Kirkpatrick SI, Reedy J, Krebs-Smith SM, Buckman DW, Dodd KW, Casavale KO, Carroll RJ. The Healthy Eating Index-2010 is a valid and reliable measure of diet quality according to the 2010 Dietary Guidelines for Americans. J Nutr 2014;144:399–407.
50. Bivoltsis A, Trapp GSA, Knuiman M, Hooper P, Ambrosini GL. Can a simple dietary index derived from a sub-set of questionnaire items assess diet quality in a sample of Australian adults? Nutrients 2018;10(4):E486.
51. Little P, Barnett J, Margetts B, Kinmonth A-L, Gabbay J, Thompson R, Warm D, Warwick H, Wooton S. The validity of dietary assessment in general practice. J Epidemiol Community Health 1999;53:165–72.
52. Cleghorn CL, Harrison RA, Ransley JK, Wilkinson S, Thomas J, Cade JE. Can a dietary quality score derived from a short-form FFQ assess dietary quality in UK adult population surveys? Public Health Nutr 2016;19:2915–23.
53. Clarys P, Deliens T, Huybrechts I, Deriemaeker P, Vanaelst B, De Keyzer W, Hebbelinck M, Mullie P. Comparison of nutritional quality of the vegan, vegetarian, semi-vegetarian, pesco-vegetarian and omnivorous diet. Nutrients 2014;6:1318–32.
54. Guenther PM, Casavale KO, Reedy J, Kirkpatrick SI, Hiza HAB, Kuczynski KJ,Kahle LL, Krebs-Smith SM.Update of the Healthy Eating Index: HEI-2010. J

Acad Nutr Diet 2013;113:569–80.

55. Epidemiology and Genetics Resarch Program. Basic steps in calculating the HEI-2010. [cited 2018, June 3]. Available from 12 Feburary 2018. NIM National Cancer Institute, Bethesda (MD). Available from: https: //epi.grants.cancer.gov/hei/calculating-hei-scores.html.

56. Pick ME, Edwards M, Moreau D, Ryan EA. Assessment of diet quality in pregnant women using the Healthy Eating Index. J Am Diet Assoc 2005;105:240–6..

57. Tsigga M, Filis V, Hatzopoulou K, Kotzamanidis C,Grammatikopoulou MG. Healthy Eating Index during pregnancy according to pre-gravid and gravid weight status. Public Health Nutr 2011;14:290–6

58. Zygmunt A, Stanczyk J. Methods of evaluation of autonomic nervous system function. Arch Med Sci 2010;6:11–8.

59. Goldsmith HH, Rothbart MK.The laboratory temperament assessment battery. Arlington (TX). 1999. Pre-Locomotor version. Available from: uta.edu/faculty/jgagne//labtab/desc_pre-loco_Lab-TAB.htm.

60. Kim H-G, Cheon E-J, Bai D-S, Lee YH, Koo B-H. Stress and heart rate variability: a meta-analysis and review of the literature. Psychiatry Investig 2018;15:235–45.

61. Appelhans BM, Luecken LJ. Heart rate variability as an index of regulated emotional responding. Rev Gen Psychol 2006;10:229–40

62. Sassi R, Cerutti S, Lombardi F, Malik M,HuikuriHV,Peng CK,Schmidt G, Yamamoto Y. Advances in heart rate variability signal analysis: joint position

statement by the e-Cardiology ESC Working Group and the European Heart Rhythm Association co-endorsed by the Asia Pacific Heart Rhythm Society. Europace 2015;17:1341–53..

63. Shaffer F, Ginsberg JP. An overview of heart rate variability metrics and norms. Front Public Health 2017;5:258.

64. Ramsay JE, Ferrell WR, Crawford L, Wallace AM, Greer IA, Sattar N. Maternal obesity is associated with dysregulation of metabolic, vascular, and inflammatory pathways. J Clin Endocrinol Metab 2002;87:4231–7.

65. World Health Organization. WHO: global database on body mass index. [cited 2018, June 3]. Geneva: WHO; 2012. Available from: who.int/nutrition/databases/bmi/en/.

66. Smarr KL, Keefer AL. Measures of depression and depressive symptoms: Beck Depression Inventory-II (BDI-II), Center for Epidemiologic Studies Depression Scale (CES-D), Geriatric Depression Scale (GDS), Hospital Anxiety and Depression Scale (HADS), and Patient Health Questionna. Arthritis Care Res. 2011;63:454–66.

67. DeCapo M, Thompson JR, Dunn G, Sullivan EL. Perinatal nutrition and programmed risk for neuropsychiatric disorders: a focus on animal models. Biol Psychiatry 2018;85(2):122–34.

68. Public Health Services. Government of Canada, Breastfeeding your baby. Ottawa (Ontario) Government of Canada; 2014 [cited 2019, Aug 19] Retrieved from

https://www.canada.ca/en/public-health/services/health-promotion/childhood-adolescence/stages-childhood/infancy-birth-two-years/breastfeeding-infant-nutrition.html

69. Mckenzie KM,Dissanayake HU,McMullan R, Caterson ID, Celermajer DS, Gordon A, Hyett J, Meroni A, Phang M, Raynes-Greenow C, et al. Quantity and quality of carbohydrate intake during pregnancy, newborn body fatness and cardiac autonomic control: conferred cardiovascular risk? Nutrients 2017;9:1375.

70. Nyaradi A, Li J, Hickling S, Foster J, Oddy WH. The role of nutrition in children's neurocognitive development, from pregnancy through childhood. Front Hum Neurosci 2013;7:97.

71. Cohen E, Wong FY, Wallace EM, Mockler JC, Odoi A, Hollis S, Horne RSC, Yiallourou SR. Fetal-growth-restricted preterm infants display compromised autonomic cardiovascular control on the first postnatal day but not during infancy. Pediatr Res 2017;82:474–82.

72. Jones NA, Field T, Fox NA, Davalos M, Lundy B, Hart S. Newborns of mothers with depressive symptoms are physiologically less developed. Infant Behav Dev 1998;21:537–41.

73. Field T, Diego M, Hernandez-Reif M, Salman F, Schanberg S, Kuhn C, Yando R, Bendell D. Prenatal anger effects on the fetus and neonate. J Obstet Gynaecol (Lahore) 2002;22:260–6.

74. Field T, Diego M, Hernandez-Reif M, Schanberg S, Kuhn C, Yando R, Bendell D.

Pregnancy anxiety and comorbid depression and anger: Effects on the fetus and neonate. Depress Anxiety. 2003;17:140–51.

75. Ponirakis A, Susman EJ, Stifter CA. Negative emotionality and cortisol during adolescent pregnancy and its effects on infant health and autonomic nervous system reactivity. Dev Psychobiol 1998;33: 163–74.

76. Suurland J, van der Heijden KB, Smaling HJA, Huijbregts SCJ, van Goozen SHM, Swaab H. Infant autonomic nervous system response and recovery: associations with maternal risk status and infant emotion regulation. Dev Psychopathol 2017;29:759–73.

77. Braeken MA,Kemp AH, Outhred T, Otte RA,Monsieur GJYJ, Jones A, Van Den Bergh BRH. Pregnant mothers with resolved anxiety disorders and their offspring have reduced heart rate variability: implications for the health of children. PLoS One 2013;8:6–12.

78. Schuetze P, Zeskind PS. Relation between maternal cigarette smoking during pregnancy and behavioral and physiological measures of autonomic regulation in neonates. Infancy 2001;2:371–83

79. Schuetze P, Eiden RD, Danielewicz S. The association between prenatal cocaine exposure and physiological regulation at 13 months of age. J Child Psychol Psychiatry Allied Discip 2009;50:1401–9.

80. Jansson LM, DiPietro JA, Elko A, Velez M. Infant autonomic functioning and neonatal abstinence syndrome. Drug Alcohol Depend 2010;109:198–204.

81. Oberlander TF, Jacobson SW, Weinberg J, Grunau RE, Molteno CD, Jacobson JL. Prenatal alcohol exposure alters biobehavioral reactivity to pain in newborns. Alcohol Clin Exp Res 2010;34:681–92

82. Fifer WP, Fingers ST, Youngman M, Gomez-Gribben E, Myers MM. Effects of alcohol and smoking during pregnancy on infant autonomic control. Dev Psychobiol 2009;51:234–42..

83. Browne CA, Colditz PB, Dunster KR. Infant autonomic function is altered by maternal smoking during pregnancy. Early Hum Dev 2000;59:209–18.

84. Le Strat Y, Dubertret C, Le Foll B. Prevalence and correlates of major depressive episode in pregnant and postpartum women in the United States. J Affect Disord 2011;135:128–38

85. Dennis CL, Falah-Hassani K, Shiri R. Prevalence of antenatal and postnatal anxiety: systematic review and meta-analysis. Br J Psychiatry 2017;210:315–23.

86. Vesga-Lopez O, Blanco C, Keyes K, Olfson M, Grant BF, Hasin DS. Psychiatric disorders in pregnant and postpartum women in the United States. Arch Gen Psychiatry 2008;65:805–15.

87. Hack M, Taylor HG, Drotar D, Schluchter M, Cartar L, Andreias L, Wilson-costello D. Chronic Conditions , Functional Limitations , and Special Health Care Needs of Extremely Low-Birth-Weight in the 1990s. JAMA. 2014;294.

88. Naef L, Srivastava L, Gratton A, Hendrickson H, Owens SM, Walker CD. Maternal high fat diet during the perinatal period alters mesocorticolimbic

dopamine in the adult rat offspring: reduction in the behavioral responses to repeated amphetamine administration. Psychopharmacology (Berl) 2008;197:83–94.

89. Ong ZY, Muhlhausler BS. Maternal “junk-food” feeding of rat dams alters food choices and development of the mesolimbic reward pathway in the offspring. FASEB J 2011;25:2167–79.

90. Grissom NM, Herdt CT, Desilets J, Lidsky-Everson J, Reyes TM. Dissociable deficits of executive function caused by gestational adversity are linked to specific transcriptional changes in the prefrontal cortex. Neuropsychopharmacology 2015;40:1353–63.

91. Mastorci F, Vicentini M, Viltart O, Manghi M, Graiani G, Quaini F, Meerlo P, Nalivaiko E, Maccari S, Sgoifo A. Long-term effects of prenatal stress: changes in adult cardiovascular regulation and sensitivity to stress. Neurosci Biobehav Rev 2009;33:191–203..

92. Lovallo WR, Farag NH, Sorocco KH, Cohoon AJ, Vincent AS. Lifetime adversity leads to blunted stress axis reactivity: studies from the Oklahoma Family Health Patterns Project. Biol Psychiatry 2012;71:344–9.

93. Piazza JR, Charles ST, Sliwinski MJ, Mogle J, Almeida D. Affective reactivity to daily stressors and long-term risk of reporting a chronic physical health condition. Ann Behav Med 2013;45: 110–20.

94. Georgieff MK. Nutrition and the developing brain. Am J Clin Nutr 2007;85:614S–

19S.

95. Marangoni F, Cetin I, Verduci E, Canzone G, Giovannini M, Scollo P, Corsello G, Poli A. Maternal diet and nutrient requirements in pregnancy and breastfeeding. An Italian consensus document. Nutrients 2016;8:629.

Chapter 5: Changes in Psychophysiological and Behavioral Measures of Emotion Regulation in Infants following Maternal Cognitive Behavioral Therapy for Postpartum Depression (Study 4)

Study 4 Overview

Title: Changes in Psychophysiological and Behavioral Measures of Emotion Regulation in Infants following Maternal Cognitive Behavioral Therapy for Postpartum Depression

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Context and Implications: Studies 1,2 and 3 addressed the need to utilize data from large cohort studies to investigate the impact of potentially modifiable, previously unmeasured confounding variables on offspring neurodevelopment. This was done to identify potential effective targets for future interventions. Additionally, in order to fully elucidate the preventative potential of the DOHaD hypothesis, we must also move toward testing the effectiveness of interventions on offspring outcomes. Further, assessing the impact of these interventions on offspring outcome variables that index vulnerability to later psychopathology (e.g., infant emotion regulation) is critical.

Therefore, in Study 4, we examined if treating mothers with major depressive disorder in the postpartum improved physiological and behavioral markers of emotion regulatory capacity in their infants. Maternal postpartum depression significantly increases the risk for emotion regulatory and psychiatry problems in offspring. However, no studies have examined if treating postpartum depression can adaptively alter physiological and behavioral systems core to infant emotion regulation. In this study, we

show that a cost-effective, first-line treatment for postpartum depression is associated with adaptive changes in infant emotion regulatory capacity evidenced across physiological and behavioral levels. Therefore, this study not only provides evidence on the potential malleability of emotion regulatory systems in infants in the first year of life, but that treating mothers is associated with reduced intergenerational transmission of psychiatric risk from mother to child.

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Conflicts of Interest: None

Submitted to: Pediatrics

Abstract

Background: Exposure to maternal postpartum depression (PPD) increases the risk for emotion regulatory and psychiatric problems in offspring. This study aimed to determine if maternal cognitive behavioral therapy (CBT) for PPD improves infant emotion regulatory capacity.

Methods: Participants were 40 infants of mothers with a primary diagnosis of major depressive disorder matched 1:1 to 40 healthy control infants of non-depressed mothers on infant age, sex and socioeconomic status. Mothers with PPD received nine weeks of group CBT. Dyads were tested at two time points. Visit 1 occurred following the first CBT session (baseline visit for control infants). Visit 2 took place after CBT (nine weeks post-baseline for controls). At both visits, infant emotion regulation was assessed using resting-state frontal EEG alpha asymmetry (FAA), heart rate variability (HRV), and maternal and partner ratings of orientation/regulation behaviors (Infant Behavior Questionnaire-Revised [Short Form]). Changes in maternal characteristics (depression, bonding, emotion regulation) from pre- to post-treatment were examined to determine if these variables explained infant changes.

Results: Following maternal PPD treatment, infants showed improved emotion regulatory capacity as evidenced by a shift from greater right to left FAA [p=0.01, d=0.60], increased HRV [p=0.003, d=0.56], and orientation/regulation behaviors reported by mothers [p=0.015, d=0.29] and partners [p=0.049, d=0.35]. After maternal treatment,

emotion regulation in these infants was similar to healthy control infants. Changes in maternal characteristics did not account for these changes.

Conclusions: Cognitive behavioral therapy, a cost-effective, first-line treatment for PPD is associated with adaptive changes in physiological and behavioral systems underlying infant emotion regulatory capacity.

Introduction

Postpartum depression (PPD) is common[1] and has significant negative effects on the lives of women and their offspring. Each case of PPD is estimated to cost $97 000 (USD) over the lifespan, with two-thirds of these costs being accounted for by the offspring[2]. Infant exposure to PPD is linked to elevated levels of emotional, behavioral, and cognitive problems in childhood [3] as well as an increased risk for psychopathology in adulthood[4].

Postpartum depression can have particularly deleterious effects on the emotion regulatory capacity of offspring [5]. Changes in emotion regulation, an individual's ability to modify emotions in the service of future goals [6], may play a key role in the transmission of risk from mothers with PPD to their children [5,7]. Indeed, early problems with emotion regulatory capacity are linked to an increased risk of psychiatric disorders [8], physical health problems and income rates below the poverty line [9].

Optimal development of emotion regulatory systems in infants relies on the quality of interactions with caregivers [8,10], and abnormal development of both central and peripheral biomarkers of these systems has been detected within the first postpartum year in the infants of depressed mothers [11]. Exposure to PPD is linked to greater activity within the right frontal hemisphere[12,13] and lower heart rate variability[14,15], two early emerging indices of emotion regulatory capacity and predictors of later psychiatric risk [16,17]. However, given the plasticity of the infant brain in the first postnatal year, interventions applied during this time may have the greatest potential to adaptively alter these emotion

regulatory systems [18]. Therefore, due to the prevalence of PPD [1], its negative impact on infant emotion regulation, and the established links between early emotion regulation and later health [8], it is important to determine whether treating mothers with PPD can improve emotion regulation in their infants.

Studies that have examined the impact of treating PPD on offspring have yielded inconsistent results with some studies suggesting that treating mothers benefits offspring [19–28], while others have not [29–36]. However, these studies measured infant outcomes using maternal informant reports and or single assessments of observed infant behavior. Assessing infant outcomes using these methods alone may not fully capture the subtle yet important changes potentially occurring in preverbal infants following maternal PPD treatment. Further, to our knowledge, only two of these studies explicitly set out to assess offspring emotion regulation following maternal PPD treatment; however, both utilized a single observational assessment method and examined offspring beyond the first postnatal year [27,28]. No studies have examined the effects of PPD treatment on infant emotion regulatory capacity by assessing the physiological and behavioral systems known to be adversely affected by PPD exposure, combined with reports from multiple informants.

Utilizing robust multimethod assessments across both physiological and behavioral levels can address the limitations of previous studies and provide a more complete picture of the potential maternal PPD treatment has to optimize infant emotion regulation. If maternal PPD treatment can alter physiological and behavioral emotion regulatory systems in infants to levels comparable to infants of non-depressed women, we

may be capable of disrupting the intergenerational transmission of risk from mothers to offspring.

We used data from 80 mother-infant dyads (40 infants of women with PPD and 40 sex- and age-matched control infants of mothers free of PPD) to examine: 1) whether maternal treatment for PPD was effective in producing improvements in infant emotion regulatory capacity examined on physiological and behavioral levels, 2) if emotion regulation after maternal treatment was comparable to healthy control infants, and 3) if changes in maternal factors (depressive symptoms, bonding and emotion regulation) accounted for putative physiological and behavioral changes in their infants.

Methods

Participants

The study sample was comprised of 80 infants and their mothers. Mothers were recruited between March 2016 and July 2019 from Hamilton, Ontario and surrounding municipalities.

Case dyads: Case infants (n=40) were born to women with a primary diagnosis of major depressive disorder diagnosed within the first postpartum year. These women were patients of the Women's Health Concerns Clinic, a specialized women's mental health clinic at St. Joseph's Healthcare Hamilton in Ontario. Major depressive disorder (MDD) diagnoses were made according to DSM-5 criteria by a mental health clinician (nurse, social worker, or psychologist) using a structured interview format and confirmed by a psychiatrist. After diagnosis, women were offered the 9-week cognitive behavioral

therapy (CBT) for PPD intervention. At the first CBT session, women were invited to participate in the present study. Mother-infant dyads were deemed ineligible if MDD was not the primary diagnosis or were diagnosed with bipolar or a schizophrenia spectrum disorder, if women were not fluent in English, or if their infant was >12 months of age.

Control dyads: Control infants (n=40) were matched 1:1 with case dyads based on infant age, sex and familial socioeconomic status (SES, total household income [Canadian Dollars (CAD)]). This group was recruited to ensure that any changes in case infants were not due to development (occurring with age), sex, or other early adverse experience [37] and to determine if putative changes in infant emotion regulation in response to maternal PPD treatment reached levels comparable to infants never exposed to PPD. Dyads were recruited from the Infant Database of the Department of Psychology, Neuroscience and Behavior at McMaster University (the same catchment area as case dyads). Dyads were ineligible if women were not fluent in English, screened positive on the major depressive episode module of the Mini International Neuropsychiatric Interview [38] or their infant was >12 months old.

Study Design

Data were collected at two points. For case dyads, Visit 1 occurred following the first CBT session and Visit 2 occurred after treatment was completed. Data acquisition from control dyads occurred at a baseline session (Visit 1) and 9-weeks after this baseline (Visit 2) (Figure 1). All testing occurred at the Child Emotion Laboratory at McMaster

University. The Hamilton Integrated Research Ethics Board approved this study. All women provided informed consent prior to participating.

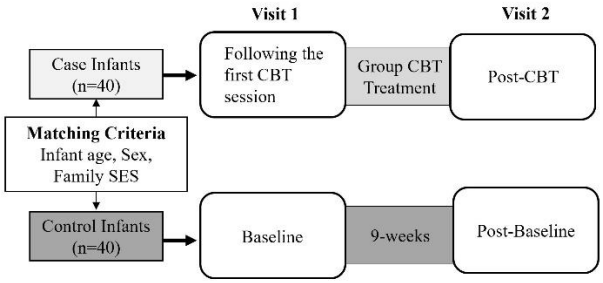

Figure 1: Study Design

Intervention (Group Cognitive Behavioral Therapy): Women with PPD received the 9-week group CBT intervention [39] delivered by two trained therapists (psychiatrists, psychologists, psychiatric nurses, and social workers). Nine two-hour sessions took place weekly. The first half consisted of core CBT content, and the second half involved a relevant discussion topic co-led by patients and therapists. To optimize the generalizability of our findings, women in the CBT group could also receive any other treatments they wished during CBT treatment.

Outcome: Infant Emotion Regulation

Multimethod assessments that combine physiological and observational methods are recommended for the measurement of emotion regulation in infancy [40]. Resting-state assessments of frontal electroencephalographic alpha asymmetry (FAA) and heart rate variability (HRV) index trait-related emotion regulation systems, as do parental reports of temperament observed across contexts [41]. Greater right FAA, lower HRV, and lower

scores on the orientation/regulation domain of temperament scale index poorer emotion regulation and vulnerability to psychopathology.

Procedure: At both visits, four minutes of resting-state physiological data were acquired in accordance with standard infant protocols [42]. Infants were seated on their mother's laps facing forward while a research assistant seated at infant eye level manipulated a toy 50cm in front of the infant's face. Mothers were instructed not to talk to their infant during this time.

Electroencephalography (EEG): EEG data were recorded using Netstation (v.4.4.1)128-electrode HydroCel sensor nets at 250Hz and referenced to the vertex (EGI Inc). Data were analyzed offline in EEGlab (14.0.0b.) (details in Supplementary Methods), re-referenced to the average and then segmented into two seconds epochs with 50% overlap. A 100% hanning window was applied to each epoch, and a Fourier transform was used to extract power within the "infant" alpha (α) band (6-9Hz) [43]. Frontal alpha asymmetry was calculated by subtracting the natural log-transformed alpha power at the left frontal hemisphere (at site F3 [channel 24]) from the right frontal hemisphere (at F4 [channel 124]) [i.e., FAA=ln(F4)-ln(F3)]. Of the 80 study infants at Visit 1, data were unanalyzable from six cases and two controls and at Visit 2 from five cases and four controls.

Heart rate variability (HRV): Pediatric ECG electrodes were placed on the infant's back. Data were acquired wirelessly using Biolab software (v 3.2.3, Mindware Ltd. Gahanna,OH). Mindware HRV (v 3.3.2) software was used to inspect data for artifacts

(details in Supplement) and to analyze data in the frequency domain. Power in the infant high-frequency band (0.24-1.04Hz)[44] was obtained. Of the 80 infants in the study, data were unanalyzable from one case Visit 1 and one control at Visit 2.

Temperament: The mother and her partner reported on infant temperament using the 91-item Infant Behavior Questionnaire-Revised (Short Form). Infant behaviors were rated over the past week using a 7-point scale [1 (never)-7 (always)].[41] *A priori,* we decided to focus on the orientation/regulation domain, which combines soothability, duration of orientation, low intensity pleasure, and cuddliness subscales. This domain predicts later effortful control, and assesses the functioning of emotion regulatory systems [45]. Higher scores indicate more regulatory behaviors. One case mother, and nine case and eight control partners did not complete the questionnaires. Cronbach α at Visit 1 was 0.88 for mothers, 0.87 for partners; and at Visit 2, Cronbach α was 0.90 for mothers and 0.78 for partners.

Maternal Change with Treatment and Infant Emotion Regulation

As we were interested in the putative mechanisms by which PPD treatment could affect offspring emotion regulation, we assessed whether maternal changes in depression, emotion regulation, and mother-infant bonding affected infant emotion regulatory capacity. Maternal depressive symptoms were assessed using the Edinburgh Postnatal Depression Scale and mother-infant bonding with the general factor scale of the Postpartum Bonding Questionnaire. The Neuroticism subscale of the revised NEO five-factor personality inventory was used to assess the construct of maternal emotion

regulation to determine if changes in infant emotion regulation were simply a reflection of changes in maternal emotion regulation. Change scores were calculated by subtracting post-CBT from pre-CBT treatment scores in the case group.

Statistical Analyses

Differences between case and control groups were assessed using chi-square and independent samples t-tests. Group by Visit interactions for each measure of infant emotion regulation were examined using repeated-measures analysis of variance (ANOVA) tests. The between-subjects factor was group, and the within-subjects factor was study visit. Planned comparisons examined between-group differences at Visit 1, and again at Visit 2 using independent samples t-tests. Within-group differences occurring from Visit 1 to Visit 2 were tested using paired t-tests.

Next, in case dyads, linear regression was used to examine if change scores in maternal characteristics occurring with treatment were associated with changes in infant outcomes. In case dyads, sensitivity analyses were conducted to examine interactions between mothers taking vs. not taking psychiatric medications on infant outcomes. Analyses were conducted in SPSS v.23. Significance was 2-tailed with $P < 0.05$.

Results

Sample Characteristics

Sample characteristics are summarized in Table 1. Infant age at enrolment was (mean [SD]) 5.7[2.6] months and 32[40%] were males. Infant birth weight was 3352.2 [464.1] grams, and all infants had been carried to term. Average household income was

$94,437 [$25,189.5] (Canadian Dollars), and 73[91%] of the women were married/common law. Of the 51 case women recruited, 2 were excluded because PPD was not the primary diagnosis and 40[78%] completed both study visits. Of the 46 controls recruited, 40[87%] completed both visits. Since our primary objective was to examine changes in emotion regulation following maternal treatment, only case dyads that completed the study were matched with a control. There were no differences between those that completed vs. those that dropped out of the study in either group (Supplementary table T1).

Table 1: Sample Characteristics

	Case (n=40)		Control (n=40)		*P* Value
Infant age, m (SD) months					
Visit 1	5.6	(2.7)	5.9	(2.6)	0.55
Visit 2	7.7	(2.7)	8.2	(2.7)	0.55
Infant sex, No (%) male	16	(40)	16	(40)	>0.99
Total household income[a] m(SD)					
<49,999	8	(20)	5	(12)	0.66
50,000-79,999	10	(25)	11	(28)	
>80,000	22	(55)	24	(60)	
EPDS Score m(SD)					
Visit 1	14.7	(5.4)	4.6	(3.4)	<0.001
Visit 2	10.6	(5.3)	4.3	(4.2)	<0.001
Maternal age, m(SD) years	32.3	(4.1)	32.7	(5.1)	0.68
Parity, No (%)					
Primiparous	21	(53)	22	(55)	>0.99
Multiparous	19	(47)	18	(45)	
Marital Status, No (%)					
Single	3	(8)	2	(5)	0.70
Separated	1	(2)	0	(0)	
Common-law	9	(22)	8	(20)	
Married	26	(68)	30	(75)	
Education, No (%)					
High school or less	5	(12)	3	(7)	0.70
College or certificate program	12	(30)	11	(28)	
University or higher	23	(58)	26	(65)	
Birthweight m(SD), grams	3329.5	(448.4)	3374.	9(475.2)	0.66
Gestational age m(SD) weeks	39.5	(2.2)	39.2	(1.1)	0.38

[a] Canadian Dollars, median household income in Ontario is $62,700

In women with PPD, a co-morbid psychiatric condition was diagnosed in 16[40%] women (mostly generalized anxiety disorder). Twenty-two [55.0%] women were taking antidepressant medication, and five increased dosage during the study. There were no differences between case and control dyads on infant age, sex, or household income. In women with PPD, a clinically significant decrease in depressive symptoms was observed following treatment (EPDSΔ= 4.1 points, p=0.001, Cohen's d=0.75).

Changes in Indices of Infant Emotion Regulation

Statistically significant Group by Study Visit interactions were observed for infant FAA [F(1,67)=7.7, p=0.007)], HRV [F(1,76)=4.7, p=0.033] (Figure 2), and the orientation/regulation domain of temperament reported by both mothers [F(1,77)=6.3, p=0.014] and partners [F(1,61)=4.3, p=0.042] (Figure 3), suggesting that each measure of infant emotion regulation changed differentially over time, depending on group (i.e., more in infants of women with PPD).

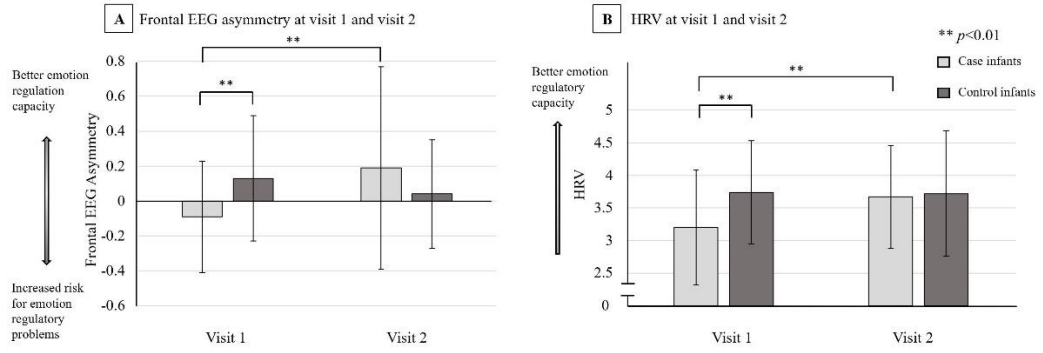

Figure 2: Changes in physiological markers of emotion regulation following maternal PPD treatment A) Frontal EEG Alpha Asymmetry changes from greater

relative right to greater relative left power in case infants **B)** Heart rate variability increases in case infants after treatment

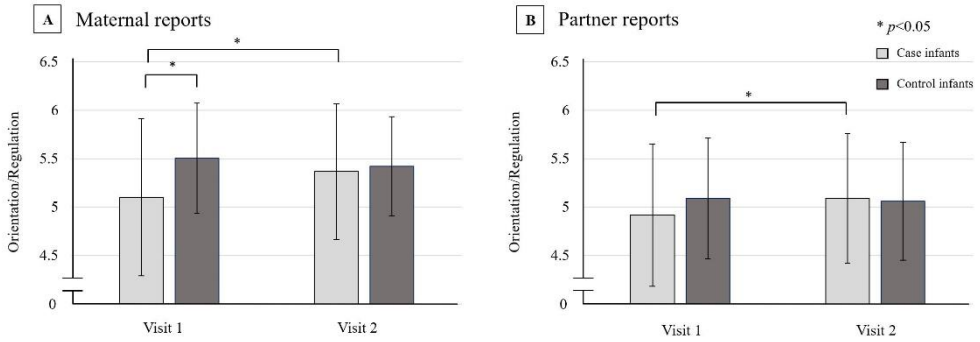

Figure 3: Orientation/Regulation Domain of Temperament: A) Infants of mothers with PPD exhibited significantly fewer orientation/regulation behaviors relative to controls at baseline. Following treatment, case infants showed improvements as reported by mothers. **B)** In case dyads, the mother's partner observed more orientation/regulation following maternal treatment.

Visit 1: Case and control infants differed significantly on FAA [M_{case}= -0.091, *SD*=0.31; $M_{control}$=0.13, *SD*=0.35, *p*=0.005, *d*=0.67], HRV [M_{case}=3.20, *SD*=0.88; $M_{control}$=3.74, *SD*=0.83, *p*=0.006, *d*=0.63] and on maternal but not paternal reported orientation/regulation [M_{case}=5.15, *SD*=0.79; $M_{control}$=5.50, *SD*=0.57, *p*=0.028, *d*=0.51].

Changes between visits: Case infants exhibited a change from greater relative right to greater relative left FAA [$M_{visit\ 1}$=-0.091, *SD*=0.31; $M_{visit\ 2}$=0.19, *SD*=0.58, *p*=0.01, *d*=0.60], increases in HRV [$M_{visit\ 1}$=3.20, *SD*=0.88; $M_{visit\ 2}$=3.67, *SD*=0.79, *p*=0.003, *d*=0.56] and orientation/regulation behaviors reported by mothers $M_{visit\ 1}$=5.15, *SD*=0.79.; $M_{visit\ 2}$=5.37, *SD*=0.71, *p*=0.015, *d*=0.29] and partners [$M_{visit\ 1}$=4.88, *SD*=0.68; $M_{visit\ 2}$=5.12, *SD*=0.69, *p*=0.049, *d*=0.35]. There were no differences between Visits 1 and 2 for control infants.

Visit 2: There were no significant differences between case and control infants.

No associations were observed between maternal depression, mother-infant bonding, or neuroticism and indices of infant emotion regulation (Table 2). Finally, there was no evidence that use or change in psychotropic medications affected infant outcomes (See Supplement for more details).

Table 2: Maternal change factors and offspring emotion regulation

	Infant Emotion Regulation Measure[a] (B, 95% CI)			
Maternal change score	ΔFAA	ΔHRV	Δ Regulation/orientation (Mother)	Δ Regulation/orientation (Partner)
Δ Maternal depression	-0.002 [-0.04;0.03]	-0.007 [-0.06;0.049]	0.02 [-0.01;0.05]	0.004 [-0.037;0.05]
Δ Mother-Infant Bonding	-0.003 [-0.03;0.024]	-0.02 [-0.06;0.03]	0.02 [-0.01; 0.04]	0.001 [-0.03;0.03]
Δ Emotion regulation	-0.02 [-0.06;0.2]	-0.01 [-0.08;0.06]	0.03 [-0.008;0.06]	-0.04 [-0.09;0.001]

[a]Higher scores=adaptive changes on both maternal and infant change scores

B: unstandardized beta

Discussion

Maternal treatment with nine weeks of group CBT for PPD was associated with adaptive changes in infant physiological and behavioral indices of emotion regulation with functioning approximating infants born to healthy control mothers. Since deviations in these emotion regulatory systems may precede the development of psychopathology [7,18], it appears that a widely acceptable, short and cost-effective CBT treatment for PPD may be capable of disrupting the intergenerational transmission of psychiatric risk from mothers to their infants. These changes did not appear to be specifically due to changes in maternal depressive symptoms, mother-infant bonding, or maternal emotion regulation.

While previous studies have examined the impact of PPD treatment on offspring within the first postnatal year, the results of these studies are inconsistent.[20,23,25,29,30,33,34,36]

However, they relied mainly on maternal reports or observational assessments, and none considered the perspective of the mother's partner. Our study is the first to assess the impact of PPD treatment on infants using a reliable, multimethod approach that utilizes objective measures of two physiological systems and reports from both mothers and partners. These methodological differences, along with our focus on emotion regulation and treatment during the first postnatal year, may have contributed to our findings of meaningful changes in offspring. The only previous studies that explicitly examined emotion regulation following maternal treatment [27,28] assessed offspring older than two years of age and did not compare them to a healthy control group. Examining emotion regulatory capacity in infants is important since deviations in these systems can emerge in the first postnatal year,[12,13,15,46,47] and since treating mothers earlier may have larger positive effects on their infants [18]. Finally, to our knowledge, only three studies compared outcomes to offspring born to non-depressed mothers [21,22,34]; however, these studies did not match infants of non-depressed mothers to the infants of depressed women that received treatment. By comparing outcomes to matched healthy control infants, the current study suggests that maternal treatment may improve functioning to levels comparable to healthy infants while ruling out that changes were due to infant age, sex or socioeconomic disadvantage.

PPD exposure appears to alter brain areas underlying in multiple aspects of emotion regulation, including the amygdala [48] and the right superior frontal cortex [49]. Our

observations of improved emotion regulation in infants could reflect more effective regulation by prefrontal networks or represent reductions in amygdala hyperactivity.

To understand how changes infant emotion regulation occurred, we examined associations between change scores in maternal characteristics thought to be involved in links between PPD exposure and infant emotion regulatory capacity. In our study, neither changes in maternal depression, nor bonding or maternal emotion regulation appeared to account for changes in infants. While the valence and quality of maternal signals can affect offspring emotion regulation, the predictability of maternal emotions and behaviors can also have an impact [50]. Treating PPD has been shown to improve maternal contingent responsiveness to infants [28,51], and anticipation of infant needs [51], suggesting that mothers may become more predictable to their infants following treatment, which may play a role in improving infant emotion regulation.

The following limitations should also be acknowledged. First, we were unable to examine if adaptive emotion regulatory changes persist beyond the study period. Second, we can only hypothesize as to the specific changes occurring in the brain areas that underlie our observations as EEG indexes brain activity at the level of the scalp. Future studies should utilize methods with greater spatial resolution (e.g., fNIRS, fMRI) to examine brain areas core to emotion regulation following treatment. Third, our design was not a randomized controlled trial; therefore, causal conclusions could not be made. However, this study took place in a setting where healthcare is universal, so it was not possible to assign women to a ‘no treatment group’. As a result, we cannot definitively

rule out that infants of mothers with PPD may have regressed to the mean (i.e., developmentally typical levels) over the treatment period. However, it is well established that exposure to early adversity results in persistent alterations in physiological development, including stable low HRV and greater right frontal EEG asymmetry[52,53]. Indeed, these measures are particularly stable in infants of depressed, relative to non-depressed mothers [47,54]. Based on this evidence, it would be unlikely for a cohort of infants of untreated clinically depressed women to display the significant, adaptive changes in emotion regulation found in our sample. Forth, while we did not observe any effect of medications on outcomes, we cannot rule out their role in infant changes.

Postpartum depression has detrimental effects on mothers, their infants and their families. Our results suggest that an acceptable, cost-effective, first-line treatment for PPD could have the potential to interrupt the intergenerational transmission of psychiatric risk, improving offspring outcomes in the short- and long-term.

References

1. Gaynes BN, Gavin N, Meltzer-Brody S, et al. Perinatal depression: prevalence, screening accuracy, and screening outcomes. *Evid Rep Technol Assess (Full Rep)*. 2005;(119):1-8.
2. Bauer A, Knapp M, Parsonage M. Lifetime costs of perinatal anxiety and depression. *J Affect Disord*. 2016;192(1):83-90. doi:10.1016/j.jad.2015.12.005
3. Grace SL, Evindar A, Stewart DE. The effect of postpartum depression on child cognitive development and behavior: A review and critical analysis of the literature. *Arch Womens Ment Health*. 2003;6(4):263-274. doi:10.1007/s00737-003-0024-6
4. Netsi E, Pearson RM, Murray L, Cooper P, Craske MG, Stein A. Association of persistent and severe postnatal depression with child outcomes. *JAMA Psychiatry*. 2018;75(3):247-253. doi:10.1001/jamapsychiatry.2017.4363
5. Maughan A, Cicchetti D, Toth SL, Rogosch FA. Early-occurring maternal depression and maternal negativity in predicting young children's emotion regulation and socioemotional difficulties. *J Abnorm Child Psychol*. 2007;35(5):685-703. doi:10.1007/s10802-007-9129
6. Thompson RA. Emotion Regulation: A Theme in Search of Definition. *Monogr Soc Res Child Dev*. 1994;59(2):25-52.
7. Goodman SH, Gotlib IH. Risk for psychopathology in the children of depressed mothers: A developmental model for understanding mechanisms of transmission.

Psychol Rev. 1999;106(3):458-490. doi:10.1037/0033-295X.106.3.458

8. Calkins SD, Dollar JM, Wideman L. Temperamental vulnerability to emotion dysregulation and risk for mental and physical health challenges. *Dev Psychopathol*. 2019;31(3):957-970. doi:10.1017/S0954579419000415
9. Moffitt TE, Arseneault L, Belsky D, et al. A gradient of childhood self-control predicts health, wealth, and public safety. *Proc Natl Acad Sci*. 2011;108(7):2693-2698. doi:10.1073/pnas.1010076108
10. Tronick E, Reck C. Infants of Depressed Mothers. *Harv Rev Psychiatry*. 2009;17(2):147-156. doi:10.1080/10673220902899714
11. Meaney MJ. Perinatal maternal depressive symptoms as an issue for population health. *Am J Psychiatry*. 2018;175(11):1084-1093. doi:10.1176/appi.ajp.2018.17091031
12. Lusby CM, Goodman SH, Yeung EW, Bell MA, Stowe ZN. Infant EEG and temperament negative affectivity: Coherence of vulnerabilities to mothers' perinatal depression. *Dev Psychopathol*. 2016;28(4pt1):895-911. doi:10.1017/S0954579416000614
13. Wen DJ, Soe NN, Sim LW, et al. Infant frontal EEG asymmetry in relation with postnatal maternal depression and parenting behavior. *Transl Psychiatry*. 2017;7(3):e1057. doi:10.1038/tp.2017.28
14. Field T, Pickens J, Fox NA, Nawrocki T, Gonzalez J. Vagal tone in infants of depressed mothers. *Dev Psychopathol*. 1995;7(2):227-231.

doi:10.1016/j.jad.2014.04.024

15. Jones NA, Field T, Fox NA, Davalos M, Lundy B, Hart S. Newborns of mothers with depressive symptoms are physiologically less developed. *Infant Behav Dev*. 1998;21(3):537-541. doi:10.1016/S0163-6383(98)90027-3

16. Baskaran A, Milev R, McIntyre RS. The neurobiology of the EEG biomarker as a predictor of treatment response in depression. *Neuropharmacology*. 2012;63(4):507-513. doi:10.1016/j.neuropharm.2012.04.021

17. Holzman JB, Bridgett DJ. Heart rate variability indices as bio-markers of top-down self-regulatory mechanisms: A meta-analytic review. *Neurosci Biobehav Rev*. 2017;74:233-255. doi:10.1016/j.neubiorev.2016.12.032

18. Wakschlag LS, Roberts MY, Flynn RM, et al. Future Directions for Early Childhood Prevention of Mental Disorders: A Road Map to Mental Health, Earlier. *J Clin Child Adolesc Psychol*. 2019;48(3):539-554. doi:10.1080/15374416.2018.1561296

19. Verduyn C, Barrowclough C, Roberts J, Tarrier N, Harrington R. Maternal depression and child behaviour problems Randomised placebo-controlled trial of a cognitive-behavioural group intervention. *Br J Psychiatry*. 2003;183:342-348. doi:10.1192/bjp.183.4.342

20. Misri S, Reebye P, Milis L, Shah S. The impact of treatment intervention on parenting stress in postpartum depressed mothers: A prospective study. *Am J Orthopsychiatry*. 2006;76(1):115-119. doi:10.1037/0002-9432.76.1.115

21. Cicchetti D, Rogosch FA, Toth SL. The efficacy of Toddler-Parent Psychotherapy for fostering cognitive development in offspring of depressed mothers. *J Abnorm Child Psychol*. 2000;28(2):135-148. doi:10.1023/A:1005118713814

22. Toth SL, Rogosch FA, Manly JT, Cicchetti D. The efficacy of toddler-parent psychotherapy to reorganize attachment in the young offspring of mothers with major depressive disorder: A randomized preventive trial. *J Consult Clin Psychol*. 2006;74(6):1006-1016. doi:10.1037/0022-006X.74.6.1006

23. Hart S, Field T, Nearing G. Depressed mothers' neonates improve following the MABI and a brazelton demonstration. *J Pediatr Psychol*. 1998;23(6):351-356. doi:10.1093/jpepsy/23.6.351

24. Onozawa K, Glover V, Adams D, Modi N, Kumar RC. Infant massage improves mother-infant interaction for mothers with postnatal depression. *J Affect Disord*. 2001;63(1):201-207. doi:10.1016/S0165-0327(00)00198-1

25. Van Doesum KTM, Riksen-Walraven JM, Hosman CMH, Hoefnagels C. A randomized controlled trial of a home-visiting intervention aimed at preventing relationship problems in depressed mothers and their infants. *Child Dev*. 2008;79(3):547-561. doi:10.1111/j.1467-8624.2008.01142.x

26. Handley ED, Michl-Petzing LC, Rogosch FA, Cicchetti D, Toth SL. Developmental cascade effects of interpersonal psychotherapy for depressed mothers: Longitudinal associations with toddler attachment, temperament, and maternal parenting efficacy. *Dev Psychopathol*. 2017;29(2):601-615.

doi:10.1017/s0954579417000219

27. Cohen NJ, Lojkasek M, Muir E, Muir R, Parker CJ. Six-month follow-up of two mother-infant psychotherapies: Convergence of therapeutic outcomes. *Infant Ment Health J*. 2002;23(4):361-380. doi:10.1002/imhj.10023

28. Stein A, Netsi E, Lawrence PJ, et al. Mitigating the effect of persistent postnatal depression on child outcomes through an intervention to treat depression and improve parenting: a randomised controlled trial. *The Lancet Psychiatry*. 2018;5(2):134-144. doi:10.1016/S2215-0366(18)30006-3

29. Ammerman RT, Altaye M, Putnam FW, Teeters AR, Zou Y, Van Ginkel JB. Depression improvement and parenting in low-income mothers in home visiting. *Arch Womens Ment Health*. 2015;18(3):555-563. doi:10.1007/s00737-014-0479-7

30. Meager I, Milgrom J. Group treatment for postpartum depression: A pilot study. *Aust N Z J Psychiatry*. 1996;30(6):852-860. doi:10.3109/00048679609065055

31. Bilszta JLC, Buist AE, Wang F, Zulkefli NR. Use of video feedback intervention in an inpatient perinatal psychiatric setting to improve maternal parenting. *Arch Womens Ment Health*. 2012;15(4):249-257. doi:10.1007/s00737-012-0283-1

32. Kersten-Alvarez LE, Hosman CMH, Riksen-Walraven JM, Van Doesum KTM, Hoefnagels C. Long-term effects of a home-visiting intervention for depressed mothers and their infants. *J Child Psychol Psychiatry Allied Discip*. 2010;51(10):1160-1170. doi:10.1111/j.1469-7610.2010.02268.x

33. Cooper PJ, Murray L, Wilson A, Romaniuk H. Controlled trial of the short- and

long-term effect of psychological treatment of post-partum depression. I. Impact on maternal mood. *Br J Psychiatry*. 2003;182:412-419. doi:10.1192/bjp.182.5.412

34. Forman DR, O'Hara MW, Stuart S, Gorman LL, Larsen KE, Coy KC. Effective treatment for postpartum depression is not sufficient to improve the developing mother-child relationship. *Dev Psychopathol*. 2007;19(2):585-602. doi:10.1017/S0954579407070289

35. Fonagy P, Sleed M, Baradon T. Randomized controlled trial of parent-infant psychotherapy for parents with mental health problems and young infants. *Infant Ment Health J*. 2016;37(2):97-114. doi:10.1002/imhj.21553

36. Clark R, Tluczek A, Wenzel A. Psychotherapy for Postpartum Depression: A Preliminary Report. *Am J Orthopsychiatry*. 2003;73(4):441-454. doi:10.1037/0002-9432.73.4.441

37. Hanson JL, Hair N, Shen DG, et al. Family poverty affects the rate of human infant brain growth. *PLoS One*. 2013;8(12). doi:10.1371/journal.pone.0080954

38. Lecrubier Y, Sheehan D V., Weiller E, et al. The Mini International Neuropsychiatric Interview (MINI). A short diagnostic structured interview: Reliability and validity according to the CIDI. *Eur Psychiatry*. 1997;12(5):224-231. doi:10.1016/S0924-9338(97)83296-8

39. Van Lieshout RJ, Yang L, Haber E, Ferro MA. Evaluating the effectiveness of a brief group cognitive behavioural therapy intervention for perinatal depression. *Arch Womens Ment Health*. 2017;20(1):225-228. doi:10.1007/s00737-016-0666-9

40. Beauchaine TP. Future Directions in Emotion Dysregulation and Youth Psychopathology. *J Clin Child Adolesc Psychol*. 2015;4416(July):1-22. doi:10.1080/15374416.2015.1038827

41. Gartstein MA, Rothbart MK. Infant Behavior Questionnaire-Revised : New evidence in support of reliability and validity Article in Child Development. *Infant Behav Dev*. 2002;26(2003):64-86. doi:10.1037/t07209-000

42. Lusby CM, Goodman SH, Bell MA, Newport DJ. Electroencephalogram patterns in infants of depressed mothers. *Dev Psychobiol*. 2014;56(3):459-473. doi:10.1002/dev.21112

43. Bell MA. Power changes in infant EEG frequency bands during a spatial working memory task. *Psychophysiology*. 2002;39(4):450-458. doi:10.1017.s0048577201393174

44. Laborde S, Mosley E, Thayer JF. Heart rate variability and cardiac vagal tone in psychophysiological research - Recommendations for experiment planning, data analysis, and data reporting. *Front Psychol*. 2017;8:1-18. doi:10.3389/fpsyg.2017.00213

45. Putnam SP, Rothbart MK, Gartstein MA. Homotypic and Heterotypic Continuity of Fine-grained Temperament during Infancy, Toddlerhood, and Early Childhood. *Infant Child Dev*. 2008;17(6):387-405. doi:10.1002/icd

46. Diego MA, Jones NA, Field T. EEG in 1-week, 1-month and 3-month-old infants of depressed and non-depressed mothers. *Biol Psychol*. 2010;83(1):7-14.

doi:10.1016/j.biopsycho.2009.09.007

47. Field T, Fox NA, Pickens J, Nawrocki T. Relative Right Frontal EEG Activation in 3- to 6-Month-Old Infants of "Depressed" Mothers. *Dev Psychol*. 1995;31(3):358-363. doi:10.1037/0012-1649.31.3.358

48. Lupien SJ, Parent S, Evans AC, et al. Larger amygdala but no change in hippocampal volume in 10-year-old children exposed to maternal depressive symptomatology since birth. *Proc Natl Acad Sci*. 2011;108(34):14324-14329. doi:10.1073/pnas.1105371108

49. Lebel C, Walton M, Letourneau N, Giesbrecht GF, Kaplan BJ, Dewey D. Prepartum and Postpartum Maternal Depressive Symptoms Are Related to Children's Brain Structure in Preschool. *Biol Psychiatry*. 2016;80(11):859-868. doi:10.1016/j.biopsych.2015.12.004

50. Glynn LM, Baram TZ. The influence of unpredictable, fragmented parental signals on the developing brain. *Front Neuroendocrinol*. 2019;53(January):100736. doi:10.1016/j.yfrne.2019.01.002

51. Puckering C, McIntosh E, Hickey A, Longford J. Mellow Babies: A group interventions for infants and mothers experienceing postnatal depression. *Couns Psychol Rev*. 2010;25(1):28-40.

52. Gentzler AL, Rottenberg J, Kovacs M, George CJ, Morey JN. Atypical development of resting respiratory sinus arrhythmia in children at high risk for depression. *Dev Psychobiol*. 2012;54(5):556-567. doi:10.1002/dev.20614

53. Goldstein BL, Shankman SA, Kujawa A, Torpey-Newman DC, Olino TM, Klein DN. Developmental changes in electroencephalographic frontal asymmetry in young children at risk for depression. *J Child Psychol Psychiatry*. 2016. doi:10.1111/jcpp.12567

54. Jones NA, Field T, Davalos M, Pickens J. EEG Stability in infants/Children of Depressed Mothers. *Child Psychiatry Hum Dev*. 1997;28(2):59-68. doi:10.1023/A

Chapter 6: Follow the leader. Elucidating the physiological mechanisms underlying adaptive mother-infant regulation of infant distress (Study 5)

Study 5 Overview

Title: Follow the leader. Elucidating the physiological mechanisms underlying adaptive mother-infant regulation of infant distress

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Context and Implications: In study 4, we found that treatment of maternal PPD with CBT was associated with improved emotion regulatory capacity in the infants of these women. PPD is also well understood to adversely affect behavior and physiology underlying mother-infant interaction patterns, particularly during moments of infant distress. Therefore, I hypothesized that CBT treatment might also be capable of adaptively altering the physiological systems that underlie mother-infant emotion regulation in the context of infant distress. During moments of distress, effective maternal regulation of infants is considered a particularly formative experience for adaptive infant self-regulation later in life.

In Study 5, we aimed to assess the physiological mechanisms underlying adaptive mother-infant synchrony patterns during infant distress. We assessed these patterns using simultaneously measured respiratory sinus arrhythmia (RSA) data, acquired on a second-by-second basis in mother-infant dyads throughout the reunion phase of the face to face still-face task. In healthy dyads free of PPD, we found that increases in maternal RSA influenced subsequent decreases in infant RSA, and this effect strengthened across the

reunion phase. We observed this same effect in the PPD dyads, but only following maternal CBT treatment. Therefore, results from study 5 show that early interventions may be capable of adaptively altering mother-infant RSA synchrony patterns during infant distress, and provide further evidence that a cost effective, short-term intervention to treat PPD may interrupt the intergenerational transmission of psychiatric risk from mother to child.

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Conflicts of Interest: None

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Abstract

This study aimed to elucidate the physiological mechanisms underlying adaptive mother-infant interaction patterns in response to infant distress. Participants were 36 healthy mothers and their typically developing infants and 36 dyads consisting of mothers with postpartum depression (PPD) and their infants. We examined whether patterns exhibited stability at two time points in healthy dyads, and whether cognitive behavioural therapy (CBT) could adaptively change patterns in PPD dyads. Respiratory sinus arrhythmia (RSA) data was acquired simultaneously in dyads to assess synchrony patterns across the reunion phase of the still-face task. We used multilevel modelling to examine if maternal (or infant) RSA predicted subsequent infant (or maternal) RSA, and if these patterns strengthened throughout the reunion phase. At both time points in healthy dyads, increases in maternal RSA predicted subsequent decreases in infant RSA, and this effect strengthened throughout the reunion. This effect was also observed in the PPD dyads, but only following CBT treatment. These findings may explain how adaptive maternal regulatory support is transmitted to infants in real time.

Introduction

Sensitive mother-infant interactions serve as the foundation for infant health and development across the lifespan[1]. The mutual regulation model (MRM) has long been used to describe the bi-directional moment-to-moment dynamics of mother-infant interaction patterns[2]. The MRM posits that mothers and their infants form a dyadic regulatory system, within which infants signal socioemotional and homeostatic needs to their mothers, and mothers provide regulatory support to their infants[3,4]. Adaptive functioning of the dyadic regulatory system across the first year of life is critical for infant neurodevelopment and subsequent emotional, behavioral and cognitive outcomes across the lifespan[1,5,6].

Optimal functioning of the dyadic regulatory system is particularly important during moments of infant distress. Due to the protracted development of prefrontal regulatory networks, infants exhibit limited self-regulatory capacity and use distress cues to signal the need for comfort and protection[7–9]. During these moments, sensitive mothers provide regulatory support to their infant to help return them to a state of calm, positive engagement[10]. Relative to non-distress moments, maternal sensitivity assessed in the presence of infant distress has been shown to better predict secure attachment, social competency and fewer behaviour problems later in life[8,11]. Therefore, effective mother-to-infant regulatory support during moments of distress is thought to provide the foundation for self-regulation across the lifespan [3]. As a result, conditions known to adversely affect a mother's ability to provide sensitive regulatory support to her infant during distress may

lead to significant long-term problems in infants across emotional, behavioral and cognitive domains [9,12].

Postpartum depression (PPD) affects up to 20% of all women in the peripartum and significantly disrupts the functioning of the dyadic regulatory system, particularly during moments of infant distress [10,13,14]. Depressed mothers display fewer caregiving behaviours and exhibit increased levels of anxiety in response to infant distress cues[15,16]. Infants also learn that their mother is unreliable and provides ineffective regulatory support[17]. Throughout the first year of life, this maladaptive functioning of the dyadic regulatory system become increasingly stable and inflexable[4] and contributes to the risks posed to offspring by PPD [12,19,20]. Therefore, examining the mechanisms underlying how the dyadic regulation system functions during infant distress is not only critical to our understanding of adaptive mother-infant interactions, but could also provide a window into how psychiatric risk is transmitted from mothers to infants, and how we might intervene to prevent this transmission.

Evidence examining the mechanisms underlying the dyadic regulatory system in the context of infant distress has largely accrued from studies that examine mother-infant synchrony patterns and from research on maternal buffering/regulatory scaffolding of infant stress and emotion regulation systems[21–25]. Synchrony patterns can be defined as the on-going relations between one dyad member's characteristics *on the subsequent* characteristics of the other dyad member as the pair interact[21]. Assessments of these patterns allows for the examination of the temporal dynamics of mother-to-infant

regulatory inputs[21,22,26,27]. However, it is not clear how these synchrony patterns function to regulate infant emotion regulatory physiology during moments of distress. Studies that examine maternal buffering/regulatory scaffolding of infant distress provide an explanation of these potential mechanisms. These studies suggest that sensitive mothers exert buffering effects on activity generated in infant stress and emotion regulatory systems during emotionally salient moments such as fear and distress[9,23,28]. However, despite this evidence, how maternal buffering/regulatory scaffolding effects are transmitted to infants on a moment-to-moment scale is not well understood. Therefore, investigating how maternal scaffolding effects are transmitted to infants by analyzing patterns of mother-infant physiological synchrony may provide a novel opportunity to elucidate the on-going functioning of the dyadic regulatory system during infant distress in real-time.

The dyadic regulatory system is thought to depend on physiological systems involved in emotion regulation and bond formation in both mothers and infants. [29] Therefore, mother-infant synchrony patterns within the parasympathetic nervous system (PSNS) may be critical to the functioning of the dyadic regulatory system during moments of distress. In adults, increased activity within the PSNS, assessed by measuring respiratory sinus arrhythmia (RSA), indexes the strength and activity of corticolimbic emotion regulatory systems critical for flexible control of emotions and behavior in response to environmental challenges [30,31]. In infants, gradual decreases in PSNS activity in the context of social interactions are thought to provide the foundation for the ability

use social interactions to effectively regulate physiological states [32]. Developing these skills is critical to adaptive emotion regulatory capacity across the lifespan[32]. As a result, it is plausible that maternal regulatory inputs may shape the development of infant emotion regulation during distress through coordinated activity in the PSNS.

Although effective emotion regulation and behavioural flexibility depend on rapid and dynamic alterations in PSNS activity in both mothers and infants, traditional methods used to assess RSA aggregate data across epochs lasting many seconds or over several minutes. Therefore, assessing activity in this system on a time scale that better reflects PSNS dynamics is needed to provide the most accurate assessment mother-infant RSA synchrony patterns [33]. Further, during moments of infant distress, sensitive mothers must alter their regulatory strategies to meet their infant's changing needs. Therefore, assessing the dynamics of mother-infant RSA synchrony patterns using statistical methods that can measure changes in mother-infant synchrony patterns throughout an interaction are needed to capture the dynamics of the dyadic regulatory system.

To our knowledge, only one other study examined associations between simultaneously assessed mother-infant RSA patterns in the context of infant distress. This study observed that increases in maternal RSA corresponded to a time-matched decrease in infant RSA[34]. However, this study did not examine whether RSA in mothers *influenced* subsequent RSA infants. Therefore, while this evidence highlighted the potential role of the PSNS in the dyadic regulation of infant distress, whether the mother-to-infant transmission of regulatory effects occur via this system is unclear. Further, this study

aggregated RSA data into 10-second epochs and did not assess the temporal dynamics of these synchrony patterns across the interaction. Therefore, examining mother-to-infant RSA synchrony effects at a resolution comparable to PSNS functioning, and determining whether these patterns change in strength across an interaction is needed. Finally, it is unknown if RSA synchrony patterns are stable across infancy in healthy dyads, or if an intervention can adaptively alter these patterns in dyads that include mothers with PPD. Taken together, a deeper understanding of how the dyadic regulatory system functions in the context of infant distress is crucial to our understanding of adaptive mother-infant interactions and may also enable us to determine whether interventions are capable of improving outcomes in dyads at risk, such as women with PPD and their infants.

This study included 36 healthy dyads free of PPD matched on infant age, sex, and familial socioeconomic status to 36 dyads lead by women diagnosed with major depression disorder in the first postpartum year. Mothers with PPD received 9-weeks of group cognitive behavioral therapy (CBT). Both groups were tested at two points (baseline and nine-weeks post baseline, before and after CBT). This study's overall goal was to elucidate the mechanisms underlying adaptive functioning of the dyadic regulatory system by examining mother-infant RSA synchrony patterns in the context of infant distress. To elucidate maternal buffering/regulatory scaffolding effects on infants in real-time, we were most interested in examining potential mother-to-infant RSA synchrony effects. We addressed these goals with three objectives. First, we examined if mother-to-infant RSA synchrony patterns in healthy dyads differed from those observed in the PPD

dyads before treatment. Second, we investigated whether these patterns were stable at both time points in the healthy dyads. Finally, we investigated whether the patterns observed in the PPD dyads following maternal CBT treatment reflected the patterns observed at both time points in the healthy dyads.

Methods

Sample

This study consisted of 72 mother-infant dyads. A cohort of 36 non-depressed healthy mother-infant dyads (referred to herein as healthy dyads) was matched to 36 dyads lead by mothers with PPD (referred to as PPD dyads) on infant sex, age, and family SES. Recruitment began in March of 2016 and completed in July 2019. Both healthy and PPD dyads were recruited from the same geographic area, including Hamilton and surrounding municipalities in southern Ontario.

Healthy dyads

The healthy dyad (n=36) group consisted of mothers who were free of PPD, fluent in English and had typically developing infants that were less than 12 months of age. Absence of current PPD was assessed using the Mini International Neuropsychiatric Interview, a validated structured psychiatric interview.[35] These dyads were recruited from the Infant Database at McMaster University's Department of Psychology Neuroscience and Behavior. Healthy dyads were recruited to examine mother-infant RSA synchrony patterns in mothers free of PPD with typically developing infants and to determine if these patterns were stable across two time points in infancy. This group also enabled us to

examine potential differences between these dyads and those with mothers with PPD (a condition known to adversely affect mother-infant interaction patterns), and if treatment was capable of adaptively modifying these patterns in PPD dyads (i.e. if patterns observed following treatment in PPD dyads reflected those observed at both visits in the healthy dyads). Finally, matching criteria were selected to try to account for potential changes that might occur as a result of the infant developmental stage or sex and to isolate the impact of maternal treatment in PPD dyads.

Postpartum depression (PPD) dyads

PPD dyads (n=36) consisted of women who had a primary diagnosis of major depressive disorder in the first year after delivery (i.e., PPD) and were patients of the Women's Health Concern Clinic at St. Joseph's Healthcare Hamilton. A mental health clinician (nurse, psychologist, or social worker) at the Women's Health Concerns Clinic diagnosed PPD using the Clinic's structured interview process according to DSM-5 criteria. A psychiatrist confirmed these diagnoses. Following the diagnosis, women with PPD were offered enrollment in the Clinic's 9-week group cognitive behavioural therapy (CBT) treatment program for PPD[36]. Women were invited to participate in the current study following the first CBT session. Dyads were ineligible to participate in the study if PPD was not the primary diagnosis, if women were diagnosed with a bipolar disorder, a personality or schizophreniform disorder, if they were unable to understand English, and/or if their infant was older than 12 months of age.

Study Design

We collected data from both healthy and PPD dyads at two time points. For healthy dyads, visit 1 occurred at a baseline session and visit 2 occurred 9-weeks after the baseline session. For PPD dyads, visit 1 took place following the first session of CBT and visit 2 data were collected after completion of treatment (9-weeks following the first CBT session). All data were collected at the Child Emotion Laboratory at McMaster University. The Hamilton Integrated Research Ethics Board approved all study protocols and each woman provided informed written consent for herself and her infant prior to completing each study visit.

CBT treatment intervention (Group Cognitive Behavioral Therapy)

Women in the PPD group received a validated 9-week group cognitive CBT intervention[36]. This treatment consisted of weekly two-hour sessions (nine sessions total), and each session was delivered by two trained therapists (psychologists, psychiatrists, social workers, and/or nurses). The first half of the program was comprised of core CBT content, and the second half consisted of psychoeducation and support with topics relevant to women with PPD. In keeping with usual treatment practice, women in the CBT group were permitted to receive any other additional treatments (e.g., psychiatric medications) while also participating in the group intervention.

Measures

Assessing the function of the dyadic regulatory system in the context of infant distress: The reunion phase of the face to face still-face task

The face to face still-face task (FFSF) is extensively used to examine the quality of mother-infant interaction patterns and provides a valid and reliable opportunity to

assess the functioning of the dyadic regulatory system during moments of infant distress[37]. Before beginning the FFSF task, we ensured that infants were in a calm but alert state and did not need to be fed or changed. Mothers placed their infants in a high-chair facing the mother at eye level and at a distance of approximately 45cm from the mother. The FFSF task proceeds in three phases, with each lasting two minutes in duration. These phases include the play phase, the still-face phase, and the reunion phase. We signaled mothers to transition between phases using small, circular red light located 1 meter above eye level on the wall opposite the mother (and completely out of the infant's view). In the play phase, we instructed mothers to play with their infants as they normally would at home. Mothers could sing, talk to, and touch their infants, but were instructed to refrain from removing their infant from the high-chair or from presenting their infant with a pacifier or any toys. In the still-face phase, mothers were instructed to maintain eye contact with their infant but to adopt an expressionless 'poker' face. Mothers were also not permitted to touch or speak to their infant during this phase. The still-face phase causes a significant disruption in the mother-infant interaction, leading to increased distress and negative affectivity in the infant[37,38]. Finally, in the reunion phase, mothers were permitted to reengage with their infants in the same way they did during the play phase. The reunion phase has been identified as a critical component of the still-face task, whereby the dyad is challenged with repairing the interaction while the infant remains distressed from the still-face phase [2,10,38]. Therefore, the reunion phase provides a valuable opportunity to assess dyadic regulatory capacity, particularly the mother's ability

to soothe her infant and provide regulatory support[39]. During this phase, mothers with PPD tend to struggle with repairing their interaction with their infants and display fewer and less flexible regulatory emotions and behaviours[40–42]. Therefore, this phase provides critical information on the quality of the dyadic regulatory system. Each dyad completed the FFSF in the same orientation in the same room at the Child Emotion Laboratory at McMaster University.

Physiological Data: Mother infant Respiratory sinus arrhythmia (RSA)

Acquisition. Electrocardiogram (ECG) data were acquired simultaneously in both mothers and their infants throughout the reunion phase using the Mindware 3000A Wireless data acquisition system (Mindware Technologies, Gahanna, OH). Prior to recording, two disposable pediatric ECG electrode stickers were attached to the infant's back (in order to prevent infants from picking at the stickers or pulling on the wires), and three adult ECG electrodes were placed on the mother's torso (right clavicle, and bilaterally below the left and right rib cage). Both dyad members were connected to the Mindware mobile ECG data acquisition unit which transmitted data wirelessly to a laptop running Biolab data acquisition software (v 3.2.3). The laptop was located in an adjacent room so that ECG signal quality could be checked by a research assistant prior to recording. The data acquisition frequency was 500Hz. Data from both mothers and infants were time-locked to one another via the Biolab software.

ECG data were cleaned and analyzed offline using Mindware HRV (v 3.3.2) analysis software. Data were visually inspected for artifacts, and artifacts were corrected

by a graduate student trained by Mindware scientists and blind to group and visit status. Mother and infant files were analyzed separately. Erroneously identified or missed R-wave peaks were manually edited or inserted where applicable. Consistent with other research examining mother-offspring physiological data, 60-second epochs of ECG data that required >10% editing were eliminated from further analysis [43]. Since we set out to test mother-infant regulatory patterns during moments of infant distress, we only considered data acquired during the reunion phase of the FFSF. Therefore, clean ECG data for both dyad members during the reunion phase were needed for inclusion in the analysis. For healthy dyads, n=2 infants and n=1 mother did not have analyzable data, and technical equiptment errors resulted in lost data for n=1 dyad. For PPD dyads, data were unanalyzable for n=4 infants, n=2 mothers, and technical errors resulted in lost data for n=4 dyads. Therefore, clean ECG data were available for 32 healthy dyads and 26 PPD dyads.

Second-by-second RSA analyses. Second-by-second respiratory sinus arrhythmia (RSA) data were analyzed using the RSAseconds toolbox in Matlab[33,44]. Cleaned ECG traces were entered into the program separately for both mothers and infants. The PSNS shifts rapidly to support adaptive emotion and behavioral responses to environmental challenges[45]. Therefore, acquiring RSA on a second-by-second scale enables us to acquire a more precise measure of mother-infant RSA synchrony patterns relative to traditional methods that aggregate RSA data across 10-120 second epochs[33]. The RSAseconds program utilizes a peak-matched multiple window (PM MW) multitaper

algorithm and a short time Fourier transform (STFT) to obtain RSA power on a much shorter time scale relative to traditional methods. This approach uses a shifting 32-second epoch to apply the PM MW and subsequently, the STFT to each epoch. Each epoch shifts by 1 second and overlaps the previous epoch by 31 seconds (thus, the first epoch contains data from 1 to 32 seconds, the next from 2 to 33, then from 3 to 34 until the end of the file). (see Gates et al., 2015; Hansson & Jönsson, 2006 for technical descriptions of these methods). This method yielded RSA data in 1-second epochs throughout the reunion phase in both mothers and infants. In keeping with North America Society of Pacing and Electrophysiology, RSA power was extracted between 0.24-1.04Hz for infants and 0.12-0.40Hz for mothers[46].

Predictor variable: Grand mean centered RSA lagged at 1 second for mothers and infants.

Grand mean centered, lagged RSA predictor variables were created for both mothers and infants (grand means were calculated separately for mothers and infants)[47]. Lagged variables were created to examine whether RSA assessed at a given time point (the grand mean centered, lagged RSA predictor variable) influenced RSA assessed at the next second. A lag length of 1-second was selected since parasympathetic influences on the heart can shift on a beat-to-beat scale in response to environmental conditions[45].

Outcome variable: log transformed raw RSA in mothers and infants.

Outcome variables for both mothers and infants consisted of log-transformed raw RSA values. These RSA outcome variables were measured 1 second after the grand-mean centered lagged, RSA predictor variables. Therefore, for both mothers and infants, grand

mean centered, lagged RSA predictor variables were used to predict subsequent log-transformed raw RSA values. This enabled us to examine mother-infant RSA synchrony patterns on a second-by-second basis.

Changes in symptoms of PPD dyads before and after CBT treatment

Edinburg Postnatal Depression Scale (EPDS). Changes in PPD symptoms were assessed at both visits using the 10-item EPDS, the most widely used measure of PPD symptoms in clinical and research settings[48]. This was used to investigate whether our 9-week group CBT treatment resulted in reductions in PPD symptoms. Women indicated the degree to which they exhibited certain emotions and behaviours in the past seven days using a 4-point scale. Higher scores indicate higher symptoms of PPD, with a maximum score of 30 points. A 4-point decrease in EPDS scores is considered clinically significant. Cronbach's α values were 0.88 at visit 1 and 0.86 at visit 2

Statistical analysis

We assessed differences in demographic characteristics between healthy and PPD groups using independent sample t-tests and chi-squared tests where appropriate. In the PPD group, a paired sample t-test was used to examine the differences in maternal depression scores before and after CBT treatment.

The Stability and Influence model

The stability and influence model was used to examine mother-infant RSA synchrony patterns on a second-by-second basis. This model uses person period pairwise format data and multilevel modelling to simultaneously assess RSA stability effects (i.e.,

autoregressive effects) and RSA influence effects (i.e., cross-lagged effects) within dyad members as the pair interact. Stability effects measure the effect that a given dyad member's grand mean centered, lagged RSA at has *their own* log-transformed raw RSA measured at the next second. Influence effects estimate the effect of a given dyad member's grand mean, lagged centered RSA on *their partner's* log-transformed raw RSA measured at the next second (e.g., the effect of a mother's lagged RSA on their infant's raw RSA measured at the next second). Stability effects are estimated while accounting for influence effects, and influence effects are estimated while accounting for stability effects. Finally, moderator variables can be added to the model to determine whether stability and influence effects change depending on a particular variable. To capture the constant adjustments and adaptations made by sensitive mothers to regulate their infant during moments of distress, we investigated whether time across the reunion phase (measured in seconds) moderated the RSA influence effects (see figure 1 for visual depiction of the effects tested in the stability and influence model).

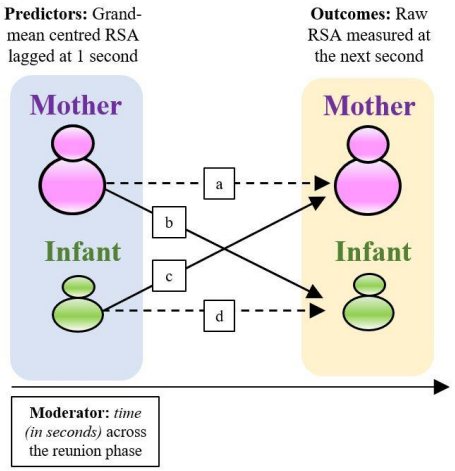


Figure 1: Stability and influence model: Dotted arrows (pathways a and d represent stability effects, and solid arrows (pathways b and c) represent influence effects. Time across the reunion phase (in seconds) can be investigated as a moderator of stability and influence effects. These pathways were examined at two study visits in healthy dyads as well as before and after maternal CBT treatment in PPD.

Using the stability and influence model to address our objectives: The overall goal of this study was to investigate the mechanisms underlying adaptive functioning of the dyadic regulatory system using mother-infant RSA synchrony patterns during infant distress. Addressing this goal required two steps. First, for both healthy and PPD dyads at both study visits, we assessed whether the RSA influence effects were moderated by dyad member (mother or infant) and time across the reunion phase using a 3-way interaction variable (See figure 5 in Thorson et. al., 2018 for the syntax used in the current study). A significant three-way interaction indicates that either the mother-to-infant and/or the infant-to-mother RSA influence effect changed in strength across the reunion phase.

The second step involved interpreting this interaction to determine whether the mother-to-infant and/or infant-to-mother RSA influence effects were moderated by time across the reunion. This was done by examining mother-to-infant RSA influence effects and infant-to-mother RSA influence effects in separate models. These interpretations were used to address our three objectives. To address objective 1, we examined whether the mother-to-infant RSA influence effect differed between the healthy and PPD dyads at visit 1. To address objective 2, we examined whether this influence effect observed in healthy dyads were stable across visit 1 and visit 2. Finally, to address objective 3, we examined whether the influence effect changed in PPD dyads following CBT treatment,

and if these patterns reflected those observed in the healthy dyads at both visits. Finally, as per the recommendations described in Thorson et. al., 2018, random effects were fully saturated in each of our models. Models were conducted using SAS University software.

Results

Demographics

Study demographics are presented in Table 1. There were no differences between the healthy and PPD dyads on any demographic variables. At visit 1, mothers were on average 32.6 (SD=4.7) years of age, and infants were 5.4 (2.5) months of age. The average infant birth weight (in grams) was 3359.12 (489.0) and, the average gestational age was 39.4 (1.7) weeks. The mean household income for the sample was $84,094.8 (32,450.0) Canadian dollars. 91.4% of women were married or in common-law relationships. In the PPD dyads, 17% were diagnosed with a comorbid anxiety disorder and 18 were taking antidepressant medication. Of the women taking antidepressants, 5 changed their dose during the CBT treatment period. Finally, in women with PPD, we observed a clinically significant decrease in EPDS scores between pre and post-treatment (change in EPDS=5.0, p=0.002).

Of the 36 PPD dyads that participated in the still-face task, clean mother and infant RSA data were available for 26 (72%) dyads. For healthy dyads 36 participated in the still-face task and mother-infant RSA data were available for 32 (88%). There were no significant differences in any of our demographic variables between dyads with and without both mother and infant RSA data.

Table 1: Sample Characteristics

	Healthy dyads (n=32)	PPD dyads (n=26)	*p*
Infant age at visit 1 (m, SD)	5.3 (2.5)	5.4 (2.5)	0.84
Infant Sex			
male (n,%)	12 (38)	11(42)	0.79
Income (n,%)			
<49 999	5 (15.6)	4 (15.4)	0.97
50-79,999	8 (25.0)	7 (26.9)	
>80,000	19 (59.4)	15 (57.7)	
Marital Status (n,%)			
Single	2 (6.3)	2 (7.7)	0.57
Separate	0 (0.0)	1 (3.8)	
Common Law	5 (15.6)	6 (23.1)	
Married	25 (78.1)	17 (65.4)	
Maternal education (n,%)			
High school or less			
College or certificate	1 (3.1)	1 (3.8)	0.70
University or higher	8 (25.0)	9 (34.6)	
	23 (71.9)	16 (61.5)	
Mother's Age (m,SD)	33.3 (5.2)	31.9 (4.1)	0.29
Parity (n,%)			
Primiparous	17 (53.1)	13 (50.0)	1.0
multiparous	15 (46.9)	13 (50.0)	
Mother's birth country (n,%)			
Canada	26 (81.3)	24 (92.3)	0.21
Elsewhere	6 (18.8)	2 (7.7)	
Birthweight (grams, m SD)	3336.5 (463.6)	3386.9 (526.6)	0.70
Gestational age (months, m, SD)	39.2 (0.82)	39.6(2.45)	0.44

Healthy Dyads: 19 had data at both visits, n=6 had data at visit 1 but not visit 2; n=5 had data at visit 2 but not visit 1) therefore, the total number of healthy dyads included in this study (that had data at one visit or both visits was n=32

PPD dyads: n=23 had data at both visits, n=2 had usable data at in visit 1 but not visit 2, n=3 had data at visit 2 but not visit 1. Therefore, the total number of PPD dyads that had data at one or both visits was n= 26.

Mother-infant RSA influence effects across the reunion phase

For healthy and PPD dyads at both visit 1 and visit 2, we observed a significant 3-way interaction between the RSA influence effect, dyad member (mother/infant), and time (in seconds across the reunion phase) on RSA assessed at the next second (Table 2).

This means that at both visits for healthy and PPD dyads, either the mother-to-infant and/or the infant-to-mother RSA influence effects strengthened across the reunion phase.

Table 2: Time by dyad member interaction on physiological influence

					95% CI	
Visit 1	dyad	Effect Estimate	*SE*	*p*	Lower	Upper
	Healthy	.0003	$6.9x10^{-5}$	<0.001	.0001	.0004
	PPD	.0001	$5.2x10^{-5}$	0.04	$4.1 x10^{-6}$	.0002
Visit 2						
	Healthy	.0002	$6.4 x10^{-5}$	0.012	$3.6 x10^{-5}$	.0003
	PPD	.0002	$5.7 x10^{-5}$	<0.001	.0001	.0004

Therefore, the next step was to interpret these interactions to determine which dyad member's influence effect was strengthening across the reunion phase (are the significant 3-way interactions accounted for by the mother-to-infant or the infant-to-mother RSA influence effect, or are both influence effects significant). Each of these interpretations were carried out separately for PPD dyads and healthy dyads at visit 1 and visit 2.

Objective 1: RSA influence patterns in healthy and PPD dyads at visit 1

Healthy dyads

Both mother-to-infant and infant-to-mother RSA influence effects were moderated by time across the reunion phase. Increases in maternal RSA influenced decreases in infant RSA measured at the next second, and this effect strengthened across the reunion phase [B=-0.00027, p=0.02]. Additionally, increases in infant RSA influenced increases in maternal RSA measured at the next second, and this effect strengthened across the reunion phase [B=0.00031, p<0.001] (Figure 2a). Therefore, at baseline, mothers

influenced infants and infants influenced mothers, and these effects strengthened across the reunion phase.

PPD Dyads

Contrary to our observations in the healthy dyads, only the infant-to-mother influence effect was observed in the PPD dyads prior to treatment at visit 1. Increases in infant RSA influenced increases in maternal RSA at the next second, and this effect strengthened across the reunion phase [B=0.00026, $p<0.001$] (Figure 2b). However, the maternal influence effect on the infant was not significant. Therefore, healthy and PPD dyads appear to exhibit different RSA synchrony patterns at visit 1, particularly in regards to the mother-to-infant RSA influence effect across the reunion phase.

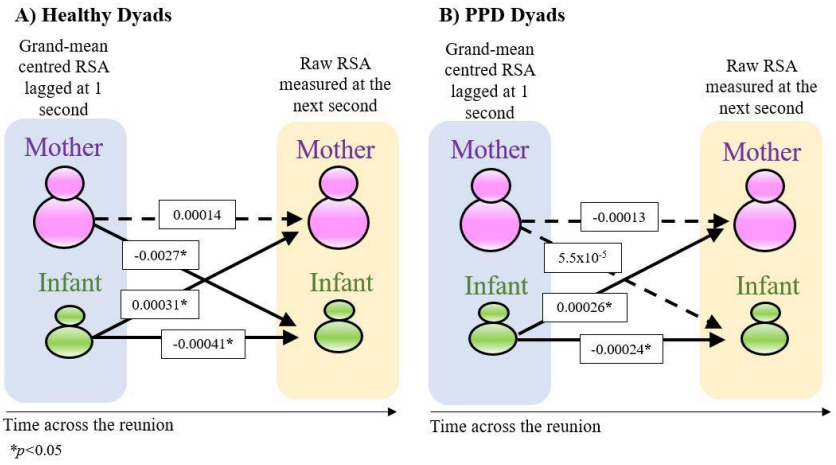


Figure 2: Mother-to-infant and infant-to-mother influence effects moderated by time across the reunion phase at visit 1 for healthy and PPD dyads. Solid arrows indicate effects that were statistically significant *A) Healthy dyads*: Increases in maternal RSA influenced decreases in subsequent infant RSA and this effect strengthened across the reunion phase. Additionally, increases in infant RSA influenced increases in subsequent maternal RSA, and this effect also strengthened across the reunion phase *B) PPD dyads:* Increases in infant RSA influenced subsequent increases maternal RSA, and this effect strengthened over the course of the reunion phase. No mother-to-infant effect was observed prior to treatment.

Objective 2: Stability of mother-infant RSA influence patterns in healthy dyads

At 9-weeks post baseline in the healthy dyads, we again observed that increases in maternal RSA influenced decreases in infant RSA at next second, and this effect strengthened across the reunion phase [B=-0.00021, p=0.02]. However, the infant-to-mother effect that was observed at visit 1 was no longer significant (Figure 3a). Therefore, at baseline and 9-weeks post baseline in healthy dyads, a similar mother-to-infant RSA synchrony pattern was observed across the reunion phase.

Objective 3: Examining if influence effects change in PPD dyads following maternal CBT treatment

Following maternal CBT treatment, the PPD dyads exhibited a significant mother-to-infant RSA influence effect that reflected the effects observed at both visits in the healthy dyads. Increases in maternal RSA influenced decreases in infant RSA at the next second, and this effect strengthened across the reunion phase [B=-0.00055, p=0.01). Additionally, following treatment, the infant-to-mother effect was no longer significant (See Figure 3b). Therefore, following maternal treatment, mother-infant RSA synchrony patterns shifted to reflect those that were observed at both visits in the healthy dyads.

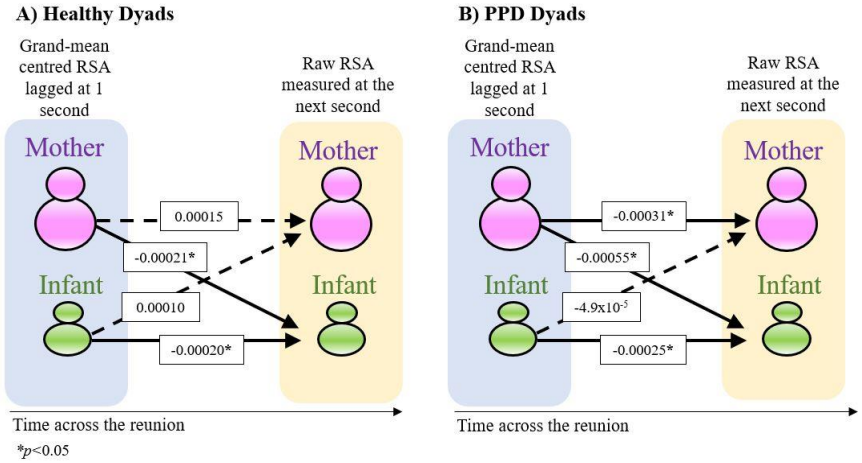


Figure 3: Mother-to-infant and infant-to-mother influence effects moderated by time across the reunion phase at visit 2. Solid arrows indicate effects that were statistically significant *A) Healthy dyads:* increases in maternal RSA influenced subsequent decreases in infant RSA, and this effect strengthened throughout the reunion phase. Infant-to-mother effects did not change. *B) PPD dyads:* Following CBT treatment, PPD dyads exhibited mother-to-infant RSA influence patterns that reflected those observed in the healthy dyads at both visits. The infant-to-mother effect was not significant.

Discussion

This study aimed to elucidate the mechanisms underlying the dyadic regulatory system during infant distress. In the healthy dyads at visit 1, increases in maternal RSA influenced subsequent decreases in infant RSA, and this effect strengthened across reunion phase. Additionally, increases in infant RSA influenced subsequent decreases in maternal RSA patterns, and this effect strengthened across the reunion phase. Conversely, before treatment in the PPD group, only an infant-to-mother RSA influence effect was detected. Therefore, at visit 1, maternal RSA influence patterns differed between healthy and PPD dyads. At visit 2, the healthy dyads exhibited a mother-to-infant RSA influence effect similar to that observed in these dyads at visit 1. Therefore, healthy dyads exhibited

similar mother-to-infant RSA synchrony patterns at both visits. Following maternal CBT treatment, the PPD dyads exhibited a mother-to-infant RSA influence effect that reflected those observed in the healthy dyads at both visits. In summary, results show that healthy dyads exhibit similar mother-to-infant RSA influence effects across two time points in infancy, and that CBT treatment in mothers with PPD may be associated with adaptive alterations in mother-infant RSA synchrony patterns.

Infants possess limited self-regulatory abilities and therefore require maternal support when they are distressed or overwhelmed. During these moments, adaptive functioning of the dyadic regulatory systems requires that mothers provide sensitive regulatory inputs to help their infants navigate through this distress. Evidence from the field of social buffering and maternal regulatory scaffolding of infant distress posits that sensitive mothers exert regulatory effects on offspring stress sensitivity and emotion regulatory systems[24,49]. Indeed, young children exhibit buffered amygdala activity and a mature inverse coupling of the amygdala-medial prefrontal cortex circuit in response to affective challenges, but only in the presence of their mothers [28]. Further, animal models have shown that the presence of a sensitive mother blocks amygdala-dependent fear learning in offspring and buffers hyperactivity in stress response systems during moments of distress[23,50]. Despite this evidence, how these regulatory effects are transmitted from mothers to their infants is not well understood. By utilizing a mother-infant synchrony framework, we were able to show that maternal regulatory scaffolding inputs may be transmitted to offspring in real-time though joint coordination of the parasympathetic

nervous system. Therefore, by combining methods from synchrony and social buffering/maternal scaffolding fields, this study provides the first evidence of the potential adaptive physiological mechanisms that underly the transmission of maternal regulatory support of infant distress in real-time. This may be a critical component of an adaptive dyadic regulatory system.

To our knowledge, only one other study examined simultaneously assessed associations between RSA patterns in mothers and infants during the FFSF. This study reported on a concurrent negative (i.e., inverse) association between mother and infant RSA during the reunion phase. In the current study, we extend upon these findings by showing maternal RSA appears to *influence* infant RSA, and that these effects strengthen across the reunion phase. Further, our study examined these effects at a resolution comparable to the functioning of the PSNS (on a second by second scale). We also provide the first evidence on the stability of these patterns at two time points in healthy dyads, and the potential malleability of these patterns in dyads of women with PPD before and after CBT treatment.

Increases maternal RSA influenced subsequent decreases in infant RSA at both study visits in healthy dyads, but only following treatment in PPD dyads. This mother-to-infant RSA influence pattern could reflect an active dyadic regulatory response to infant distress. This active response likely involves the mother's ability to accurately read infant cues and provide regulatory support, but also depends on the infant's ability to reengage with the mother. At the beginning of the reunion phase, we suspect that the mother

immediately increases activity in multiple prefrontal areas, the anterior cingulate and the superior frontal gyrus. Greater activity in these regions is indexed by increased RSA[31], is associated with accurately deciphering infant cues, and with initiating and maintaining appropriate regulatory behaviours[51,52]. Since these brain areas are not fully developed in infants, our results may suggest that during moments of distress activity from higher-order networks in mothers is transmitted to infants via coupling of the PSNS to provide the infant with regulatory capabilities that they do not yet possess on their own. This is in keeping with why mothers and infants are theorized to form the dyadic regulatory system in the first place[2–4]. The increases in maternal RSA influence decreases in infant RSA at the next second. This may reflect the infant's response to the regulation provided by the mother since gradual RSA decreases reflect the infants ability to mobilize physiological resources needed to engage in social interactions[32,53]. The maturation of the PSNS, particularly the development of RSA withdrawal (e.i., RSA decreases in response to socioemotional challenge) plays a critical role in the development of emotion regulation in the context of social interactions[32,45]. Therefore, it is plausible that maternal regulatory scaffolding exerts its effects on infants via this system. Finally, we also observed that this effect strengthened throughout the reunion phase. Evidence suggests that the infant amygdala retains distress information even after distressing stimuli are no longer present[54,55]. Therefore, while a particular maternal behaviour can temporarily soothe a distressed infant; distress cues subsequently return unless the mother can adjust her regulatory strategies to meet these changing infant needs. As a result, maternal

adaptations to infant cues may improve throughout the reunion and explain why the mother-to-infant influence effect strengthened. In summary, our results may suggest that maternal high order regulatory networks could be doing the 'heavy lifting' of providing the regulatory scaffolding support of infants in distress. This subsequently provides the infant with opportunities to reengage with the mother.

Before treatment in the PPD dyads, we observed a direct infant-to-mother effect that strengthened throughout of the reunion phase. Given the importance of maternal emotion regulatory scaffolding of infant distress[24], the fact that the mother-to-infant effect did not change across the reunion might indicate an inflexible, maladaptive dyadic regulatory system. We speculate that this infant-to-mother effect may reflect a passive dyadic response to the reunion phase. Indeed, increasing RSA in both mothers and infants may suggest that the dyad is relieved by the cessation of the stressful still-face phase, but do not initiate an active recovery response. Mothers with PPD consistently lack efficacy in mounting an effective regulatory response to infant distress[17]. This may be driven by hypoactivity in the left orbitofrontal cortex, which has been observed in women with PPD in the context of negative infant stimuli[51,56]. Since left OFC activity is needed to initiate approach behaviours in the presence of negative stimuli[57], hypoactivity in this region in women with PPD may be associated with a decreased likelihood that these mothers will attempt to mount a regulatory response to infant distress cues. This may also decrease the likelihood that the infant will look to the mother for regulatory support[7]. Taken together, prior to treatment, mothers appear to be incapable of initiating and an active regulatory

response. Additionally, infants are not provided with opportunities to reengage in the interaction and may not look to the mother for regulatory support.

Following maternal treatment for PPD, dyads exhibited a mother-to-infant RSA influence effect that was similar to the effects observed in healthy dyads at both time points during infancy. Previous studies examining mother-infant interaction patterns following maternal treatment for PPD have reported on improved maternal responsiveness and better anticipation of the infant's needs[58,59]. Therefore, rather than passively reacting to infant signals, mothers may be more capable of mobilizing an active regulatory response to infant distress. Finally, over the course of CBT treatment, infants may be gradually exposed to a mother who is able to meet their needs more consistently. Due to the plasticity of the infant PSNS during the first year of life, this system can reorganize and update its circuitry in response to changing socioemotional environmental conditions[7,32]. As a result, infants may begin to seek out and utilize socioemotional inputs from the mother when distressed[32]. Therefore, our evidence suggests that treating maternal PPD may equip mothers with the skills needed to read infant cues and actively regulate their distressed infant, and infants may become more receptive to this support provided by mothers.

The results of this study should be interpreted in the context of the following limitations. First, we were unable to assess whether the mother-to-infant RSA synchrony patterns observed at both visits in the healthy dyads and following treatment in PPD dyads were present beyond visit 2. However, evidence suggests that mothers exhibit

buffering effects on offspring corticolimbic circuitry for up to 10 years, so we may expect to see these patterns during age-appropriate distress simulations throughout childhood, but not into adolescence[28]. Future studies should include longer follow-up periods to examine these physiological synchrony patterns beyond infancy. Second, since parasympathetic activity indexes multiple neural circuits, we could only speculate on the neurophysiological networks that give rise to our observed patterns. Future studies should consider acquiring dyadic RSA data in combination with methods that have a high degree of spatial resolution. Indeed, hyperscanning techniques using functional near-infrared spectroscopy (fNIRS) could enable researchers to explicitly test whether RSA influence effects are driven by activity within maternal higher-order regulatory brain areas. Third, we were unable to observe what happens to RSA influence patterns in the moments following the reunion phase when the mother is permitted to hold her infant, provide a pacifier, toy or feed her infant. Since mother-infant interaction patterns differ depending on context (e.g., resting play, feeding, distress)[8,21,60] investigating what happens to RSA influence patterns during these contexts would be an important objective for future studies. Finally, we were unable to rule out whether changes in synchrony patterns observed following CBT in PPD dyads were due to factors other than treatment. However, decades of evidence have shown that impaired mother-infant interaction patterns become increasingly stable and inflexible in dyads lead by mothers with PPD[3,4,10,61]. Additionally, adverse mother-offspring RSA synchrony patterns have been observed in late childhood in mothers with a history of depression during a child's life[61].

Based on this evidence, we believe that it would be highly unlikely for a cohort of dyads led by mothers with untreated, clinically significant PPD to spontaneously begin to exhibit similar mother-to-infant RSA influence patterns that were observed at two time points in infancy in healthy dyads.

Mother-infant interactions serve as the foundation for infant health and development across the lifespan. Moments of infant distress are emotionally salient and cue the mother that the infant requires safety, protection and comfort. Therefore, the functioning of the dyadic regulatory system during these moments is critical. Analyses of synchrony provide a window into how mother-infant interactions unfold in real time and research on social buffering/maternal scaffolding has reported on the effects of maternal regulatory scaffolding on infant stress and emotion regulatory physiology. This study combined methods from these two field to examine how maternal regulatory inputs are transmitted to infants on a moment-to-moment basis. In healthy dyads at two-time points in infancy, increases in maternal RSA influenced subsequent decreases in infant RSA, and this effect strengthened over the course of the reunion phase. This effect was also observed in the PPD dyads, but only after the mothers received CBT treatment for PPD. Therefore, we show for the first time that ongoing real-time coordination of the parasympathetic nervous system in mother-infant dyads may underly adaptive functioning of the dyadic regulatory system during infant distress. Given that effective maternal regulation of infant distress is a particularly formative experience for the development of self-regulation across the lifespan, this research not only provides

important evidence on how healthy mothers actively regulate their infant's distress in real time, but also shows that interventions may be capable of adaptively altering this system, and may interrupt the intergenerational transmission of risk.

References

1. Cole, P. M., Martin, S. E. & Dennis, T. A. Emotion Regulation As A Scientific Construct-Methodological Challenges And Directions For Child Development Research. *Child Dev.* **75**, 317–333 (2004).
2. Gianino, A. & Tronick, E. . The mutual regulation model: The infant's self and interactive regulation and coping and defensive capacities. (1988) doi:10.1016/j.paid.2014.03.014.
3. DiCorcia, J. A. & Tronick, E. D. Quotidian resilience: Exploring mechanisms that drive resilience from a perspective of everyday stress and coping. *Neurosci. Biobehav. Rev.* **35**, 1593–1602 (2011).
4. Tronick, E. & Beeghly, M. Infants' meaning-making and the development of mental health problems. *Am. Psychol.* **66**, 107–119 (2011).
5. Raby, K. L., Roisman, G. I., Fraley, C. R. & Simpson, J. The Enduring Predictive Significance of Early Maternal Sensitivity: Social and Academic Competence Through Age 32 Years. *Child Dev.* **86**, 695–708 (2015).
6. Bernier, A., Calkins, S. D. & Bell, M. A. Longitudinal Associations between the Quality of Mother-Infant Interactions and Brain Development across Infancy. *Child Dev.* **87**, 1159–1174 (2016).
7. Atzil, S., Gao, W., Fradkin, I. & Barrett, L. F. Growing a social brain. *Nat. Hum. Behav.* **2**, 624–636 (2018).
8. Leerkes, E. M., Weaver, J. M. & O'Brien, M. Differentiating Maternal Sensitivity

to Infant Distress and Non-Distress. *Parenting* **12**, 175–184 (2012).

9. Tottenham, N. Early Adversity and the Neotenous Human Brain. *Biol. Psychiatry* **16**, 1–10 (2019).
10. Tronick, E. & Reck, C. Infants of Depressed Mothers. *Harv. Rev. Psychiatry* **17**, 147–156 (2009).
11. Leerkes, E. M. *et al.* Differential Effects of Maternal Sensitivity to Infant Distress and Nondistress on Social- Emotional Functioning Published by : Wiley on behalf of the Society for Research in Child Development Stable URL : http://www.jstor.org/stable/29738652 Differential. *Wiley behalf Soc. Res. Child Dev.* **80**, 762–775 (2009).
12. Field, T. The Effects of Mother's Physical and Emotional Unavailability on Emotion Regulation. *Monogr. Soc. Res. Child Dev.* **59**, 208–227 (1994).
13. Gaynes, B. N. *et al.* Perinatal depression: prevalence, screening accuracy, and screening outcomes. *Evid. Rep. Technol. Assess. (Full. Rep).* 1–8 (2005).
14. Field, T., Healy, B., Goldstein, S. & Guthertz, M. Behavior-State Matching and Synchrony in Mother-Infant Interactions of Nondepressed Versus Depressed Dyads. *Dev. Psychol.* **26**, 7–14 (1990).
15. Akbari, E., Gonzalez, A., Dundin, A., Steiner, M. & Flemming, A. Depressed Mothers Show Increased Anxiety and Negativity and Differential Salivary Cortisol in Response to Infant Cries. *J. Depress. Anxiety* **s3**, (2015).
16. Esposito, G., Manian, N., Truzzi, A. & Bornstein, M. H. Response to infant cry in

clinically depressed and non-depressed mothers. *PLoS One* **12**, 1–15 (2017).

17. Tronick, E. Emotions and Emotion Communication in Infants. *Am. Psychol.* **44**, 112–119 (1989).
18. Field, T., Diego, M. & Hernandez-Reif, M. Depressed mothers' infants are less responsive to faces and voices. *Infant Behav. Dev.* **32**, 239–244 (2009).
19. Goodman, S. H. *et al.* Maternal Depression and Child Psychopathology: A Meta-Analytic Review. *Clin. Child Fam. Psychol. Rev.* **14**, 1–27 (2011).
20. Murray, L., Fiori-cowley, A. & Hooper, R. The Impact of Postnatal Depression and Associated Adversity on Early Mother-Infant Interactions and later Infant Outcome. *Child Dev.* **67**, 2512–2526 (1996).
21. Feldman, R. Parent-infant synchrony and the construction of shared timing; physiological precursors, developmental outcomes, and risk conditions. *J. Child Psychol. Psychiatry Allied Discip.* **48**, 329–354 (2007).
22. Feldman, R. Infant Biological Foundations Synchrony and Developmental Outcomes. *Curr. Dir. Psychol. Sci.* **16**, 340–345 (2007).
23. Gunnar, M. R., Hostinar, C. E., Sanchez, M. M., Tottenham, N. & Sullivan, R. M. Parental buffering of fear and stress neurobiology: Reviewing parallels across rodent, monkey, and human models. *Soc. Neurosci.* **10**, 474–478 (2015).
24. Tottenham, N. Social scaffolding of human amygdala-mPFCcircuit development. *Soc. Neurosci.* **10**, 489–499 (2015).
25. Hostinar, C. E., Sullivan, R. M. & Gunnar, M. R. Psychobiological mechanisms

underlying the social buffering of the hypothalamic-pituitary-adrenocortical axis: A review of animal models and human studies across development. *Psychol. Bull.* **140**, 256–282 (2014).

26. Feldman, R. The Neurobiology of Human Attachments. *Trends Cogn. Sci.* **xx**, 1–20 (2016).
27. Feldman, R. Sensitive periods in human social development: New insights from research on oxytocin, synchrony, and high-risk parenting. *Dev. Psychopathol.* **27**, 369–395 (2015).
28. Gee, D. G. *et al.* Maternal buffering of human amygdala–prefrontal circuitry during childhood but not adolescence. *Psychol. Sci.* **25**, 2067–2078 (2014).
29. Beauchaine, T. P. Future Directions in Emotion Dysregulation and Youth Psychopathology Future Directions in Emotion Dysregulation and Youth Psychopathology. *J. Clin. child Adolesc. Psychol.* **44**, 875–896 (2015).
30. Thayer, J. F. & Lane, R. D. A model of neurovisceral integration in emotional regulation and dysregulation. *J. Affect. Disord.* **61**, 201–216 (2000).
31. Thayer, J. F., Åhs, F., Fredrikson, M., Sollers, J. J. & Wager, T. D. A meta-analysis of heart rate variability and neuroimaging studies: Implications for heart rate variability as a marker of stress and health. *Neurosci. Biobehav. Rev.* **36**, 747–756 (2012).
32. Porges, S. W. & Furman, S. A. The Early Development of the Autonomic Nervous System Provides a Neural Platform for Social Behaviour: A Polyvagal Perspective.

Infant Child Dev. **20**, 106–118 (2011).

33. Gates, K. M., Gatzke-Kopp, L. M., Sandsten, M. & Blandon, A. Y. Estimating time-varying RSA to examine psychophysiological linkage of marital dyads. *Psychophysiology* **52**, 1059–1065 (2015).

34. Ostlund, B. D., Measelle, J. R., Laurent, H. K., Conradt, E. & Ablow, J. C. Shaping emotion regulation: attunement, symptomatology, and stress recovery within mother–infant dyads. *Dev. Psychobiol.* **59**, 15–25 (2017).

35. Lecrubier, Y. *et al.* The Mini International Neuropsychiatric Interview (MINI). A short diagnostic structured interview: Reliability and validity according to the CIDI. *Eur. Psychiatry* **12**, 224–231 (1997).

36. Van Lieshout, R. J., Yang, L., Haber, E. & Ferro, M. A. Evaluating the effectiveness of a brief group cognitive behavioural therapy intervention for perinatal depression. *Arch. Womens. Ment. Health* **20**, 225–228 (2017).

37. Tronick, E. Z., Als, H., Adamson, L. & Brazelton, B. T. The Infant's Response to Entrapment between Contrdictory Messages in Face-to-Face Interaction. *American Academy of Child Psychiatry* 1–13 (1978).

38. Mesman, J., van IJzendoorn, M. H. & Bakermans-Kranenburg, M. J. The many faces of the Still-Face Paradigm: A review and meta-analysis. *Dev. Rev.* **29**, 120–162 (2009).

39. Coppola, G., Aureli, T., Grazia, A. & Ponzetti, S. Reunion Patterns in the Still-Face Paradigm as Predicted by Maternal Sensitivity and Dyadic Coordination.

Infancy **21**, 453–477 (2015).

40. Weinberg, M. K., Olson, K. L., Beeghly, M. & Tronick, E. Z. Making up is hard to do, especially for mothers with high levels of depressive symptoms and their infant sons. *J. Child Psychol. Psychiatry Allied Discip.* **47**, 670–683 (2006).

41. Reck, C. *et al.* Interactive coordination of currently depressed inpatient mothers and their infants during the postpartum period. *Infant Ment. Health J.* **32**, 542–562 (2011).

42. Field, T. *et al.* Still-face and separation effects on depressed mother-infant interactions. *Infant Ment. Health J.* **28**, 314–323 (2007).

43. Lunkenheimer, E., Tiberio, S. S., Skoranski, A. M., Buss, K. A. & Cole, P. M. Parent-child coregulation of parasympathetic processes varies by social context and risk for psychopathology. *Psychophysiology* (2017) doi:10.1111/psyp.12985.

44. Hansson, M. & Jönsson, P. Estimation of HRV spectrogram using multiple window methods focussing on the high frequency power. *Med. Eng. Phys.* **28**, 749–761 (2006).

45. Porges, S. W. The polyvagal theory: Phylogenetic substrates of a social nervous system. *Int. J. Psychophysiol.* **42**, 123–146 (2001).

46. Laborde, S., Mosley, E. & Thayer, J. F. Heart rate variability and cardiac vagal tone in psychophysiological research - Recommendations for experiment planning, data analysis, and data reporting. *Front. Psychol.* **8**, 1–18 (2017).

47. Thorson, K. R., West, T. V. & Mendes, W. B. Measuring Physiological Influence

in Dyads: A Guide to Designing, Implementing, and Analyzing Dyadic Physiological Studies. *Psychol. Methods* **23**, 595–616 (2017).

48. Cox, J. ., Holden, J. M. & Sagovsky, R. Detection of Postnatal Depression Development of the 10-item Edinburg Postnatal Depression Scale. *Br. J. Psychiatry* **150**, 782–786 (1987).

49. Hofer, M. A. Psychobiological roots of early attachment. *Curr. Dir. Psychol. Sci.* **15**, 84–88 (2006).

50. Opendak, M. *et al.* Neurobiology of maternal regulation of infant fear: the role of mesolimbic dopamine and its disruption by maltreatment. *Neuropsychopharmacology* **44**, 1247–1257 (2019).

51. Laurent, H. K. & Ablow, J. C. A cry in the dark: Depressed mothers show reduced neural activation to their own infant's cry. *Soc. Cogn. Affect. Neurosci.* **7**, 125–134 (2012).

52. Moses-Kolko, E. L. *et al.* Abnormally Reduced Dorsomedial Prefrontal Cortical Activity and Effective Connectivity With Amygdala in Response to Negative Emotional Faces in Postpartum Depression. *Am. J. Psychiatry* **167**, 1373–1380 (2010).

53. Graziano, P. & Derefinko, K. Cardiac vagal control and children's adaptive functioning: A meta-analysis. *Biol. Psychol.* **94**, 22–37 (2013).

54. Posner, M. I., Rothbart, M. K., Sheese, B. E. & Voelker, P. Control Networks and Neuromodulators of Early Development. *Dev. Psychol.* **48**, 827–835 (2012).

55. Harman, C., Rothbart, M. & Posner, M. Distress and attention interactions in early infancy. *Motiv. Emot.* **21**, 27–43 (1997).

56. Pawluski, J. L., Lonstein, J. S. & Fleming, A. S. The Neurobiology of Postpartum Anxiety and Depression. *Trends Neurosci.* **40**, 106–120 (2017).

57. Roelofs, K., Minelli, A., Mars, R. B., van Peer, J. & Toni, I. On the neural control of social emotional behavior. *Soc. Cogn. Affect. Neurosci.* **4**, 50–58 (2009).

58. Puckering, C., McIntosh, E., Hickey, A. & Longford, J. Mellow Babies: A group interventions for infants and mothers experienceing postnatal depression. *Couns. Psychol. Rev.* **25**, 28–40 (2010).

59. Stein, A. *et al.* Mitigating the effect of persistent postnatal depression on child outcomes through an intervention to treat depression and improve parenting: a randomised controlled trial. *The Lancet Psychiatry* **5**, 134–144 (2018).

60. Atzil, S., Hendler, T. & Feldman, R. The brain basis of social synchrony. *Soc. Cogn. Affect. Neurosci.* **9**, 1193–1202 (2014).

61. Woody, M. L., Feurer, C., Sosoo, E. E., Hastings, P. D. & Gibb, B. E. Synchrony of physiological activity during mother–child interaction: moderation by maternal history of major depressive disorder. *J. Child Psychol. Psychiatry Allied Discip.* **57**, 843–850 (2016).

Chapter 7: Conclusion

Summary

Mental disorders are prevalent and have a significant negative impact on individuals, their families, and the healthcare system. Problems with emotion regulation in infancy and early childhood appear to increase the risk for mental disorders later in life (Beauchaine, 2015; Wakschlag et al., 2019). The DOHaD hypothesis posits that exposure to adverse environmental conditions during the perinatal period can adversely affect neurodevelopment, leading to problems with emotion regulatory capacity and increased risk for psychopathology (Gluckman, Hanson, & Buklijas, 2010). Since many of these adverse environmental conditions may be modifiable, the DOHaD hypothesis has immense preventative potential. Unlocking this potential is critical to reduce the impact of, or even prevent the development of mental disorders.

The studies that comprise this thesis aimed to elucidate the preventative potential of the DOHaD hypothesis as it pertains to emotion regulatory problems and psychopathology. Results from Studies 1 and 2 indicated that a previously unmeasured risk factor (prenatal diet quality) increased the risk of cognitive, emotion and behavioural problems in offspring. In Study 3, poorer prenatal diet quality was associated with adverse development of the autonomic nervous system, a core physiological regulatory system critical to adaptive functioning across organ systems (Thayer & Sternberg, 2006). Adverse functioning of this system may increase susceptibility to multiple mental disorders and could even increase the risk for all-cause mortality (Thayer & Sternberg,

2006). Evidence from these three studies highlights the potential that interventions designed to improve maternal prenatal diet might have on optimizing offspring neurodevelopment. In Studies 4 and 5, we examined the effects of treating maternal PPD with cognitive behavioural therapy (CBT) on offspring emotion regulation and mother-infant interaction patterns. In Study 4, following maternal CBT treatment for PPD, we observed improvements in infant emotion regulatory capacity, assessed across physiological and behavioural levels. Finally, in Study 5, we showed that intervening on maternal PPD may also be capable of adaptively altering mother-infant physiological synchrony patterns. Taken together, the results from the studies that comprise this thesis highlight the importance of the prenatal and early postnatal period as a time during which potentially modifiable risk factors can be identified and intervened upon to reduce the risk of mental disorders in offspring across the lifespan.

Overall implications for the DOHaD hypothesis

DOHaD research has become a powerful influence on the development of global initiatives striving to optimize the health of individuals, families and communities (Penkler, Hanson, Biesma, & Müller, 2019). However, these large-scale initiatives have generally focused on reducing risk for cardiovascular and metabolic diseases. Results from the studies that comprise this thesis demonstrate that modifiable prenatal and early postnatal risk factors adversely affect infant emotion regulation (and its underlying physiology) and potentially increase risk for mental disorders later in life. This work also shows that intervening on modifiable risk factors might have the potential to optimize

offspring neurodevelopment and reduce the risk of mental disorders in the short and long term. Therefore, these results address limitations of DOHaD research that have limited the progress towards development and implementation of larger, public health interventions that aim to optimize neurodevelopment to reduce the risk of mental disorders across the lifespan.

Elucidating the role of modifiable risk factors

One of the most important limitations of DOHaD as it pertains to neurodevelopment has been the inability to assess the impact of modifiable confounding variables on offspring outcomes (Gage, Munafò, & Davey Smith, 2016). Indeed, most of the evidence linking prenatal and early postnatal adversity to offspring neurodevelopmental outcomes accumulated from large cohort studies that were not designed to assess offspring outcomes related to psychopathology and or neurodevelopment. Therefore, these cohorts often lack data on variables that may confound the links between exposures such as maternal metabolic complications and offspring neurodevelopmental outcomes. As a result, it is unclear whether maternal metabolic conditions are causally related to adverse offspring neurodevelopment, or if these conditions serve as markers for the actual causal factors (Van Lieshout, Taylor, & Boyle, 2011). We defined a confounding variable as a variable linked to both the exposure and outcome, but not on the causal pathway (Gage et al. 2016). We observed that the associations between maternal prenatal metabolic complications and offspring cognitive, emotional and behaviour problems were due to confounding variables, particularly prenatal diet quality.

Interventions designed to improve maternal diet and quality during pregnancy may be more feasible than say, reducing maternal body mass index to the normal range before pregnancy, especially since over half of all pregnancies in Canada are unplanned (Black et al., 2015). In summary, this evidence provides a more complete picture of the mechanisms involved in the associations between maternal prenatal metabolic complications and offspring neurodevelopment. Further, this work suggests that overall prenatal diet quality may be a more feasible, effective target for future interventions.

Perinatal interventions could reduce the risk for mental disorders

Studies 4 and 5 further support the preventative potential of the DOHaD hypothesis by showing that interventions designed to target PPD, a well-recognized risk factor for offspring mental disorders, are capable of reorganizing infant neurodevelopment and could reduce the risk for mental disorders later in life. By observing adaptive changes in infant emotion regulatory capacity (Study 4) and in the physiological systems essential to adaptive mother-infant interactions (Study 5), these studies not only provide evidence on the malleability of emotion regulatory systems in infancy, but also highlight our potential to interrupt the intergenerational transmission of psychiatric risk from mother to offspring.

Mechanisms: Unhealthy maternal diet and offspring neurodevelopment

In the first three studies, we observed that prenatal diet quality appears to account for significant variance in cognitive and behavioural problems in offspring. Research from non-human animal models have shown that prenatal exposure to a high-fat, high-

sugar diets results in altered development of regulatory systems in the brain, particularly those that mediate satiety and reward sensitivity. Exposure to high-fat diets has been shown to alter set-points within the arcuate and paraventricular nuclei of the hypothalamus, resulting in a higher threshold for satiety and changes in the rewarding effects of food intake (Barson, Morganstern, & Leibowitz, 2011; Bouret et al., 2008; Vogt et al., 2014). Occurring in parallel to these changes, exposure to western style diets (e.g., diets high in saturated fats, salt and sugars) alters the development of the mesocorticolimbic dopamine circuitry. Decreased dopamine D2 receptor availability, decreased levels of tyrosine hydroxylase (the rate limiting enzyme in dopamine production) and hypomethylation of the genes encoding the dopamine transporter (DAT) in the ventral striatum, the nucleus accumbens and the medial and orbitofrontal cortex have been observed (Naef, Moquin, Gratton, & Walker, 2013; Ong & Muhlhausler, 2011; Vucetic, Kimmel, Totoki, Hollenbeck, & Reyes, 2010). These changes result in a hypodopaminergic state and are thought to give rise to a phenotype that prefers immediate rewards over delayed ones. This is thought to decrease the likelihood that an individual will expend effort to obtain rewards as well as the ability to inhibit reward seeking behaviour in the presence of hedonistic stimuli (Gatzke-Kopp, 2011). This altered relationship with rewarding stimuli can lead to impulsive behaviors that can impair functioning in developmentally appropriate challenges (e.g., sitting still in a classroom setting, sharing with peers, waiting their turn) (Cole, Ram, & English, 2019). Therefore, these changes in reward and satiety systems increase the risk for externalizing problems,

poorer cognitive development. These changes may also play a role in the development of internalizing problems, particularly if the individual's problems with response inhibition and delayed gratification lead to negative attention from parents and teachers, and adversely affect their interactions with peers (Nigg, 2006). Further, due to the hierarchical development of the brain, adverse development of the lower order mesocorticolimbic and regulatory satiety systems may also affect the development of higher order structures involved in regulating attentional biases and reward-based decision making (Gatzke-Kopp, 2011; Nigg, 2006; Tottenham, 2019). Adverse development of the lower order reward and satiety networks may override regulatory areas such as the medial and orbitofrontal cortices, adversely affecting the balance between prepotent drives and regulatory responses (Cole et al., 2019). Further, dysregulated development of emotion regulatory regions, including ventromedial prefrontal cortex, following prenatal exposure to high fat diets has also been observed (Grissom, Herdt, Desilets, Lidsky-Everson, & Reyes, 2014). This may result in poorer top-down emotion and behavioural regulation. In summary, exposure to unhealthy prenatal diets appears to program multiple lower and higher order neural networks, which may increase the risk for offspring emotion dysregulation and psychopathology.

The predictive adaptive response (PAR) provides an explanation for how and why prenatal exposure to poor overall maternal diets (high in fats and sugars) alter the development of reward, satiety and emotion regulatory systems. Prenatal exposure to high-fat, high-sugar diets may signal to the fetus that the postnatal environment is rich in

resources and energy-dense foods and hedonistic stimuli are plentiful and relatively easy to obtain. Given the importance of consuming energy-dense food sources for survival in paleolithic environments (Eaton & Konner, 1985), it is plausible that evolution would have selected for neural systems that could be programmed by prenatal exposure to the nutritional environment (Gatzke-Kopp, 2011). Therefore, prenatal exposure to high-fat, high-sugar diets may program a 'take things while you can' phenotype that may have increased the chances of survival to sexual maturity (Volkow & Baler, 2015). This phenotype may have been an adaptive response to primitive resource rich environments. However, in today's society, optimal functioning requires the delay of gratification and response inhibition. Therefore, this mismatch between the programmed phenotype and environmental conditions may present as emotion regulatory problems and increase the risk for later psychopathology.

Exposure to high-fat, high-sugar prenatal diets could also increase the risk of damage to regulatory brain areas in the developing fetus. Indeed, intrauterine conditions resulting from high-fat, high-sugar diets may be associated with elevated markers of inflammation and oxidative stress, factors known to adversely affect multiple neurodevelopmental processes (Graham et al., 2017). Further, adequate maternal intake of particular nutrients is needed for fetal neurogenesis, synaptogenesis and the formation neural networks (Georgieff, 2007). Therefore, exposure to high levels of fats and sugars, as well as the potential deficiency of important micronutrients may also lead to adverse development within regulatory systems in the developing brain.

Mechanisms: Postpartum depression

In Studies 4 and 5, we observed improvements in infant emotion regulatory capacity and mother infant interactions following maternal CBT treatment for PPD. In Study 4, we observed adaptive changes in infant emotion regulation assessed across physiological and behavioural levels. While the mechanisms underlying these changes are not known, alterations in brain areas that serve as the foundation for approach/withdrawal motivational tendencies and the acquisition of socioemotional skills in infancy are likely candidates (Fox et al., 1995; Harmon-Jones & Gable, 2017; Quigley & Moore, 2018). Changes in mothers following PPD treatment may adaptively alter infant amygdala activity, the regulatory capacity of the medial prefrontal cortex and functional connectivity between the amygdala and prefrontal cortex. This may enable infants to exhibit more approach related behaviours within their social environment (particularly towards the mother). This explanation is in line the observed switch from greater right, to greater left frontal EEG asymmetry as well as increased resting-state parasympathetic tone in infants following maternal treatment for PPD.

In Study 5, we report on a potential mechanism through which adaptive maternal regulatory scaffolding is transmitted to infants to regulate distress in real time. Following CBT treatment, mothers appear to be more effective at providing regulatory support to their infants during distress. This may be mediated by alterations in maternal higher order regulatory networks following treatment, which may enable mothers to simultaneously regulate their own distress, accurately decipher infant cues, and to maintain appropriate

behaviours to better regulate their infant during moments of distress (Laurent & Ablow, 2012; Moses-Kolko et al., 2010; Pawluski, Lonstein, & Fleming, 2017). Additionally, following maternal CBT treatment, infants may also be more receptive to maternal regulatory support.

Changes in infant neurodevelopment observed following maternal PPD treatment may occur via a PAR, or by neural reorganization following damage to emotion regulatory circuity. Indeed, infants deprived of sensitive and supportive caregivers exhibit stronger coupling of the amygdala-medial prefrontal cortex (mPFC) circuit. In the context of the PAR, the formation of this circuitry may occur to increase the offspring's ability to navigate their environments independent of the caregiver (Tottenham, 2015). However, this also results in a greater adult-like retention of fear memories and elevated levels of withdrawn behaviour (Callaghan, Sullivan, Howell, & Tottenham, 2014). In primitive environments, this phenotype may increase chances of survival to sexual maturity in offspring forced to 'go it alone'; however, these alterations in the amygdala-mPFC circuity are thought to play a significant role in the development of affective disorders across the lifespan (Gabard-Durnam et al., 2018; Tottenham, 2019). Despite this evidence, this circuitry remains plastic during the first year of life. Therefore, changes to the infant's socioemotional environment that may occur throughout the course of maternal PPD treatment, may update and reorganize this circuitry. Indeed, due to the protracted development of the infant prefrontal cortex, it is more effective for infants to solicit regulatory support from their mother, rather than utilize metabolically demanding,

primitive sympathetic systems to self-regulate (Tronick & Beeghly, 2011). Therefore, inputs from more sensitive, supportive mothers may program reorganizations within this circuitry in their infants.

Alterations in this neural circuitry before and after maternal CBT treatment may also reflect damage, and subsequent recovery of these circuits. Indeed, in keeping with experience-expectant theories of neurodevelopment, neural circuits that mediate emotion regulatory capacity require environmental inputs for normal development (Greenough, Black, & Wallace, 1987; Pine & Fox, 2015). When expected environmental conditions are not experienced, this may result in damage to these neural circuits. Therefore, unsupportive and inconsistent maternal behaviours characteristic of mothers with PPD may result in damage to the development of emotion regulatory circuitry in their offspring. However, positive changes to the infant's socioemotional environment have immense potential to reorganize these plastic neural networks, particularly during the first year of life (Wakschlag et al., 2019). Taken together regardless of whether exposure to PPD alters the infant brain via the PAR, damage to emotion regulatory circuitry, or a combination of the two, the immense neuroplasticity of the infant brain in the first postnatal year affords us with the opportunity modify risk factors (e.g., PPD) and potentially optimize infant brain development to reduce the intergenerational transmission of psychiatric risk.

Bringing the work together: Relations between perinatal depression and unhealthy diet quality

Why might both overall prenatal diet quality and PPD increase risk for outcomes associated with adverse development of emotion regulation in offspring? While evidence suggests that these two exposures can be considered as independent risk factors, there is also evidence that these factors are interrelated and adversely affect overlapping neurodevelopmental processes involved in emotion regulatory capacity in offspring (Lucassen et al., 2013; Monk, Georgieff, & Osterholm, 2013). Indeed, depression has been shown to increase the intake of unhealthy foods and dysregulate hormones involved in nutrient absorption and metabolism (Kiecolt-Glaser, 2010). Additionally, an unhealthy diet may play a role in elevated levels of depression (e.g., Sarris et al., 2015). Both factors have been shown to exhibit adverse effects on offspring reward, cognitive, stress and emotion regulation systems (Lucassen et al., 2013; Monk et al., 2013). Indeed, in two large cohort studies, maternal perinatal unhealthy diet was associated with symptoms of depression, and both were linked to elevated levels of emotion dysregulation and adverse cognitive outcomes across childhood (Barker, Kirkham, Ng, & Jensen, 2013; Pina-Camacho, Jensen, Gaysina, & Barker, 2015). Therefore, while often considered to be independent of one another, perinatal depression and the early nutritional environment might be interrelated. These associations might be important to consider when investigating ways to improve offspring neurodevelopment at the population level.

Examining the common elements of prenatal diet quality and PPD: Exploring the role of predictability

Investigating offspring outcomes following exposure to unhealthy diet and PPD in a single thesis (two common exposures that may be interrelated but are generally

investigated separately in the DOHaD literature) provides an opportunity to hypothesize on whether there may be a certain characteristic common to both exposures that might account for similar variance in offspring brain development. Most DOHaD studies describe adverse environmental exposures according to their type, the timing and duration of the exposure, and the intensity (e.g., the absolute level of 'stress') of the exposure. However, I propose that adverse exposures can also be described in terms of their *predictability*. I define predictability as the degree to which a given adverse exposure varies in frequency over time, and how consistent or inconsistent these variations are. Therefore, high predictability refers to a given adverse exposure that is frequent and predictable/consistent over time, and low predictability refers to an exposure that is frequent and unpredictable/inconsistent over time (e.g., Belsky, Schlomer, & Ellis, 2012; Ellis, Boyce, Belsky, & Bakermans-kranenburg, 2011). I argue that both PPD and unhealthy prenatal diets can vary in their degree of predictability (i.e., In two women with the same level of PPD symptoms occurring at the same time in the postnatal period, one mother's emotions and behaviours may be more unpredictable/inconsistent relative to the other mother. Additionally, dietary patterns in certain women may be more 'unpredictable' relative to patterns observed in other women). Considering this predictability dimension of adverse exposures may enhance our ability to explain how exposures affect neurodevelopment. Since the goal of this thesis is to address the preventative potential of the DOHaD hypothesis, highlighting potential mechanisms not previously considered in the DOHaD literature may provide new ways to interpret

existing evidence and stimulate novel research questions aimed at moving us closer to preventing the development of mental disorders.

This predictability component of exposures is not integrated into current mechanisms underlying DOHaD (with few exceptions e.g., Ellison, 2005). However, the importance of predictable signals in the prenatal and early postnatal periods is well recognized in classic human development and neuroscience literature as well as in studies investigating the development of stress physiology. Bowlby's foundational research on attachment states that sensitive mothers provide a safe and *predictable* base upon which infants and children build trusting and affectionate relationships (Bowlby, 1969). Erik Erikson postulated that the foundation for all later development requires feelings of physical comfort and *minimization of uncertainty* (Erikson, 1959). Classic neurophysiological experiments by Donald Hebb revealed that neural circuits strengthen following exposure to repeated and consistent stimuli (Stanton, 1996). Finally, studies in both humans and animal models often utilize an 'unpredictable' adverse stimulus to examine the effects of stress on neurodevelopment (St-Cyr & McGowan, 2018). Taken together, while the concept of predictability has not received much attention when describing the effects of adverse exposures in the DOHaD literature, its importance has long been recognized in other fields. The following sections briefly outline the components of unhealthy prenatal diet and PPD, which might be considered 'unpredictable' and how accounting for this may stimulate the development of novel

research questions to help us move closer to preventing the development of mental disorders (See Figure 1).

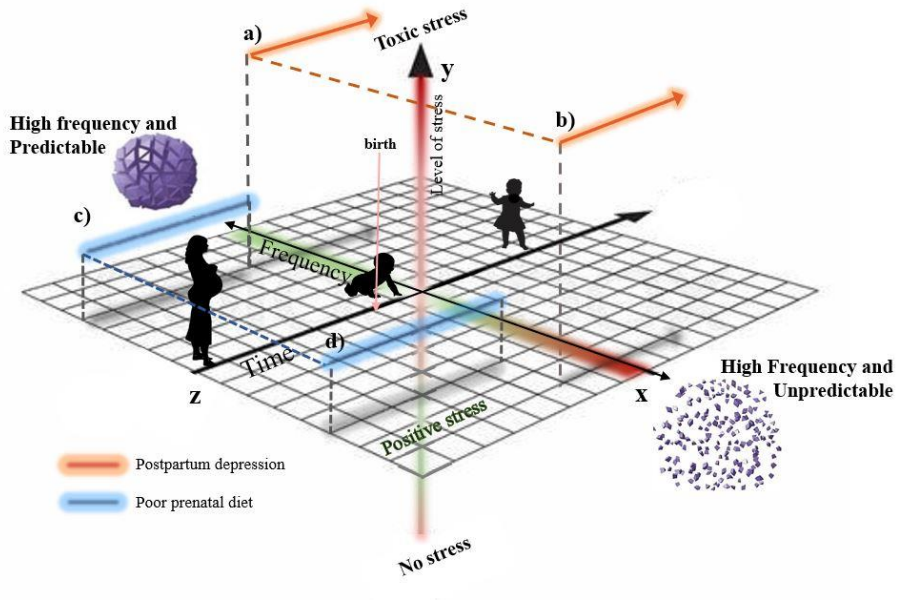


Figure 1: Proposed model that considers the type, timing, duration, intensity, and predictability of exposures. Exposure type can be charted according to its timing, duration, intensity, and predictability (PPD, and poor prenatal diet exposures are charted in Figure 1). ***z-axis****: Exposure timing*. Indicates when and long the fetus or infant is exposed to a given adverse condition. ***y-axis:*** *Intensity (absolute levels) of stress.* Indicates the intensity of the exposure. Red indicates exposure to increasingly 'toxic' levels of stress. This axis also acknowledges stress-inoculation, which posits that exposure to mild deviations is expected for optimal neurodevelopment. ***x-axis*****:** *Predictability of exposure:* Green indicates that the exposure is frequent and predictable. Red indicates that the exposure is frequent and unpredictable. **a)** Exposure to PPD is intense but relatively predictable (e.g., if the mother is consistently withdrawn) while these infants are exposure to more predictable/consistent maternal emotions and behaviours, elevated maternal withdrawn behaviours still increase risk for adverse neurodevelopment and psychopathology. **b)** Exposure to PPD is intense and unpredictable (e.g., if mother exhibits high levels of emotional lability). **c)** Maternal prenatal diet is consistently unhealthy. **d)** Maternal prenatal diet is unhealthy and includes irregular meal intake patterns and/or large fluctuations in fats and sugar intake between meals.

Predictability dimension of prenatal diet quality

Traditionally assessments of prenatal diet quality utilize indices of overall diet quality (e.g., the Healthy Eating Index-2010) or statistical methods such as confirmatory factor analysis using data from a food frequency questionnaire (Guenther et al., 2014; Pina-Camacho et al., 2015). However, while these methods can assess the intensity of the unhealthy diet exposure, they do not account for potential large fluctuations in intake of saturated fats, salt and sugars between meals. Frequent exposure to these large fluctuations may be considered unpredictable to the developing brain, and over time adversely affect the developing brain's ability to organize neural circuitry (Glynn & Baram, 2019). Set-points critical to the establishment of homeostatic, stress and emotion regulatory systems begin to form prenatally and require consistent environmental inputs for normal development (Phillips, 2007; Schlotz & Phillips, 2009; Thayer & Sternberg, 2006). Further, evidence suggests that the frequency, irregularity, and clock-time (when during the day) of when meals are consumed interact with circadian rhythms and effect physiology (see Asher & Sassone-Corsi, 2015; Pot, Almoosawi, & Stephen, 2016 for reviews). Studies have reported on links between meal irregularity and increased pro-inflammatory cytokines, insulin resistance, hyperlipidemia and metabolic dysfunction (Pot et al., 2016). Further, irregular maternal prenatal meal frequency patterns appear to adversely affect offspring, increasing risk for preterm birth, macrosomia and the development of offspring circadian systems (Ainscough et al., 2020; Canaple, Gréchez-Cassiau, Delaunay, Dkhissi-Benyahya, & Samarut, 2018; Englund-Ögge et al., 2017). To my knowledge, no studies have examined the links between irregular prenatal maternal

meal patterns or large fluctuations in fats and sugars between meals, and offspring emotion regulatory circuity. However, given the necessity of consistent prenatal signals on infant brain development, combined with increasing prevalence of eating outside the home, skipping or delaying meals, this may be an important factor to investigate in future studies.

Predictability dimension of postpartum depression

While the valence and quality of maternal emotions and behaviors play a significant role in the link between PPD exposure and adverse offspring outcomes, recent studies have highlighted the importance of maternal predictability on offspring neurodevelopment (Baram et al., 2012; Glynn & Baram, 2019). Indeed, fragmented, unpredictable maternal emotions and behaviors adversely affect cognitive and emotional development in offspring (Glynn & Baram, 2019; Howland et al., 2020; Molet et al., 2016). The infant brain is an open dynamic system that gradually organizes sensory information together into meaningful concepts. This process is critically dependent on the predictability of maternal environmental signals (e.g., Grossmann, 2015). When infants are able to create these ‘meanings’ from predictable and consistent environmental signals, they develop more flexible emotion regulatory capacity and able to adaptively recover from stressors or challenges (DiCorcia & Tronick, 2011; Tronick & Beeghly, 2011). Conversely, when environmental inputs are unpredictable, it is more difficult for the infant brain to detect and form connections between having their socioemotional and homeostatic needs met and stimuli from the social environment (e.g., the presence and

behaviours of a caregiver). Indeed, caregiver mood that swings rapidly between negative and positive increases the likelihood that the infant will enter states of disequilibrium, which undermine their ability to detect consistencies between caregiver behaviours and having their homeostatic and socioemotional needs met. As a result, infants are at increased risk for emotion regulatory problems (Atzil, Gao, Fradkin, & Barrett, 2018; Tronick & Beeghly, 2011). This evidence suggests that neurodevelopment in infants exposed to unpredictable, emotionally labile mothers with PPD might differ from infants exposed to mothers with PDD who are more consistently withdrawn (e.g., Diego, Field, Jones, & Hernandez-Reif, 2006). As a result, infant responses to maternal PPD treatment might differ depending on changes in maternal predictability. Therefore, due to the importance of predictable environmental signals for optimal offspring brain development, investigating whether maternal PPD treatment can alter maternal predictability could be an important objective of future studies.

Limitations

The findings presented in this thesis should be interpreted in the context of the following limitations. Each of these studies used observational designs; therefore, causal conclusions cannot be made from this work. Studies 1,2 and 3 were unable to examine whether offspring outcomes were linked to the quality of the prenatal diet during gestation alone, or throughout the woman's life. Indeed, since women are born with a finite number of oocytes, exposure to poor diet quality prior to pregnancy might also play a role in her offspring's neurodevelopment. The samples that comprise Studies 1, 2 and 3

were well educated and of higher socioeconomic status. Therefore, it is important for future studies to include a wider range of socioeconomic and educational backgrounds to increase the generalizability of these findings. Studies 1,2 and 3 also did not have data on maternal prenatal exercise behaviours; another modifiable risk factor known to play a role in offspring neurodevelopment. Indeed, there may be other unmeasured confounding variables that could have played a role in our findings (e.g., shared genetics, maternal exercise).

For Studies 4 and 5, despite observing changes in two physiological networks underlying emotion regulation and mother-infant interaction patterns, we were unable to assess what brain areas might account for these changes. Indeed, frontal EEG asymmetry assesses neurophysiological activity at the scalp and heart rate variability indexes the functional integration of multiple neural networks involved in emotion regulation. Additionally, while it would have been ideal to conduct a randomized controlled trial, these studies took place in a setting where healthcare is universal; therefore, it would have been unethical to withhold treatment from women with PPD. Therefore, also were unable to include a depressed control group of women that did not receive CBT treatment. However, we believe that including a depressed control group would not have provided additional value for two reasons. First, the evidence linking adverse environmental conditions to enduring alterations neurodevelopment across the lifespan is well established (Krebs-Smith, Guenther, Subar, Kirkpatrick, & Dodd, 2010; McLaughlin, Fox, Zeanah, & Nelson, 2011; Tottenham, 2019; van den Bergh et al., 2017). Indeed,

experience-expectant neurodevelopment posits that environmental inputs exert strong effects on the trajectory of neurodevelopment; therefore, our observed adaptive neurodevelopmental changes in infants would only occur if treatment had positive effects on the infant's socioemotional environment (e.g., maternal emotions and behaviours, or even her predictability) (Bock, Rether, Gröger, Xie, & Braun, 2014; Cicchetti & Toth, 1998; Greenough et al., 1987). Second, the randomized controlled trials conducted in institutionalized infants consistently show that infants removed from institutionalized care exhibit improved socioemotional and behavioural functioning, relative to those that remained institutionalized (Nelson, 2017; Nelson & Gabard-Durnam, 2020; Nelson, Zeanah, & Fox, 2019). This evidence shows that only infants exposed to changed environmental conditions exhibit improved neurodevelopment. Therefore, it would be highly unlikely to observe significant adaptive neurophysiological changes (of medium to large effect size) in infants, and in the interaction patterns between infants and mothers with untreated clinical depression.

Future research

Future research should be conducted to further examine the preventative potential of the DOHaD hypothesis. Critical steps forward could be made by i) advancing study designs and statistics, ii) using a more diverse range of neurophysiological and statistical methods to assess outcomes and offspring response to interventions and iii) by integrating the concept of predictability into studies testing the preventative potential of DOHaD.

First, researchers should replicate and extend upon the findings presented in this thesis using more advanced study designs and statistical methods, including those that allow for causal conclusions. For example, large cohort studies could elucidate the role of variables of interest using directed acyclic graphs to operationally determine which variables should be considered confounding variables and which may lie on the causal pathway. Interventions studies that target maternal lifestyle factors could also be investigated. Since neuroplasticity decreases with age, prenatal interventions have tremendous potential to optimize offspring neurodevelopmental outcomes (e.g., Van Lieshout & Krzeczkowski, 2016). Additionally, future studies examining the effects of treating PPD on offspring neurodevelopment and mother-infant interactions could also benefit from experimental designs. For example, studies could randomize women with PPD treatments delivered by public health nurses or peers and assess the impact of these interventions on offspring outcomes. In settings where randomization to 'no-treatment' conditions are unethical, future studies could randomize groups of women to different treatment conditions (e.g., interpersonal psychotherapy, mindfulness based cognitive behavioral therapy, treatment as usual in a clinical setting). This could further test the preventative potential of the DOHaD hypothesis by assessing if changes to environmental conditions via multiple pathways (different types of interventions) can alter the similar emotion regulatory circuitry in infants.

Second, future studies should attempt to improve the methods used to assess offspring outcomes. This involves moving beyond the general reliance on self and informant report questionnaires. Future DOHaD research should consider integrating with

the novel research domain criteria (RDoC) framework. The RDoC framework aims to understand the dimensions of mental disorders in terms of dysfunction across certain behavioural and physiological systems (Cuthbert & Insel, 2013). Therefore, integrating this framework within DOHaD research could enable us to examine the effects of adverse exposures on particular domains of functioning (e.g., negative or positive valence systems, regulatory/arousal systems) and at multiple levels of analysis (e.g., genetic, epigenetic, neurophysiological, behavioural levels). This approach may also provide us with more precise ways to assess the effectiveness of interventions (e.g, rather than examining if a prenatal maternal diet intervention improves scores on an informant reported questionnaire that assesses child behaviour problems, we could examine whether these interventions effect the development and functioning in offspring positive valence systems, assessed across physiological, behavioural and informant reported levels).

Future studies should also utilize other neurophysiological analysis techniques to elucidate the mechanisms that may account for changes in offspring emotion regulation following treatment of maternal PPD. Indeed, dyadic hyperscanning techniques using functional near infrared spectroscopy (fNIRS) would be an effective way to examine activity within specific cortical areas that may play a role in the effects of maternal PPD treatment on infant emotion regulation and mother-infant interactions. Finally, advanced statistical techniques such as group-based trajectory modelling could be used to determine whether certain sub-groups of infants respond differently depending on particular changes in their mothers following PPD treatment.

Lastly, it would be interesting to further investigate the concept of exposure predictability on offspring neurodevelopment. Studies could consider exploring ways to assess the timing and irregularity of maternal food intake patterns on offspring neurodevelopment. This could enable us to potentially differentiate between the effects of poor overall maternal diet quality and the randomness and unpredictability of maternal food intake. Studies could also assess if prenatal and early postnatal interventions can improve the predictability of the exposure (e.g., by examining if PPD treatment can reduce maternal symptoms of depression and if maternal emotions and behaviours become more predictable).

Finally, none of this evidence will make a substantial impact unless effective knowledge translation plans are developed and implemented. Moving forward, it is critical that DOHaD researchers effectively communicate their evidence not only in research settings, but to health care providers and policy makers in order for this evidence to have a lasting impact on individuals, families and communities.

Conclusion

Mental disorders affect one in five Canadians and have significant adverse effects on an individual's health and well-being across the lifespan. Optimal emotion regulatory capacity is a critical component of mental health and its development in infancy and early childhood depends heavily on prenatal and early postnatal environmental conditions. The DOHaD hypothesis posits that adverse prenatal and early postnatal environmental conditions results in increased risk for psychopathology across the lifespan. Therefore,

unlocking the immense preventative potential of the DOHaD hypothesis is critical. The work presented in this thesis highlights the importance of the prenatal and early postnatal periods as a time during which important and potentially modifiable risk factors can be identified and intervened upon to reduce the risk of psychiatric problems across the lifespan. Efforts to elucidate the preventive potential of the DOHaD hypothesis could have an immense public health impact may be our best chance to reduce the impact of, or even prevent the development of mental disorders across the lifespan.

References

Ainscough, K. M., Kennelly, M. A., Lindsay, K. L., O'Brien, E. C., O'Sullivan, E. J., Mehegan, J., … McAuliffe, F. M. (2020). An observational analysis of meal patterns in overweight and obese pregnancy: exploring meal pattern behaviours and the association with maternal and fetal health measures. *Irish Journal of Medical Science*, *189*(2), 585–594. https://doi.org/10.1007/s11845-019-02099-0

Asher, G., & Sassone-Corsi, P. (2015). Time for food: The intimate interplay between nutrition, metabolism, and the circadian clock. *Cell*, *161*(1), 84–92. https://doi.org/10.1016/j.cell.2015.03.015

Atzil, S., Gao, W., Fradkin, I., & Barrett, L. F. (2018). Growing a social brain. *Nature Human Behaviour*, *2*(9), 624–636. https://doi.org/10.1038/s41562-018-0384-6

Baram, T. Z., Davis, E. P., Obenaus, A., Sandman, C. A., Small, S. L., Solodkin, A., & Stern, H. (2012). Fragmentation and unpredictability of early-life experience in mental disorders. *American Journal of Psychiatry*, *169*(9), 907–915. https://doi.org/10.1176/appi.ajp.2012.11091347

Barker, E. D., Kirkham, N., Ng, J., & Jensen, S. K. G. (2013). Prenatal maternal depression symptoms and nutrition, and child cognitive function. *The British Journal of Psychiatry*, *203*(6), 417–421. https://doi.org/10.1192/bjp.bp.113.129486

Barson, J., Morganstern, I., & Leibowitz, S. (2011). Similarities in hypothalamic and mesocorticolimbic circuits regulating the overconsumption of food and alcohol. *Physiology and Behavior*, *104*(1), 128–137. https://doi.org/10.1038/jid.2014.371

Beauchaine, T. P. (2015). Future Directions in Emotion Dysregulation and Youth Psychopathology Future Directions in Emotion Dysregulation and Youth Psychopathology. *Journal of Clinical Child and Adolescent Psychology*, *44*(5), 875–896. https://doi.org/10.1080/15374416.2015.1038827

Belsky, J., Schlomer, G. L., & Ellis, B. J. (2012). Beyond cumulative risk: Distinguishing harshness and unpredictability as determinants of parenting and early life history strategy. *Developmental Psychology*, *48*(3), 662–673. https://doi.org/10.1037/a0024454

Black, A., Guilbert, E., Hassan, F., Chatziheofilou, I., Lowin, J., Filonenko, A., & Trussell, J. (2015). The Cost of Unintended Pregnancies (CoUP) in Canada: Estimating Direct Cost, Role of Imperfect Adherence, and the Potential Impact of Increased Use of Long-Acting Reversible Contraceptives. *Canadian Journal of Obstetrics and Gynaecology*, *37*(12), 1087–1097. https://doi.org/10.1016/j.gde.2016.03.011

Bock, J., Rether, K., Gröger, N., Xie, L., & Braun, K. (2014). Perinatal programming of emotional brain circuits: an integrative view from systems to molecules. *Frontiers in Neuroscience*, *8*(11), 1–16. https://doi.org/10.3389/fnins.2014.00011

Bouret, S. G., Gorski, J. N., Patterson, C. M., Chen, S., Levin, B. E., & Simerly, R. B. (2008). Hypothalamic Neural Projections Are Permanently Disrupted in Diet-Induced Obese Rats. *Cell Metabolism*, *7*(2), 179–185. https://doi.org/10.1016/j.cmet.2007.12.001

Bowlby, J. (1969). *Attachment and loss* (Vol.1). New York: Basic Books. https://doi.org/10.1242/jeb.107086

Callaghan, B. L., Sullivan, R. M., Howell, B., & Tottenham, N. (2014). The international society for developmental psychobiology Sackler symposium: Early adversity and the maturation of emotion circuits-A cross-species analysis. *Developmental Psychobiology*, *56*(8), 1635–1650. https://doi.org/10.1002/dev.21260

Canaple, L., Gréchez-Cassiau, A., Delaunay, F., Dkhissi-Benyahya, O., & Samarut, J. (2018). Maternal eating behavior is a major synchronizer of fetal and postnatal peripheral clocks in mice. *Cellular and Molecular Life Sciences*, 1–15. https://doi.org/10.1007/s00018-018-2845-5

Cicchetti, D., & Toth, S. L. (1998). The Development of Depression in Children and Adolescents The Peer Ethology Project (PEP) View project. *American Psychologist*, *53*(2), 221. https://doi.org/10.1037/0003-066X.53.2.221

Cole, P. M., Ram, N., & English, M. S. (2019). Toward a Unifying Model of Self-regulation: A Developmental Approach. *Child Development Perspectives*, *13*(2), 91–96. https://doi.org/10.1111/cdep.12316

Cuthbert, B. N., & Insel, T. R. (2013). Toward the future of psychiatric diagnosis: the seven pillars of RDoC. *BMC Medicine*, *11*(1), 126. https://doi.org/10.1186/1741-7015-11-126

DiCorcia, J. A., & Tronick, E. D. (2011). Quotidian resilience: Exploring mechanisms that drive resilience from a perspective of everyday stress and coping. *Neuroscience*

and Biobehavioral Reviews, *35*(7), 1593–1602. https://doi.org/10.1016/j.neubiorev.2011.04.008

Diego, M. A., Field, T., Jones, N. A., & Hernandez-Reif, M. (2006). Withdrawn and intrusive maternal interaction style and infant frontal EEG asymmetry shifts in infants of depressed and non-depressed mothers. *Infant Behavior and Development*, *29*(2), 220–229. https://doi.org/10.1016/j.infbeh.2005.12.002

Eaton, S. B., & Konner, M. (1985). Paleolithic Nutrition A consideration of its nature and current implications. *The New England Journal of Medicine*.

Ellis, B. J., Boyce, W. T., Belsky, J. A. Y., & Bakermans-kranenburg, M. J. (2011). Differential susceptibility to the environment : An evolutionary – neurodevelopmental theory, *23*, 7–28. https://doi.org/10.1017/S0954579410000611

Ellison, P. T. (2005). Evolutionary perspectives on the fetal origins hypothesis. *American Journal of Human Biology*, *17*(1), 113–118. https://doi.org/10.1002/ajhb.20097

Englund-Ögge, L., Birgisdottir, B. E., Sengpiel, V., Brantsæter, A. L., Haugen, M., Myhre, R., … Jacobsson, B. (2017). Meal frequency patterns and glycemic properties of maternal diet in relation to preterm delivery: Results from a large prospective cohort study. *PLoS ONE*, *12*(3), 1–18. https://doi.org/10.1371/journal.pone.0172896

Erikson, E. (1959). *Identity and the life cycle: Selected Papers*. (G. Klien, Ed.) (1st ed.). New York: W.W. Norton Company.

Fox, N. A., Rubin, K. H., Calkins, S. D., Marshall, T. R., Coplan, R. J., Porges, S. W., …

Stewart, S. (1995). Frontal Activation Asymmetry and Social Competence at Four Years of Age Published by : Wiley on behalf of the Society for Research in Child Development Stable URL : http://www.jstor.org/stable/1131909 REFERENCES Linked references are available on JSTOR fo. *Child Development*, *66*(6), 1770–1784.

Gabard-Durnam, L. J., O'Muircheartaigh, J., Dirks, H., Dean, D. C., Tottenham, N., & Deoni, S. (2018). Human amygdala functional network development: A cross-sectional study from 3 months to 5 years of age. *Developmental Cognitive Neuroscience*, *34*(June), 63–74. https://doi.org/10.1016/j.dcn.2018.06.004

Gage, S. H., Munafò, M. R., & Davey Smith, G. (2016). Causal Inference in Developmental Origins of Health and Disease (DOHaD) Research. *Annual Review of Psychology*, *67*(1), 576–585. https://doi.org/10.1146/annurev-psych-122414-033352

Gatzke-Kopp, L. M. (2011). The canary in the coalmine: The sensitivity of mesolimbic dopamine to environmental adversity during development. *Neuroscience and Biobehavioral Reviews*, *35*(3), 794–803. https://doi.org/10.1016/j.neubiorev.2010.09.013

Georgieff, M. K. (2007). Nutrition and the developing brain. *The American Journal of Clinical Nutrition*, *85*(Suppl), 614S-619S. https://doi.org/10.1038/266751a0

Gluckman, P. D., Hanson, M., & Buklijas, T. (2010). A conceptual framework for the developmental origins of health and disease. *Journal of Developmental Origins of*

Health and Disease, *1*(1), 6–18. https://doi.org/10.1017/S2040174409990171

Glynn, L. M., & Baram, T. Z. (2019). The influence of unpredictable, fragmented parental signals on the developing brain. *Frontiers in Neuroendocrinology*, *53*(January), 100736. https://doi.org/10.1016/j.yfrne.2019.01.002

Graham, A. M., Rasmussen, J. M., Rudolph, M. D., Heim, C. M., Gilmore, J. H., Styner, M., … Buss, C. (2017). Maternal Systemic Interleukin-6 During Pregnancy Is Associated With Newborn Amygdala Phenotypes and Subsequent Behavior at 2 Years of Age. *Biological Psychiatry*, (28), 1–11. https://doi.org/10.1016/j.biopsych.2017.05.027

Greenough, W. T., Black, J. E., & Wallace, C. S. (1987). Experience and Brain Development. *Child Development*, *58*(3), 539–559.

Grissom, N. M., Herdt, C. T., Desilets, J., Lidsky-Everson, J., & Reyes, T. M. (2014). Dissociable Deficits of Executive Function Caused by Gestational Adversity are Linked to Specific Transcriptional Changes in the Prefrontal Cortex. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology*, *40*(August), 1–25. https://doi.org/10.1038/npp.2014.313

Grossmann, T. (2015). The development of social brain functions in infancy. *Psychological Bulletin*, *141*(6), 1266–1287. https://doi.org/10.1037/bul0000002

Guenther, P. M., Kirkpatrick, S. I., Reedy, J., Krebs-Smith, S. M., Buckman, D. W., Dodd, K. W., … Carroll, R. J. (2014). The Healthy Eating Index-2010 is a valid and reliable measure of diet quality according to the 2010 Dietary Guidelines for

Americans. *The Journal of Nutrition*, *144*(3), 399–407. https://doi.org/10.3945/jn.113.183079

Harmon-Jones, E., & Gable, P. A. (2017). On the role of asymmetric frontal cortical activity in approach and withdrawal motivation: An updated review of the evidence. *Psychophysiology*, *1*. https://doi.org/10.1111/psyp.12879

Howland, M. A., Sandman, C. A., Davis, E. P., Stern, H. S., Phelan, M., Baram, T. Z., & Glynn, L. M. (2020). Prenatal maternal mood entropy is associated with child neurodevelopment. *Emotion*. https://doi.org/10.1037/emo0000726

Kiecolt-Glaser, J. K. (2010). Stress, food, and inflammation: psychoneuroimmunology and nutrition at the cutting edge. *Psychosomatic Medicine*, *72*, 365–369. https://doi.org/10.1097/PSY.0b013e3181dbf489

Krebs-Smith, S. M., Guenther, P. M., Subar, A. F., Kirkpatrick, S. I., & Dodd, K. W. (2010). Americans Do Not Meet Federal Dietary Recommendations. *The Journal of Nutrition*, *140*(10), 1832–1838. https://doi.org/10.3945/jn.110.124826

Laurent, H. K., & Ablow, J. C. (2012). A cry in the dark: Depressed mothers show reduced neural activation to their own infant's cry. *Social Cognitive and Affective Neuroscience*, *7*(2), 125–134. https://doi.org/10.1093/scan/nsq091

Lucassen, P. J., Naninck, E. F. G., Goudoever, J. B. Van, Fitzsimons, C., Joels, M., & Korosi, A. (2013). Perinatal programming of adult hippocampal structure and function ; emerging roles of stress , nutrition and epigenetics. *Trends in Neurosciences*, *36*(11), 621–631. https://doi.org/10.1016/j.tins.2013.08.002

McLaughlin, K. A., Fox, N. A., Zeanah, C. H., & Nelson, C. A. (2011). Adverse rearing environments and neural development in children: The development of frontal electroencephalogram asymmetry. *Biological Psychiatry*, *70*(11), 1008–1015. https://doi.org/10.1016/j.biopsych.2011.08.006

Molet, J., Heins, K., Zhuo, X., Mei, Y. T., Regev, L., Baram, T. Z., & Stern, H. (2016). Fragmentation and high entropy of neonatal experience predict adolescent emotional outcome. *Translational Psychiatry*, *6*(August 2015), e702. https://doi.org/10.1038/tp.2015.200

Monk, C., Georgieff, M. K., & Osterholm, E. A. (2013). Research Review: Maternal prenatal distress and poor nutrition - Mutually influencing risk factors affecting infant neurocognitive development. *Journal of Child Psychology and Psychiatry and Allied Disciplines*, *54*(2), 115–130. https://doi.org/10.1111/jcpp.12000

Moses-Kolko, E. L., Perlman, S. B., Wisner, K. L., James, J., Tova Saul, A., & Phillips, M. L. (2010). Abnormally Reduced Dorsomedial Prefrontal Cortical Activity and Effective Connectivity With Amygdala in Response to Negative Emotional Faces in Postpartum Depression. *American Journal of Psychiatry*, *167*, 1373–1380. Retrieved from http://ajp.psychiatryonline.org/doi/pdf/10.1176/appi.ajp.2010.09081235

Naef, L., Moquin, L., Gratton, A., & Walker, C. (2013). Reduced anticipatory dopamine responses to food in rats exposed to high fat during early development. *International Journal of Obesity (2005)*, *37*(6), 885–888. https://doi.org/10.1038/ijo.2012.153

Nelson, C. A. (2017). Hazards to Early Development: The Biological Embedding of Early

Life Adversity. *Neuron*, *96*(2), 262–266. https://doi.org/10.1016/j.neuron.2017.09.027

Nelson, C. A., & Gabard-Durnam, L. J. (2020). Early Adversity and Critical Periods: Neurodevelopmental Consequences of Violating the Expectable Environment. *Trends in Neurosciences*, *43*(3), 133–143. https://doi.org/10.1016/j.tins.2020.01.002

Nelson, C. A., Zeanah, C. H., & Fox, N. A. (2019). How early experience shapes human development: The case of psychosocial deprivation. *Neural Plasticity*, *2019*. https://doi.org/10.1155/2019/1676285

Nigg, J. T. (2006). Temperament and developmental psychopathology, *4*, 395–422. https://doi.org/10.1111/j.1469-7610.2006.01612.x

Ong, Z. Y., & Muhlhausler, B. S. (2011). Maternal "junk-food" feeding of rat dams alters food choices and development of the mesolimbic reward pathway in the offspring. *The FASEB Journal*, *25*(7), 2167–2179. https://doi.org/10.1096/fj.10-178392

Pawluski, J. L., Lonstein, J. S., & Fleming, A. S. (2017). The Neurobiology of Postpartum Anxiety and Depression. *Trends in Neurosciences*, *40*(2), 106–120. https://doi.org/10.1016/j.tins.2016.11.009

Penkler, M., Hanson, M., Biesma, R., & Müller, R. (2019). DOHaD in science and society: Emergent opportunities and novel responsibilities. *Journal of Developmental Origins of Health and Disease*, *10*(3), 268–273. https://doi.org/10.1017/S2040174418000892

Phillips, D. I. W. (2007). Programming of the stress response: A fundamental mechanism

underlying the long-term effects of the fetal environment? *Journal of Internal Medicine*, *261*(5), 453–460. https://doi.org/10.1111/j.1365-2796.2007.01801.x

Pina-Camacho, L., Jensen, S. K., Gaysina, D., & Barker, E. D. (2015). Maternal depression symptoms , unhealthy diet and child emotional – behavioural dysregulation. *Psychological Medicine*, *45*, 1851–1860. https://doi.org/10.1017/S0033291714002955

Pine, D. S., & Fox, N. A. (2015). Childhood Antecedents and Risk for Adult Mental Disorders. *Annual Review of Psychology*, *66*(1), 459–485. https://doi.org/10.1146/annurev-psych-010814-015038

Pot, G. K., Almoosawi, S., & Stephen, A. M. (2016). Meal irregularity and cardiometabolic consequences: Results from observational and intervention studies. *Proceedings of the Nutrition Society*, *75*(4), 475–486. https://doi.org/10.1017/S0029665116000239

Quigley, K. M., & Moore, G. A. (2018). Development of cardiac autonomic balance in infancy and early childhood: A possible pathway to mental and physical health outcomes. *Developmental Review*, *49*(February), 41–61. https://doi.org/10.1016/j.dr.2018.06.004

Sarris, J., Logan, A. C., Akbaraly, T. N., Amminger, G. P., Balanzá-Martínez, V., Freeman, M. P., … Jacka, F. N. (2015). Nutritional medicine as mainstream in psychiatry. *The Lancet Psychiatry*, *2*(3), 271–274. https://doi.org/10.1016/S2215-0366(14)00051-0

Schlotz, W., & Phillips, D. I. W. (2009). Fetal origins of mental health: Evidence and mechanisms. *Brain, Behavior, and Immunity*, *23*(7), 905–916. https://doi.org/10.1016/j.bbi.2009.02.001

St-Cyr, S., & McGowan, P. O. (2018). Adaptation or pathology? The role of prenatal stressor type and intensity in the developmental programing of adult phenotype. *Neurotoxicology and Teratology*, *66*(December 2017), 113–124. https://doi.org/10.1016/j.ntt.2017.12.003

Stanton, P. K. (1996). LTD, LTP, and the sliding threshold for long-term synaptic plasticity. *Hippocampus*, *6*(1), 35–42. https://doi.org/10.1002/(SICI)1098-1063(1996)6:1<35::AID-HIPO7>3.0.CO;2-6

Thayer, J. F., & Sternberg, E. (2006). Beyond heart rate variability: Vagal regulation of allostatic systems. *Annals of the New York Academy of Sciences*, *1088*, 361–372. https://doi.org/10.1196/annals.1366.014

Tottenham, N. (2015). Social scaffolding of human amygdala-mPFCcircuit development. *Social Neuroscience*, *10*(5), 489–499. https://doi.org/10.1080/17470919.2015.1087424

Tottenham, N. (2019). Early Adversity and the Neotenous Human Brain. *Biological Psychiatry*, *16*, 1–10. https://doi.org/10.1016/j.biopsych.2019.06.018

Tronick, E., & Beeghly, M. (2011). Infants' meaning-making and the development of mental health problems. *American Psychologist*, *66*(2), 107–119. https://doi.org/10.1037/a0021631.Infants

van den Bergh, B. R. H., van den Heuvel, M. I., Lahti, M., Braeken, M., de Rooij, S. R., Entringer, S., … Schwab, M. (2017). Prenatal developmental origins of behavior and mental health: the influence of maternal stress in pregnancy. *Neuroscience & Biobehavioral Reviews*, (November 2016). https://doi.org/https://doi.org/10.1016/j.neubiorev.2017.07.003

Van Lieshout, R. J., & Krzeczkowski, J. E. (2016). Just DO(HaD) It! Testing the clinical potential of the DOHaD hypothesis to prevent mental disorders using experimental study designs. *Journal of Developmental Origins of Health and Disease*, 1–9. https://doi.org/10.1017/S2040174416000441

Van Lieshout, R. J., Taylor, V. H., & Boyle, M. H. (2011). Pre-pregnancy and pregnancy obesity and neurodevelopmental outcomes in offspring: A systematic review. *Obesity Reviews*, *12*(501), 548–559. https://doi.org/10.1111/j.1467-789X.2010.00850.x

Vogt, M. C., Paeger, L., Hess, S., Steculorum, S. M., Awazawa, M., Hampel, B., … Brüning, J. C. (2014). Neonatal insulin action impairs hypothalamic neurocircuit formation in response to maternal high-fat feeding. *Cell*, *156*(3), 495–509. https://doi.org/10.1016/j.cell.2014.01.008

Volkow, N. D., & Baler, R. D. (2015). NOW vs LATER brain circuits: Implications for obesity and addiction. *Trends in Neurosciences*, *38*(6), 345–352. https://doi.org/10.1016/j.tins.2015.04.002

Vucetic, Z., Kimmel, J., Totoki, K., Hollenbeck, E., & Reyes, T. M. (2010). Maternal

high-fat diet alters methylation and gene expression of dopamine and opioid-related genes. *Endocrinology*, *151*(10), 4756–4764. https://doi.org/10.1210/en.2010-0505

Wakschlag, L. S., Roberts, M. Y., Flynn, R. M., Smith, J. D., Krogh-Jespersen, S., Kaat, A. J., … Davis, M. M. (2019). Future Directions for Early Childhood Prevention of Mental Disorders: A Road Map to Mental Health, Earlier. *Journal of Clinical Child and Adolescent Psychology*, *48*(3), 539–554. https://doi.org/10.1080/15374416.2018.1561296

Appendix

Chapter 2: Maternal Metabolic Complications in Pregnancy and Offspring Behavior Problems at Two Years of Age (Study 1)

Supplemental table e1: Study overview for Edmonton sub-cohort of CHILD

		Prenatal		Birth	Post-natal				
	Q: Questionnaire P: Physiological Testing B: Biological Sample E: Environmental assessment	Recruitment CLINIC	36 wk CLINIC	HOSPITAL	3 mo HOME	6 mo PHONE	9 mo PHONE	1 yr CLINIC	2 yr
Mother	Q: Health and Medications	✓						✓	
	Q: Environment	✓			✓	✓		✓	
	Q: Nutrition[a]	✓			✓			✓	
	Q: Activity	✓							
	Q: Stress	✓	✓					✓	
	Q: Socio-economic status	✓						✓	
	Q: Delivery			✓					
	Q: Sleep							✓	
	P: Skin Prick Testing							✓	
	B: Breast Milk				✓				
Father	Q: Health	✓							
	Q: Environment	✓							
	Q: Socio-economic status	✓						✓	

	Q: Sleep	✓							
	P: Skin Prick Testing	✓							
Infant	Q: Health and Medications				✓	✓	✓	✓	✓
	Q: Infection				✓	✓	✓	✓	✓
	Q: Activity				✓	✓		✓	
	Q: Solid Food Introduced[a]				✓	✓		✓	
	Q: Pediatric Sleep Questionnaire				✓	✓	✓	✓	
	Q: Brief Infant Sleep Questionnaire				✓	✓	✓	✓	
	Q: CBCL[b]								✓
	Q: BSID-III[c]							✓	
	P: Polysomnography							✓	
	P: Skin Prick Testing							✓	
	P: Height and Weight			✓				✓	

[a] Sleep and neurobehavioral measures

[b] Child Behavioural Checklist

[c]BSID-III: Bayley scale of infant development version III

Supplemental Table e2 Study inclusion and exclusion criteria for the CHILD study

Inclusion Criteria

1. Pregnant women aged 18 years and older
2. Residence in reasonable proximity to the study centre.
3. Able to read and speak English
4. Willing to provide informed consent
5. Willing to consent to cord blood collection by the study
6. Planning to give birth at the recruitment centre

7. Infants born at or after 35.5 weeks
8. Must be able to provide a valid address and telephone number and names and phone numbers of two alternate contact individuals

Exclusion Criteria

1. Children with major congenital abnormalities or respiratory distress syndrome (RDS)
2. Infants born before 35.5 weeks gestation
3. Expectation of moving away from a recruitment area within 1 year
4. Children of multiple births
5. Children resulting from in-vitro fertilization
6. Children who do not spend at least 80% of nights in the home index

Supplemental Table e3 Unadjusted Associations between Maternal Metabolic Complications during Pregnancy and CBCL 1½ -5 Externalizing and Internalizing Problems

Predictors (B, 95-CI)	Externalizing	Internalizing
BMI (Continuous)	0.14 (0.02; 0.27)*	0.10 (0.01; 0.20)*
BMI (Categorical)[a]		
Normal-Overweight/Obese	1.34 (0.01; 2.69)*	1.24 (0.24; 2.23)*

[a] Normal (18.5-24.9) vs. Overweight/Obese (BMI >25).

* $p<0.05$

Supplemental Table e4 Adjusted Associations between Maternal Metabolic Complications during Pregnancy and CBCL 1½ -5 Externalizing and Internalizing Problems

Predictors (B, 95-CI)	Externalizing	Internalizing
BMI (Continuous)	0.05 (-0.06; 0.16)	0.001 (-0.07; 0.07)
Smoking	1.52 (-5.21; 2.18)	3.32 (5.7; 0.74)
HEI total	-0.19 (-0.28; -0.11)	-0.07 (-0.14; 0.01)
SES	-0.46 (-0.85;-0.06)	-0.39 (-0.73;-0.05)
Depression	0.18 (0.08;0.27)	0.12 (0.05;0.20)
Breastfeeding duration	-0.10 (-0.21; 0.01)	-0.09 (-0.18; 0.01)
BMI (Categorical)[a]		
Normal- Overweight/Obese	-0.03 (-1.43; 1.50)	0.57 (-048; 1.62)
Smoking	2.59 (-6.35; 1.18)	3.06 (5.78; 0.35)
HEI total	-0.17 (-0.28;-0.07)	-0.06 (-0.14; 0.10)
SES	-0.61 (-1.32;-0.19)	-0.38 (-0.72;-0.04)
Depression	0.14 (0.03;0.25)	0.12 (0.05; 0.23)

Breastfeeding duration	0.13 (-0.25; 0.003)	-0.09 (-0.18; 0.003)

Adjusted for SES, maternal smoking, maternal diet (measured by HEI total score), maternal depression at 12 months postpartum and breastfeeding duration.

[a] Normal (BMI=18.5-24.9) vs. Overweight/Obese (BMI >25).

Supplemental Table e5 *Post-hoc* analyses of semi-partial correlations accounting for the associations between BMI, GDM and offspring behaviour problems.

Model		Semi-partial correlation coefficients (r_s, *p*-value)	
BMI (continuous)		Externalizing	Internalizing
	HEI-total	-0.19(<0.01)	-0.10 (0.075)
	Empty calories[a]	-0.19 (<0.01)	-0.11 (0.052)
	Depression	0.14 (<0.05)	0.18 (<0.01)
	SES	-0.14 (<0.05)	-0.13 (<0.05)
	Smoking	0.08 (N.S.)	0.12 (<0.05)
	Breastfeeding	-0.11 (N.S.)	-0.10 (N.S.)
	BMI	0.02 (N.S.)	0.04 (N.S.)

[a] The HEI-2010 component that remained significant when added to the fully adjusted model *in place* of HEI-total.

Chapter 3: Neurodevelopment in 3–4 year old children exposed to maternal hyperglycemia or adiposity in utero (Study 2)

Supplementary Table 1: Demographic comparisons between those with and without BMI and hyperglycemia data

	BMI[a] (n=757)	BMI Missing (n=51)	Tested for hyperglycemia (n=594)	Hyperglycemia data missing (n=214)
Education (n,%)				
High School or less	75 (9.9)	8 (15.7)	63 (10.6)	20 (9.4)
College Educated/ University Degree	680 (90.1)	43 (84.3)	530 (89.4)	193 (90.6)
Marital Status				
Married/Common	727 (96)	50 (98)	573 (96.5)	204 (95.3)
law	4 (0.7)	0	3 (0.5)	1 (0.5)
Divorced/Separated	25 (3.3)	1 (2.0)	17 (3.0)	9 (4.2)
Single				
Maternal age (M. SD)	32.8 (4.8)	32.0 (4.8)	32.7 (4.8)	32.9 (4.8)
Household Income (n,%)				
Less than 50 000	**103 (13.6)**	**14 (27.5)**	88 (14.8)	29 (13.6)
50 001-100 000	**321 (42.4)**	**24 (47.1)**	249 (41.9)	96 (44.9)
Greater than 100 000	**333 (44.0)**	**13 (25.5)**	257 (43.3)	89 (41.6)
Smoking (n,%)				
Never	493 (65.1)	36 (70.6)	380 (64)	149 (69.6)
Former	201 (26.6)	11 (21.6)	160 (26.9)	52 (24.3)
Current/Quit in pregnancy	63 (8.3)	4 (7.8)	54 (9.1)	13 (6.1)
Birth Country				
Canada	622 (82.2)	39 (76.5)	482 (81.1)	179 (83.6)

Elsewhere	135 (17.8)	12 (23.5)	112 (18.9)	35 (16.4)
HOME total (M,SD)	**47.4 (4.3)**	**46.1 (4.0)**	47.3 (4.4)	47.5 (4.0)
Depression (M,SD)	5.5 (4.2)	6.4(4.8)	5.6 (4.3)	5.3 (4.1)
Stress (M,SD)	16.1 (4.6)	16.9 (4.9)	16.2 (4.6)	16.1 (4.6)
Diet Quality (M,SD)	72.3 (7.89)	70.6 (7.16)	**71.8 (8.0)**	**73.5 (7.3)**
Breastfeeding (M, SD)	5.5 (2.1)	5.1 (2.1)	5.41 (2.3)	5.7 (1.6)
Presence of hyperglycemia (n,%)	**39 (7.1)**	**8 (17.3)**		
Infant Sex (n,%) Male	377 (49.8)	28 (54.9)	303 (47.7)	102 (47.7)
Gestational Age (M,SD)	39.0 (1.7)	38.6 (2.4)	38.9 (1.7)	39.1 (1.8)
Birth Weight (g) (M,SD)	3440.82 (521.4)	3459.55 (649.1)	3432.4 (537.8)	3469.1 (507.6)

[a] Includes women with an underweight BMI (n=20) whom are not included in table 1.

Boldface= $p<0.05$

Chapter 4: Maternal Pregnancy Diet Quality Is Directly Associated with Autonomic Nervous System Function in 6-Month-Old Offspring

Supplementary Table 1: Characteristics of mother-infant dyads enrolled in MIREC vs. the MIREC-ID (current study's) sub-study sample. (MIREC n=1583, MIREC-ID n=400)

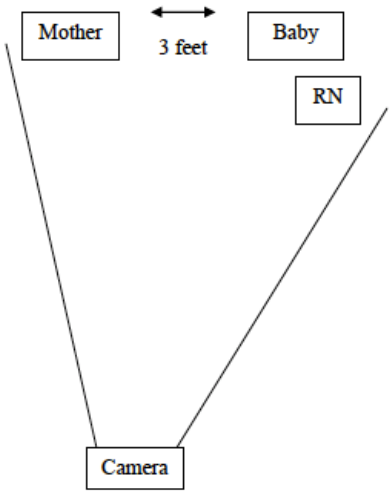
	Original MIREC Cohort (n=1583)	MIREC-ID sample (n=400)	*P*
Diet Quality	71.9±8.7	72.2 ±8.4	0.5
Age	32.3 ±5.2	31.8±4.7	0.1
Marital Status *n,* (%)			
Married/Common law	1507 (95.2)	382 (95.2)	0.84
Divorced/Separated	7 (0.4)	3 (0.8)	
Single	69 (4.4)	15 (3.8)	
Household Income $[1] *n,*(%)			
<50 000	276 (17.4)	71 (17.8)	<0.01
50 001-100 000	600 (37.9)	186 (46.5)	
>100 000	707 (44.7)	143 (35.8)	
Education *n,* (%)			
High School or less	230 (14.5)	50 (12.6)	0.31
College Educated/ University Degree	1353 (85.5)	348 (87.4)	
Country of Birth *n,*(%)			
Canada	1263 (79.8)	349 (87.3)	0.01
Elsewhere	320 (20.2)	51 (12.7)	
Smoking *n,*(%)			
Never	937 (59.2)	265 (66.3)	0.01
Former	442 (27.9)	102 (25.5)	
Current/Quit in pregnancy	204 (12.9)	33 (8.3)	
Pre-Pregnancy BMI (kg/m^2) *n,*(%)			
Underweight	45 (3.1)	7 (1.9)	0.16

Normal	896 (61.0)	210 (56.9)	
Overweight	318 (21.7)	86 (23.3)	
Obese	209 (14.2)	66 (17.9)	
Birth weight, g	3385.7±625.0	3497.7 ±497.4	<0.01
Gestational age (weeks)	38.2±4.1	39.2 ±1.4	<0.01
Male *n*, (%)	810 (53.4)	205 (51.2)	0.45

[1] Canadian Dollars

Supplementary Table 2: Description of procedures during which heart rate variability was assessed

Task	Description
Anthropometric assessment	HRV was obtained during the final two measurements of the anthropometric assessment. This included the following measures: a) crown-rump length assessment: a measurement of the distance from the top of the head to the bottom of the buttock, and b) digit length assessment: measured in order to calculate ratio of index and ring finger lengths
Tilt procedure	When the infant was in a calm state, belts were fitted well to minimize infant movement. During the tilt maneuver, the cradle was gently raised and fixed to the angle of 60°, heads-up position for 5 minutes. Then, the baby was placed in a horizontal position for 5 minutes before moving back to the heads-up position for 5 minutes. The baby was then brought back to the horizontal position and removed from the tilt device.
Arm Restraint	Before the infant arrived, the setting was arranged in accordance with the diagram below.

	The mother was seated on a chair 3 feet on the left of where the baby would be positioned (baby's right side). The baby was seated in a  chair and the research nurse was kneeling in front (slightly on the right side) of the baby. The camera was positioned on the tripod at the proper distance so that both the baby's and the mother's bodies were captured on video. The baby was allowed to play freely for 5 minutes, then the baby's arms were held for 2 minutes gently by their side by the research assistant (RN). Following this, the baby was then allowed to play freely for 5 minutes

Chapter 5: Changes in Psychophysiological and Behavioral Measures of Emotion Regulation in Infants following Maternal Cognitive Behavioral Therapy for Postpartum Depression

Methods

Electroencephalogram (EEG)*:* EEG data were recorded using Netstation (Version 4.4.1). Infants were fitted for a 128-electrode HydroCel sensor net, which was subsequently connected to an Amp 300 DC-coupled amplifier (Phillips Inc [Formerly Electrogeodesics Incorporated], Eugene, OR). Scalp impedances were kept below 50KOhms during acquisition. EEG signals were digitized at 250Hz and referenced to Cz (vertex). In keeping with infant EEG research procedure, eye electrodes had been removed from the nets (channels 125, 126,127,128) prior to recording.

Data were analyzed offline in Matlab using EEGlab (v14.0.0b) by a trained graduate student blind to group status. The signal was band-passed filtered from 1 Hz (high pass) to30Hz (low pass). Non-usable channels were removed via a visual inspection. Channels were replaced using spherical spline interpolation. Sections of data contaminated by large artifacts were removed by visual inspection. The data were re-referenced to an average reference then were segmented into epochs of 2 seconds duration with 50% overlap. Data were further examined for any remaining artifact (fluctuations) and removed from further analyses. At Visit 1, there were an average of 68.9 (SD=28.4) artifact-free, usable EEG epochs and 77.7 (SD=39.9) at Visit 2. There were no significant differences in the number of clean epochs between case and control infants at either visit. A 100% hanning window function was applied to each epoch before undergoing a fast Fourier transform to obtain the power spectrum.

Power within the "infant" alpha band (6 to 9Hz)[1] was extracted. To be consistent with the majority of studies that examined the effects of PPD on infant frontal asymmetry[2–8], our frontal asymmetry measure was calculated by subtracting the natural log-transformed alpha power at the left frontal hemisphere (at site F3 [channel 24]) from the right frontal hemisphere (at F4 [channel 124]) [i.e., FAA=*ln*(F4)-*ln*(F3)].

Heart Rate Variability: Prior to recording, disposable pediatric ECG electrodes were placed on the infant's back. Electrocardiogram (ECG) data were recorded continuously (sampling rate 500Hz) using the Mindware 3000A mobile data acquisition unit (Mindware Technologies Ltd.

Gahanna, OH), which transmitted data wirelessly to a laptop computer running Biolab (3.3.2) acquisition software.

Mindware HRV 3.2.3 was used to inspect and analyze ECG data. Each 60 second epoch of data were visually inspected for artifacts (large movements, software misidentifications of R-wave peaks). Mis-identified or missing heartbeats were manually corrected as needed by a graduate student blind to group status. Data were excluded from analysis if epochs had more 10% of R-waves estimated or if >50% of the epoch was missing. For each cleaned 60 second epoch, a fast Fourier transform was used to obtain power within high frequency band (for infants: 0.24 to1.04 Hz[9]), which is believed to reflect parasympathetic nervous system activity[10].

Supplementary Table 1: Differences between those that competed the study vs. dyads that did not complete both visit

	Cases			Controls		
	Completed Study (n=40)	Did not complete study (n=11)	*P* Value	Completed Study (n=40)	Did not complete study (n=6)	*P* Value
Infant age at visit 1, m (SD), months	5.6 (2.7)	6.3 (3.17)	0.42	5.9(2.6)	5.8	0.96
Infant sex, No. (%), male	16.0 (40)	7.0 (64)	0.20	16.0 (40)	2.0 (33)	>0.99
Total household income[a] m(SD) <49,999 50,000-79,999 >80,000	 8 (20) 10 (25) 22 (55)	 2 (20) 3 (30) 6 (60)	0.94	 5 (13) 11 (27) 24 (60)	 2 (33) 2 (33) 2 (33)	0.33
Maternal age, m(SD), years	32.4(4.2)	30.7(4.8)	0.29	32.7(5.1)	34.8(4.6)	0.45
Parity, No. (%), Primiparous	 21(53)	 7 (64)	0.48	 22 (55)	 2 (33)	0.59

Multiparous	19 (47)	4 (36)		18 (45)	4 (66)	
Marital Status, No. (%),						
Single	3 (8)	0 (0)	0.17	2 (5)	0 (0)	0.70
Separated	1 (2)	1 (9)		0 (0)	0 (0)	
Common-law	9 (22)	5 (45)		8 (20)	3 (50)	
Married	26 (68)	5 (45)		30 (75)	3 (50)	
Education, No. (%),						
High school or less	5 (12)	1(9)	0.76	3 (7.5)	1 (17)	0.69
College or certificate program	12 (30)	2 (18)		11 (27.5)	3 (33)	
University or higher	23 (58)	8 (73)		26 (65.0)	2(17)	
Birthweight m(SD), grams	3329.4 (442.0)	3276.2 (346.78)	0.72	3374. 9 (489.0)	3206.8 (950.0)	0.54
Gestational age m(SD), weeks	39.5 (2.2)	38.2 (2.5)	0.078	39.2(1.1)	39.8 (0.95)	0.38

[a]Canadian Dollars

Sensitivity analyses: The impact of maternal medications on offspring emption regulation

Repeated measures ANOVAs were used to determine if infant emotion regulation outcomes differed for women on medications vs. not on medications between Visit 1 and Visit 2. There were no significant between-subjects effects on frontal EEG asymmetry [$F(1,32)=0.07$, $p=0.49$]; heart rate variability [$F(1,35)=0.06$,$p=0.72$] and on orientation/regulation behaviors reported on by mothers [$F(1,37)=0.002$,$p=0.99$] and partners [$F(1,29)=0.03$,$p=0.69$]. Therefore, medication did not impact our results. Further, significant within-subjects effects on frontal EEG asymmetry [$F(1,32)=8.37$, $p=0.007$]; high frequency heart rate variability [$F(1,35)=10.3$,$p=0.003$] and on orientation/regulation behaviors reported on by mothers [$F(1,37)=6.2$,$p=0.017$] and partners [$F(1,29)=4.19$,$p=0.05$] indicated that improvements were observed in infants regardless of whether women were taking medication.

There were also no differences between women whom started medications within 1 month before the start of group vs. those on a stable medication regimen for >1 month on frontal EEG asymmetry [$F(1,18)=0.10$,$p=0.76$]; high frequency heart rate variability [$F(1,19)=0.2$,$p=0.63$] and on orientation/regulation behaviors reported on by mothers [$F(1,20)=0.2$,$p=0.89$] and partners [$F(1,15)=0.62$,$p=0.44$]. Finally, no differences were observed when the 5 women whom had to the dose of their medications during group were removed from analysis. While no differences were observed, we acknowledge that we may not have had the power to detect these differences.

References

1. Bell MA. Power changes in infant EEG frequency bands during a spatial working memory task. *Psychophysiology*. 2002;39(4):450-458. doi:10.1017.s0048577201393174
2. Lusby CM, Goodman SH, Bell MA, Newport DJ. Electroencephalogram patterns in infants of depressed mothers. *Dev Psychobiol*. 2014;56(3):459-473. doi:10.1002/dev.21112
3. Diego MA, Jones NA, Field T. EEG in 1-week, 1-month and 3-month-old infants of

depressed and non-depressed mothers. *Biol Psychol*. 2010;83(1):7-14. doi:10.1016/j.biopsycho.2009.09.007

4. Field T, Fox NA, Pickens J, Nawrocki T. Relative Right Frontal EEG Activation in 3- to 6-Month-Old Infants of "Depressed" Mothers. *Dev Psychol*. 1995;31(3):358-363. doi:10.1037/0012-1649.31.3.358
5. Diego MA, Field T, Jones NA, Hernandez-Reif M. Withdrawn and intrusive maternal interaction style and infant frontal EEG asymmetry shifts in infants of depressed and non-depressed mothers. *Infant Behav Dev*. 2006;29(2):220-229. doi:10.1016/j.infbeh.2005.12.002
6. Dawson G, Frey K, Panagiotides H, Yamada E, Hessl D, Osterling J. Infants of depressed mothers exhibit atypical frontal electrical brain activity during interactions with mother and with a familiar, nondepressed adult. *Child Dev*. 1999;70(5):1058-1066. doi:10.1111/1467-8624.00078
7. Dawson G, Klinger LG, Panagiotides H, Hill D, Spieker S. Frontal Lobe Activity and Affective Behavior of Infants of Mothers with Depressive Symptoms. *Child Dev*. 1992;63(3):725-737.
8. Jones NA, Field T, Fox NA, et al. Infants of intrusive and withdrawn mothers. *Infant Behav Dev*. 1997;20(2):175-186. doi:10.1016/S0163-6383(97)90020-5
9. Bar-Haim Y, Marshall PJ, Fox N a. Developmental changes in heart period and high-frequency heart period variability from 4 months to 4 years of age. *Dev Psychobiol*. 2000;37(1):44-56. doi:10.1002/1098-2302(200007)37:1<44::AID-DEV6>3.0.CO;2-7 [pii]
10. Laborde S, Mosley E, Thayer JF. Heart rate variability and cardiac vagal tone in psychophysiological research - Recommendations for experiment planning, data analysis, and data reporting. *Front Psychol*. 2017;8:1-18. doi:10.3389/fpsyg.2017.00213

Chapter 6: Follow the leader. Elucidating the physiological mechanisms underlying adaptive mother-infant regulation of infant distress

Supplementary Table 1: Differences between those with and without data clean data

	Cases			Controls		
	Complete data (n=26)	No data (n=10)	*p*	Complete data (n=32)	No data (n=4)	
Infant age	5.4(2.5)	6.3(2.9)	0.39	5.3(2.5)	8.8(2.1)	0.009
Infant sex (n,%) Male	11(42.3)	5(50.0)	0.72	12(37.5)	3(75.0)	0.30
Income (n,%) <49,999 50-79,999 >80,000	4(15.4) 7(26.9) 15(57.7)	2(20.0) 2(20.0) 6(60.0)	0.89	5(15.6) 8(25.0) 19(59.4)	1(25.0) 1(25.0) 2(50.0)	0.89
Marital Status (n,%) Single Separate Common Law Married	2(7.7) 1(3.8) 6(23.1) 17(65.5)	1(10.0) 1(10.0) 4(40.0) 4(40.0)	0.57	2(6.3) 0(0.0) 5(15.6) 25(78.1)	0 0 0 4(100.0)	0.58
Maternal Education (n,%) High School or less	1(3.8)	2(20.0)	0.14	1(3.1)	1(25.0)	0.19

College or Certificate University or higher	9(34.6) 16(61.5)	2(20.0) 6(60.0)		8(25) 23(71.9)	1(25.0) 2(50.0)	
Mother's age (m,SD)	31.9(4.1)	33.7(4.2)	0.24	33.3(5.2)	30.7(4.2)	0.75
Parity (n,%) Primiparous multiparous	13(50.0) 13(50.0)	5(50.0) 5(50.0)	0.54	17(53.1) 15(47)	3(75.0) 1(25.0)	0.39
Mother's birth country (n,%) Canada Elsewhere	24(92.3) 2(7.6)	12(85.7) 1(7.1)	0.72	26(81.2) 6(18.8)	7(77.7) 2(22.2)	0.51
Gestational age (m,SD)	39.6(2.45)	38.5(2.4)	0.18	39.2(0.82)	39.9(1.25)	0.09
Birthweight (g) (m,SD)	3386.9(526.6)	3319.9(350.7)	0.67	3336.5(463.6)	3434.7(674.4)	0.63