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Effects of a Pregnancy Lifestyle Intervention on Emotion Regulation in Infants Responding to a Toy Removal Task at One-Year of Age

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Abstract

Poor infant emotion regulation (ER) has been linked with increased psychopathological risk in infancy and childhood. The Developmental Origins of Health and Disease Hypothesis suggests the prenatal period as the earliest point of intervention, where fetal exposure to healthy lifestyle changes can promote optimal ER in infancy. Infant electroencephalography (EEG; n=13) and heart rate variability (HRV; n=18) were measured at rest and in response to a toy removal task, to determine the ER of infants whose mothers participated in a nutrition and exercise intervention during pregnancy. Results demonstrated positive left frontal alpha asymmetry at rest (0.08± 0.66) and in response to stress (0.27 ± 0.72; p=0.167), with a significant increase in HRV (RMSSD: Z= -2.90, p<0.004; RSA: Z= -3.22, p< 0.001). These data suggest optimal infant ER, demonstrating the potential positive value of investing in lifestyle interventions during the pre- and postnatal period for early child development.

Keywords

"Infant emotion regulation, electroencephalography, heart rate variability, pregnancy lifestyle interventions, infant temperament, fetal neurodevelopment, developmental origins of health and disease"

Lay Summary

Research shows that the behaviour of the mother during pregnancy can affect brain development of the infant. Brain development can impact how an infant manages emotions such as anger or sadness. This is known as emotion regulation. If an infant manages emotions well there is evidence that this will continue throughout life. However, if an infant cannot manage emotions this may be harmful later in life. This study looked at how the nutrition and exercise choices of the mother affect the brain health of their infant. It is important because these choices impact the infant from early life to older age. To test this, women participated in a nutrition and exercise intervention during pregnancy. One year after these women gave birth, the emotion regulation of their infants was tested by recording brain and heart activity patterns. Results demonstrated that the infants of women who participated in a nutrition and exercise program while pregnant managed their emotions well. This research is important because it could help women protect the brain health of their infants before they are born. In the future, researchers and doctors may use these findings to inform women about healthy behaviour during pregnancy that will positively impact the health of their infant.

Co-Authorship Statement

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Glossary of Important Terms

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1. Ventromedial prefrontal cortex- *vmPFC*
2. Ventral anterior cingulate- *vACC*
3. Ventrolateral prefrontal cortex- *vlPFC*
4. Dorsolateral prefrontal cortex- *dlPFC*
5. Pre-supplemental motor area- *pre SMA*
6. Supplemental motor area- *SMA*
7. Anterior middle cingulate cortex- *aMCC*
8. Ventrolateral prefrontal cortex- *VLPFC*
9. Dorsolateral prefrontal cortex- *DLPFC*
10. Central autonomic network- *CAN*

Chapter 1 : Review of the Literature

1 Introduction

The following chapter will begin by introducing emotions and emotion regulation, the ways in which emotion regulation can be measured (physiologically via electroencephalography; EEG and electrocardiogram, ECG; and informant based via a questionnaire) and the stages of fetal neurodevelopment related to emotion circuitry in the brain. The theory of developmental plasticity, fetal programming, and the developmental origins of health and disease (DOHaD) hypothesis will also be outlined. As well, a case will be made for why pregnancy is a critical period to intervene with lifestyle interventions that aim to improve health outcomes for both mother and baby. The chapter will conclude with a summary and rationale for the current study.

1.1 Emotion and Emotion Regulation

1.1.1 What is an emotion?

Throughout history, emotions, which consist of a sequence of internal modifications that lead to external behaviour, have been essential to human survival (Sheppes, Suri, & Gross, 2015). Emotions comprise a substantial range of responses that vary in intensity, duration, complexity, difficulty, and confidentiality (Gross, 2014). In response to environmental events, individuals draw on their emotions to promptly recruit several systems to take action (Thayer & Lane, 2000). Emotions, therefore, function as a way to measure how an individual adapts to continuously shifting environmental challenges (Thayer & Lane, 2000). To effectively manage goal-directed behaviour, emotions are a necessary response for self-regulation (Thayer & Lane, 2000). A collection of methods related to emotion exist in order to to appraise the value of events regarding our security, comfort, and safety (Dixon et al., 2017). Although largely dependent on the indivdiual and particular set of circumstances, there exists central characteristics of an emotion (Gross, 2014).

1.1.2 Central elements of an emotion

When an emotion takes place, the circumstances in which it occurs will be appraised by an individual to determine whether they relate to a pertinent and active goal (Gross, 2015). The significance underlying the goal evaluated by the appraisal process can range from complex and deliberate to straightforward and involuntary (Gross, 2014). The basic value of the goal is what drives emotion (Gross, 2014). Dependent on this valuation, the emotion will shift with changes in the significance of the circumstances or changes in the circumstances themselves (Gross, 2014).

While emotions comprise several elements related to appraisal, they also involve the impulse to act, modifications in physiology, and are influenced by personal feelings (Dixon et al., 2017). Emotions are therefore multidimensional as they consist of coordinated behavioural, social, experiential, and physiological modifications (Gross, 2014). Experiencing an emotion is subjective and while this regularly leads to "feeling," emotions also incite action (Gross, 2014). Often, the multi-modal reaction that results in emotion facilitates goal achievement related to the objective that initiated the original emotional response (Gross, 2014).

There are a set of commonalities shared between emotions (Sheppes, Suri, & Gross, 2015). These include the circumstance being addressed, which must be understood as fundamental to social, cultural, and individual objectives in order to produce an emotion (Sheppes, Suri, & Gross, 2015). Additionally, all emotions comprise a myriad of coordinated changes in physiology, experience, and behaviour (Sheppes, Suri, & Gross, 2015). These changes can be illustrated by expressing how long the reaction lasts, the regularity of the reaction over a particular time period, as well as the type and consequences of the reaction (Sheppes, Suri, & Gross, 2015). Finally, these mutual attributes include the ability to adapt emotions to

align with the demands of a particular circumstance, which enables the capacity for emotion regulation (Sheppes, Suri, & Gross, 2015).

1.1.3 The 'modal model' of emotion: Generating an emotion

Based on the 'modal model', for an emotion to occur, an individual-circumstance interaction is required (Gross, 2014). This interaction must be considered significant to current goals after appraisal and produce a synchronized response involving multiple systems that can adapt if need be (Gross, 2014). Ultimately, the response must modify the initial individual-circumstance interaction (Gross, 2014)

The sequence of steps through which emotions are produced, illustrated in Figure 1, include: 1. Addressing the circumstance; 2. Determining emotional signficance; and 3. Generating a reaction that is behaviourally, practically, and biologically coordinated (Sheppes, Suri, & Gross, 2015; Gross, 2015). To initiate the process of producing emotions, the circumstance must be considered significant psychologically, which can occur internally or externally (Gross, 2014). An external circumstance may include an animal entering a camping tent, while feeling like a failure when starting a new job can be considered an internal mentally relevant situation (Gross, 2014). Regardless of the nature of the circumstance, it will be addressed in a variety of ways, which depend on both the indivdiual and the evaluation of the circumstance in relation to pertinent goals (Gross, 2014). Together with the evaluation of the situation, an emotional, behavioural, physiological, and practical response will ensue (Gross, 2014). Taken together to comprise an emotional response, these changes will likely loop back, modifying the circumstance that produced the initial response (Gross, 2014).

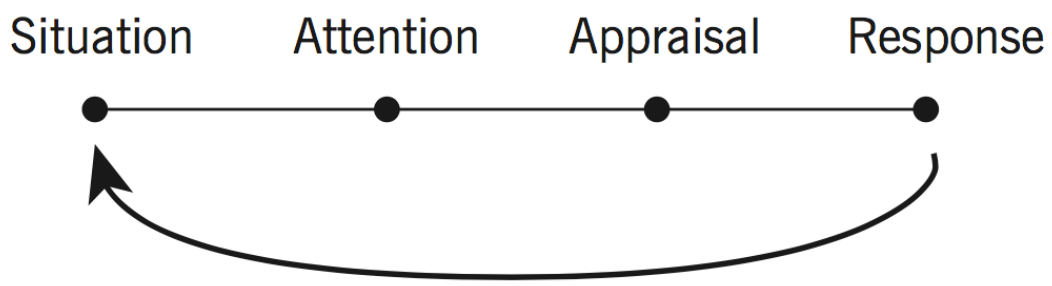


Figure 1. The modal model of emotion (Gross, 2014)

1.1.4 Regulating an emotion

Emotion regulation can be defined as the way in which an individual appraises, regulates, and adapts their emotions to reach long-term goals (Etkin, Büchel, & Gross, 2015; Tau & Peterson, 2010). The selection of a suitable emotional response requires developing a capacity to perceive stimuli and the emotional consequences (Ahmed, Bittencourt-Hewitt, & Sebastian, 2015). When a response is selected, a physiological reaction takes places, indicating that emotion regulatory systems are active (Williams et al., 2015). Thus, as a multi-dimensional concept, emotion regulation requires the coordination of cognitive appraisal capacity, the attention processes of an individual, as well as physiological arousal in response to stimuli (Ahmed, Bittencourt-Hewitt, & Sebastian, 2015; Thompson, 1994). Emotion regulation, therefore focuses on the way in which emotions are regulated or the regulation of emotions as opposed to regulation by emotions (Gross, 2014).

1.1.5 The relationship between emotion regulation and generation

Emotion regulation and generation are related in that regulation occurs when a goal is set in motion that mobilizes factors that affect emotion generation (Sheppes, Suri, & Gross, 2015). The methods through which both the regulation and generation of emotions function share similar elements, such as goal activation (Sheppes, Suri, & Gross, 2015). Despite sharing commonalities with regards to the activation of goals, the objective of these goals varies depending on whether regulation or generation is taking place (Sheppes, Suri, & Gross, 2015). For example, when generating emotions, the objective often depends on external and internal

consequences (Sheppes, Suri, & Gross, 2015). However, the goal of regulating an emotion is to stimulate a change in the framework that was used to initially generate the emotion (Sheppes, Suri, & Gross, 2015).

1.1.6 Central elements and brain regions of emotion regulation

There are several fundamental elements of emotion regulation, the first being the initiation of a goal to alter the development of emotion generation (Gross, 2014). When the initiation of a goal occurs within the individual, it is considered intrinsic emotion regulation (Gross, 2014). Whereas, extrinsic regulation occurs when a goal is initiated in someone else and as a result, the regulation of emotion from another occurs (Gross, 2014). While it can be valuable to distinguish between intrinsic and extrinsic emotion regulation, there are some circumstances in which both exist concurrently (Gross, 2014). For example, it is possible to regulate the emotion of another person, considered extrinsic regulation, with the goal of composing oneself (Gross, 2014).

There are several methods through which emotions can be regulated and these range from implicit to explicit (Gross, 2014). Rather than conceptualizing implicit and explicit emotion regulation as two fixed categories, they can be regarded as existing on a continuum (Ahmed, Bittencourt-Hewitt, & Sebastian, 2015; Etkin, Büchel, & Gross, 2015). Implicit emotion regulation is considered an unconscious process, occurring automatically, whereas explicit, requires a conscious effort to regulate emotional reactions and involves a certain measure of active management (Ahmed, Bittencourt-Hewitt, & Sebastian, 2015; Etkin, Büchel, & Gross, 2015). Two types of implicit emotion regulation include the management of conflict related to emotion and fear suppression (Etkin, Buchel, & Gross, 2015). The ventromedial prefrontal cortex (vmPFC) and the ventral anterior cingulate (vACC) are brain regions primarily concerned with implicit regulation (see Figure 2) (Etkin, Buchel, & Gross, 2015).

Furthermore, reappraisal, a widespread explicit approach to emotion regulation, involves deliberately modifying the significance of a stimulus that provokes emotion (Etkin, Buchel, & Gross, 2015). Specific brain areas that have been linked with reappraisal include the ventrolateral prefrontal cortex (vlPFC), the dorsolateral prefrontal cortex (dlPFC), and the parietal cortex, which together comprise the frontoparietal executive network (Etkin, Buchel, & Gross, 2015). The pre-supplemental motor area, and the supplemental motor area (SMA) as well as the insula are also implicated in the reappraisal process (see Figure 2) (Etkin, Buchel, & Gross, 2015). Both implicit and explicit regulation are therefore considered fundamental elements of emotion management as they are responsible for changing the trajectory that an emotion might take (Gross, 2014).

The influence of emotion regulation on the dynamics of an emotion is the third and final fundamental element of emotion regulation (Gross, 2014). Emotion dynamics occur in practical, biological, and social circumstances and include: the "latency, rise time, magnitude, duration, and offset of responses" (Gross, 2014). The role that emotion regulation plays to modulate these dynamics is contingent upon the objective of the individual (Gross, 2014). For example, it is possible that the latency can increase as a result of emotion regulation, while the magnitude may decrease (Gross, 2014). Furthermore, if the way in which an individual experiences an emotion and the subsequent physiological reaction do not align with an appropriate change in facial expression, emotion regulation may impact how the elements of an emotional reaction correspond with one another (Gross, 2014). While emotion regulation is often diverse among individuals, the initiation of a goal for regulation, the methods through which emotions can be monitored, and the dynamics of the emotion are three commonly observed elements (Gross, 2014).

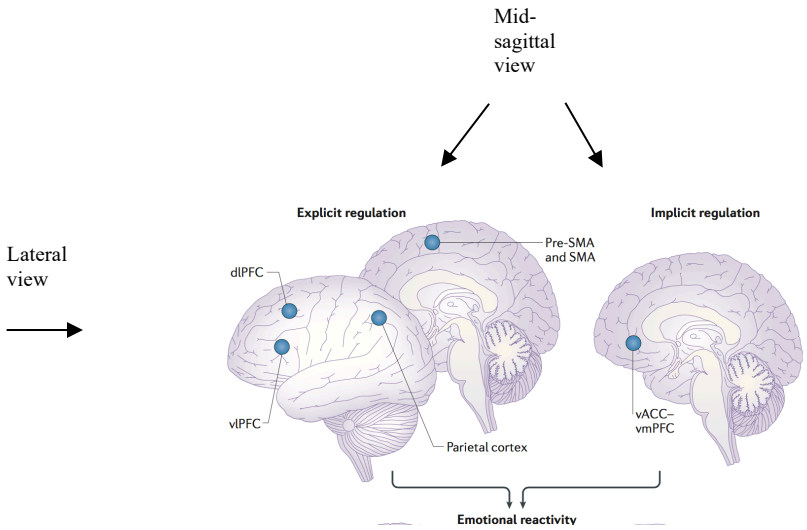


Figure 2. Regions of the brain involved in emotion regulation

dlPFC: dorsolateral prefrontal cortex, vlPFC: ventrolateral prefrontal cortex, pre-SMA & SMA: supplementary motor area, vACC-vmPFC: ventromedial prefrontal cortex-ventral anterior cingulate (vACC) (Etkin, Buchel, & Gross, 2015)

Several brain regions work to stimulate and regulate emotion, including the brainstem and prefrontal cortex (Dixon et al., 2017). The ventromedial prefrontal cortex consists of interrelated areas found in the orbital prefrontal cortex and the lower medial prefrontal cortex, both of which are thought to regulate adverse emotions (Hiser & Koenigs, 2018). Additional cortical regions implicated in emotion generation include the dorsal anterior cingulate (dACC) and the anterior insula (AI), while subcortical regions involved include the ventral striatum, amygdala, and periaqueductal grey (see Figure 3) (Etkin, Buchel, & Gross, 2015).

The subcortical amygdala and ventral striatum function to generate emotion and are also essential for indicating emotional stimulation and excitation (Kohn et al., 2014). The AI and the anterior middle cingulate cortex (aMCC) act as a link between the subcortical structures to the vlPFC, which is believed to represent the initiation of emotion regulation (Kohn et al., 2014). The vlPFC plays a functional role in perceiving, processing, and evaluating emotions (Kohn et al., 2014). As well, it is responsible for signaling to the dlPFC a need for regulation to begin (see

Figure 3) (Kohn et al., 2014). To execute the regulatory process, the superior temporal gyrus, pre supplementary motor area, and angular gyrus are activated by the dlPFC (Kohn et al., 2014). This forward signal from the dlPFC will then result in the production of an emotionally regulated state (Kohn et al., 2014).

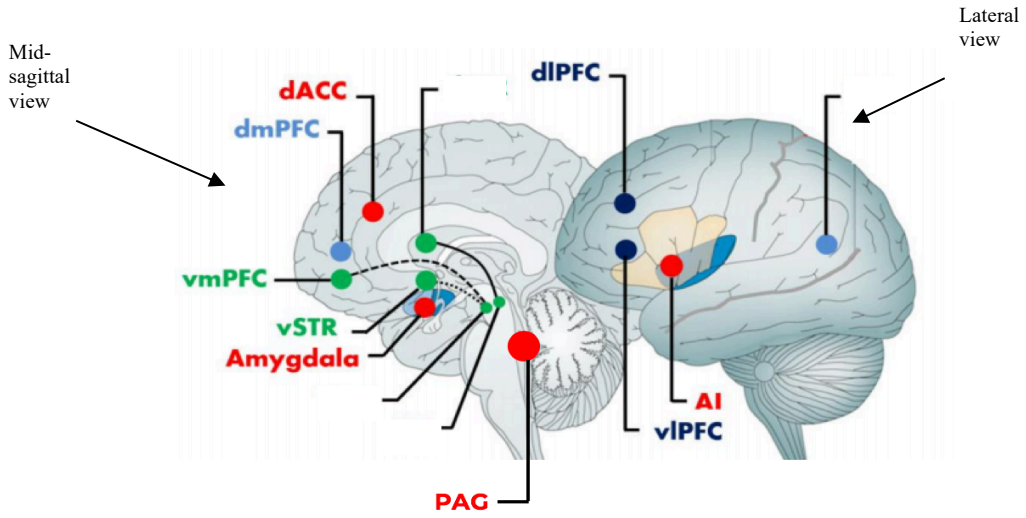


Figure 3. Overview of brain regions involved in emotion regulation

dlPFC: dorsolateral prefrontal cortex, AI: anterior insula, vlPFC: ventrolateral prefrontal cortex PFC: prefrontal cortex, PAG: periaqueductal grey, vSTR: ventral striatum, vmPFC: ventromedial prefrontal cortex, dmPFC: dorsomedial prefrontal cortex, dACC: dorsal anterior cingulate cortex (Kreuger, 2019)

Furthermore, the central autonomic network (CAN) is considered an important functional unit of the central nervous system (CNS), playing a role to integrate behavioural, visceromotor, and neuroendocrine responses related to self-regulation and flexibility (Thayer & Lane, 2000). Composed of the prefrontal cortex, the amygdala, the hypothalamus, and the medulla, the CAN is under tonic inhibitory control (Thayer & Lane, 2000). Together, these neural structures

function, processing input and output data to various degrees, which helps explain the multi-dimensional nature of emotion and emotion regulation (Etkin, Buchel, & Gross, 2015).

The prenatal development of complex neural networks, specifically the corticolimbic circuitry, influences childhood brain growth and maturation (Etkin, Büchel, & Gross, 2015; Swartz & Monk, 2013; Tau & Peterson, 2010). This therefore highlights the importance of ensuring an optimal intrauterine environment for healthy development and the way in which these brain structures develop prenatally will be explored further (Swartz & Monk, 2013).

1.1.7 Neurodevelopment and emotion regulation

Fetal neurodevelopment is characterized by the rapid proliferation, migration, and differentiation of neurons (Monk, Georgieff, & Osterholm, 2013). The process of neurulation initiates the development of precursor cells of the brain and spinal cord, composing the CNS (Rice & Barone, 2000). The notochord, a cellular rod outlining the primitive axis of the embryo, stimulates the overlying ectodermal tissue to produce the neural plate (Rice & Barone, 2000). The neural plate will then fold along its central axis, establishing the neural groove with adjacent neural folds (Rice & Barone, 2000). This occurs at approximately gestational day 18 (Rice & Barone, 2000). As the neural folds travel towards one another and merge, the neural tube begins to form (Rice & Barone, 2000). This occurs at approximately gestational day 21 in close proximity to the anterior end of the notochord (Rice & Barone, 2000). Three vesicles are then produced from the rostral end of the neural tube, which form the forebrain, the midbrain, and the hindbrain at approximately gestational day 28 (Tau & Peterson, 2010). The brain develops in a caudal-to-rostral manner, with the neural tube starting to close near the hindbrain, overlying the notochord and moving in an anterior to posterior direction (Rice & Barone, 2000). Neural tube development is complete between gestational days 26-28 (Rice & Barone, 2000).

Regions of the brain involved in regulating complex cognitive functions include the frontal lobes of the cerebral cortex, which are concerned with strategy development, planning, problem solving, organizing memories, focusing, inhibiting extraneous stimuli, and self-regulation (Bryan et al., 2004; Nyaradi et al., 2013). The corticolimbic circuity, comprised of the prefrontal cortex and the amygdala, are responsible for processing environmental stimuli related to emotion (Swartz & Monk, 2013). The prefrontal cortex functions to monitor emotional responses and process higher order emotional stimuli, while the amygdala senses relevant emotional or social cues, including indicators of threat and demonstrative facial expressions (Swartz & Monk, 2013). The amygdala and prefrontal cortex undergo regional neurogenesis between 4-19 weeks' gestation and 6-19 weeks' gestation respectively (Rice & Barone, 2000). The peak of neuronal migration occurs between 12-20 weeks' gestation and the majority of this process is complete by 26-29 weeks (Tau & Peterson, 2010) (see Figure 4 for a summary of important milestones during neurodevelopment). Exposure of the developing brain to insults such as recreational drugs, nutritional deficiencies, and toxins can disturb processes like myelination and synaptogenesis, increasing susceptibility to cognitive, neuropsychiatric, and neurodevelopmental consequences (Tau & Peterson, 2010).

To identify how potential insults *in utero* may impact brain structure and function, it is important to understand the conventional model of neurodevelopment as well as the developmental origins of health and disease hypothesis (DOHaD) (Gluckman et al., 2008;Swartz & Monk, 2013; Thompson, 1994). Perturbations to the neurodevelopmental model impacting the intrauterine environment such as maternal psychological stress, biological elements of disease, and undernutrition, can increase psychopathology risk in the offspring as well as the likelihood

of developing disorders related to emotion dysregulation (Monk et al., 2013; Tau & Peterson, 2010; Thompson, 1994).

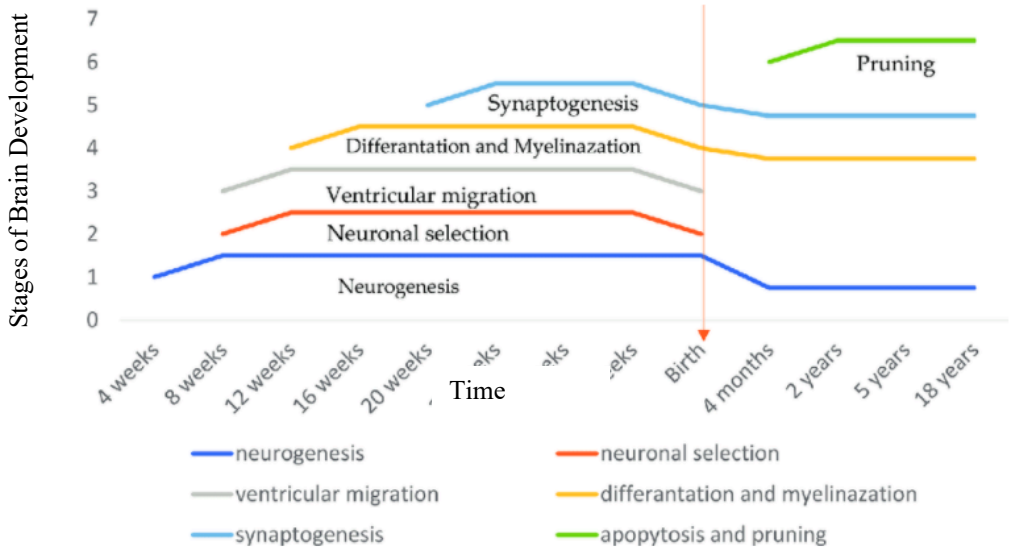


Figure 4. Timeline of neurodevelopment

Neuronal selection and ventricular migration occur before birth with neurogenesis, synaptogenesis, and differentiation and myelinazation taking place both before and after birth. The important milestones for emotion regulation occur between 4-19 gestational weeks (amygdala neurogenesis) and 6-19 weeks gestation (prefrontal cortex neurogenesis) with fine tuning occurring throughout infancy into childhood and adulthood. (Uytun, 2018)

1.1.7.1 The Developmental Origins of Health and Disease hypothesis

The DOHaD suggests that a poor intrauterine environment can lead to metabolic and physiological adaptations, increasing long-term disease risk in the adult (Gluckman et al., 2008). A collection of independent studies conducted in the 1980s promoted the relationship between developmental influences and sensitivity to disease later in life (Barker & Osmond, 1986; Gennser, Rymark, & Isberg, 1988; Wadsworth, Cripps, Midwinter, & Colley, 1985). Specifically, these observations demonstrated a link between low birth weight and increased risk of future cardiovascular and metabolic conditions (Gluckman, Hanson, & Low, 2011). This correlation has since been extended to include cognitive disorders, neurological diseases,

osteoporosis, hypertension, hyperlipidemia, and type 2 diabetes in relation to poor intrauterine environment exposure (De Boo & Harding, 2006; Gluckman, Hanson, & Low, 2011).

1.1.7.2 The Developmental Origins of Behaviour, Health, and Disease hypothesis

While the DOHaD suggests that early life events play a significant role to influence the development process, increasing risk for psychopathological issues later in life, incorporating additional elements related to behaviour and early brain development establishes these as essential targets to promote disease prevention (Gluckman et al., 2008; Shonkoff, 2010; Van Den Bergh, 2011). The addition of these elements leads to a reformed DOHaD, known as the 'Developmental Origins of Behaviour, Health, and Disease' (DOBHaD) (Van Den Bergh, 2011). The idea of the DOBHaD hypothesis is beneficial as it extends our consideration to include how growth and development of the brain influences behaviour and how these processes react to adverse stimuli prenatally or during early postnatal life (Van Den Bergh, 2011). This understanding may be useful to direct our attention towards the root cause of disease, informing a potential course of action for both treatment and prevention (Van Den Bergh, 2011).

From both the DOHaD and the extended DOBHaD, it is evident that interacting with harmful stimuli during vulnerable periods of *in utero* development may result in changes, which alter the 'programming' of organs and tissue (De Boo & Harding, 2006). The long-term effects of 'programming' can result in a predisposition to behavioural issues, trouble learning, psychopathology risk, and delayed cognitive development (Van Den Bergh, 2011)**.** Therefore, 'programming' has important implications for the health of the developing fetus as maternal exposures and behaviour during pregnancy can affect development, influencing risk for a variety of prevalent diseases before birth (De Boo & Harding, 2006).

1.1.7.3 Psychopathology and fetal neurodevelopment

The process of emotion management, regulation, and response is impacted by functional and structural changes to the brain that can occur during development (Swartz & Monk, 2013). Exposure to psychological or physiological insult during pregnancy may lead to enhanced postnatal psychopathological risk, given that a large proportion of neuroanatomical circuity related to cognition and emotion mature *in utero* (Van Lieshout & Krzeczkowski, 2016).

Deviation from the standard trajectory of brain development, including reduced amygdala activation in response to emotional stimuli or a lack of growth in prefrontal-cortex amygdala connectivity, may result in an increased risk of developing a range of future problems including criminal conviction, mental disorders, and substance dependence (Beauchaine, 2015; Daly et al., 2015; Moffitt et al., 2011). The dysregulation of emotions that originate in the prefrontal area has also been correlated with clinical issues such as substance abuse, anxiety, and binge eating through to adulthood (Gross, 1998). To prevent these clinical concerns, intervening during gestation and the early years of life are beneficial to mitigate potential risk for dysregulation as both developmental and environmental experiences play a role in the establishment of emotion regulation (Beauchaine, 2015, Gross, 1998).

1.1.8 Pregnancy and the perinatal period

To promote healthy emotion regulation and prevent adverse mental health outcomes, pregnancy presents the earliest opportunity to intervene (Van Den Bergh, 2011). Maternal lifestyle behaviours during pregnancy have been demonstrated to influence future health outcomes of the child (Van Den Bergh, 2011). To prevent the predisposition to adverse physical and mental health outcomes, the prenatal and early postnatal periods may be targeted through modifiable lifestyle behaviours (Van Den Bergh, 2011). Furthermore, the perinatal period presents a unique opportunity to modify regulatory systems, leading to a ‘reprogramming’ effect,

which can influence susceptibility to poor mental and physical health outcomes in infancy, childhood, and adulthood (Van Den Bergh, 2011).

Excess stress exposure during development has been demonstrated in animal research to alter the programming of the CNS and peripheral nervous system (PNS), both of which play important regulatory roles in the body (Van Den Bergh, 2011). Modifications have been observed in structures of the brain that make up the limbic system, including the amygdala and the hippocampus as well as the prefrontal cortex (Van Den Bergh, 2011). As a result, the way in which an individual responds to environmental stressors together with changes in physiology may be the source of future psychopathological issues (Van Den Bergh, 2011). Therefore, the perinatal period has important implications to shape the capacity of an infant to regulate emotions, which may determine susceptibility to mental health issues later in life (Van Den Bergh, 2011). Adversity experienced *in utero* and during early postnatal years can influence neurodevelopment and predispose infants and children to increased psychopathological risk, due to 'plasticity,' which facilitates developmental adaptations in response to early environmental stimuli (Barker, 2003; Gluckman et al., 2008; Van Den Bergh, 2011).

1.1.8.1 Developmental plasticity

Developmental plasticity explains how environmental changes during sensitive periods of development *in utero* can result in a variety of physical or morphological traits that can be attributed to one genotype (Barker, 2003). Evolutionarily, 'plastic' development is beneficial as it facilitates the production of phenotypes more suitable to the environment in which the fetus and subsequently the infant grows (Barker, 2003). While plasticity enables the fetus to make short term modifications in response to environmental changes, these adaptations can be naturally selected, leading to longer-term intergenerational effects (Barker, 2003). The potential for these changes to persist together with the decreased ability of the developing brain to make

modifications in response to the environment over time, supports the possible benefit of prenatal interventions (Barker, 2003; Van Lieshout & Krzeczkowski, 2016). Based on the theory of developmental plasticity, it is conceivable that physical activity and nutrition interventions introduced during pregnancy may be beneficial to support cognitive, behavioural, and self-regulatory development (Monk et al., 2013; Nyaradi et al., 2013).

1.1.8.2 The impact of nutrition and exercise interventions on emotion regulation

Healthy eating and regular exercise during pregnancy may play a role in the cognitive and behavioural maturation of the offspring, contributing to the development of infant emotion regulation (Monk et al., 2013; Nyaradi et al., 2013; Van Lieshout & Voruganti, 2008; Yeung et al., 2017). Research related to maternal nutrition and brain development *in utero* has focused on specific nutrient deficiencies, such as protein or iodine (Bath et al., 2013; Grantham-McGregor & Baker-Henningham, 2005). It is important to consider overall maternal diet as nutrient deficits rarely occur in isolation and foods are often consumed in combination to ensure optimal neurodevelopment (Christian et al., 2015; Hamadani et al., 2002; Leung et al., 2011; Jacka et al., 2013; Smithers et al., 2012). Studies investigating the effects of overall diet quality on fetal brain development have therefore been more effective than examining single nutrients and have demonstrated improvements in cognitive scores up to 3 years of age (Nyaradi et al., 2013; Tamura et al., 2002)

Furthermore, evidence from animal models demonstrated that the consumption of a high fat, high sugar diet during pregnancy may result in increased offspring aggression, impulsivity, and reward sensitivity, which are essential components of emotion regulation (Ong & Muhlhausler, 2011; Sullivan et al., 2010). Diets high in sugar and fat are linked to changes in the hippocampus and mesocorticolimbic dopamine system, whereby modifications in the hedonistic

set points occur, which is related to issues in emotion regulation (Naef et al., 2013; Vucetic et al., 2010). While little work has been done to investigate the effect of maternal diet quality on offspring emotion regulation, observational studies have established a relationship between poor maternal diet during pregnancy and increased risk of issues with emotion regulation in the offspring (Jacka et al., 2013). Nutrition interventions administered during pregnancy that focus on encouraging overall diet quality may have the potential to improve offspring emotion regulation (Nyaradi et al., 2013; Tamura et al., 2002).

With regards to the impact of maternal exercise during pregnancy on cognitive development, three case-control studies investigated the effects of reducing or maintaining exercise levels during gestation in women who were active pre-pregnancy (Clapp, Lopez, & Harcar-Sevcik, 1999; Clapp et al., 1998). It was determined that women who maintained their exercise routines had children with more optimal emotion regulation and cognitive scores up to five years of age (Clapp, Lopez, & Harcar-Sevcik, 1999; Clapp et al., 1998). A recent study investigating emotion regulation of one-month old infants determined that women who exercised during pregnancy had offspring with more adaptive emotion regulation, further demonstrating the beneficial effects of exercise on infant cognitive development (May, Scholtz, Suminski, & Gustafson, 2014).

It is essential to maintain a healthy weight during pregnancy via nutrition and exercise interventions as there is evidence to suggest that downstream effects can lead to advantageous adaptations to environmental stressors for both mother and baby (Ferraro, Gaudet, & Adamo, 2012). Lifestyle interventions during pregnancy can help provide a more optimal intrauterine environment that can establish a healthy trajectory for the child before birth (Ferraro, Gaudet, & Adamo, 2012). To reduce the risk of adverse maternal and fetal health outcomes and improve

infant cognitive development and emotion regulation, interventions introduced during gestation may present an ideal opportunity in the prevention of chronic diseases (Arabin & Baschat, 2017)

Maternal nutrition and exercise during pregnancy are key modifiable determinants to promote optimal neuroanatomical and neurochemical processes that underlie emotion regulation (Tau & Peterson, 2010, Monk et al., 2013; Nyaradi et al., 2013). Targeting maternal diet as a whole is beneficial as foods are rarely consumed in isolation and may interact synergistically to positively influence neurodevelopment (Nyaradi et al., 2013). Exercise maintenance during pregnancy is also a promising point of intervention due to its beneficial impact on infant emotion regulation and cognition (Clapp, 1996; Clapp et al., 1999, 1998). Given that nutrition and exercise during pregnancy play a role in 'programming' fetal metabolic traits as well as brain development this relationship may be an additional target that could be strengthened by lifestyle interventions delivered during gestation (Van Den Bergh, 2011). To determine whether lifestyle interventions (nutrition and exercise) engaged in during gestation 'program' fetal brain development to subsequently influence regulatory outcomes in infancy, emotion regulation in the early years of life must be explored.

1.1.9 Infant emotion regulation

Infant emotion regulation is largely influenced by prenatal and intrauterine exposures that affect brain development and subsequently mental, physical and behavioural health outcomes (Van Den Bergh, 2011). Established in the early years of life with developmental plasticity and programming, infant emotional and social development depend on the capacity of an individual to regulate emotions successfully (Ekas, Lickenbrock, & Braungart-Rieker, 2013). Consistent with commonly accepted definitions, infant emotion regulation is described as more than just the management of emotions, but as a state of constant flux working to assess time-based and comprehensive elements of an emotional event (Perry & Calkins, 2018). Playing a central role in

the ability of an infant to function, emotion regulation can be conceptualized as a collection of methods working at behavioural, social, and biological domains (Perry & Calkins, 2018). The investigation of emotion regulation as it progresses is critical for understanding both typical and uncharacteristic infant development (Perry & Calkins, 2018). Consisting of both regulatory and responsive elements that work to influence one another, the fundamentals of emotion regulation function to increase, preserve, change, or obstruct both the strength and valence of an emotion with the goal of achieving objectives (Perry & Calkins, 2018). According to a biopsychosocial perspective, emotion regulatory processes describe multi-faceted reactions that are biological in nature, include interactive behaviour, and occur within a social context (Perry & Calkins, 2018).

Considering the development of infant emotion regulation within a biological, behavioural, and social context facilitates the understanding that these elements are reciprocal, working to influence and consequently moderate one another (Perry & Calkins, 2018). In the biological sphere, early emotion regulation is characterized by reflexive physiological responses (Perry & Calkins, 2018). Constant changes occurring throughout physiological systems enables the capacity to manage emotions behaviourally (Perry & Calkins, 2018). In early infancy, methods for regulation related to attention start to mature, laying the foundation for the development of a more deliberate and multi-faceted capacity to manage emotions (Perry & Calkins, 2018).

Specific physiological systems underlying infant emotion regulation include the executive attention network, consisting of components of the prefrontal cortex and the anterior cingulate cortex (ACC) found in the medial frontal lobe (Perry & Calkins, 2018). In the voluntary management of attention, the executive attention network is fundamental to facilitate the development of the emotion regulatory capacity of the infant (Perry & Calkins, 2018). In

order to manage and organize inputs from other neural networks concerned with the regulation of emotion and cognition, activity of the ACC and elements of the prefrontal cortex are essential (Perry & Calkins, 2018). To moderate emotional stimulation, increased organization of synaptic networks within the executive attention network could improve the rate at which infants attend to their surroundings, potentially enabling them to focus on particular elements of the environment (Perry & Calkins, 2018). Consequently, there may be improvement in the cognitive adaptability of the infant and capacity to select the alternate, less predominant but more favourable response (Perry & Calkins, 2018). For example, this type of restraint may be beneficial in a situation where an infant must delay engaging with an attractive object and control their enthusiasm to do so (Perry & Calkins, 2018). Underlying biological mechanisms are therefore critical to the development of adaptable successful emotion regulation (Perry & Calkins, 2018).

1.1.9.1 Milestones related to emotion regulation in infancy

Significant modifications in the behavioural capacity of the infant to regulate emotions occurs from birth to one year of age (Ekas, Lickenbrock, & Braungart-Rieker, 2013). Initially, with the goal of regulating emotions, infants often depend on inborn responses and reflexes like sucking or turning their heads throughout their first few months (Ekas, Lickenbrock, & Braungart-Rieker, 2013). At approximately 3-months infants begin to participate in basic behavioural regulatory actions to soothe themselves (Ekas, Lickenbrock, & Braungart-Rieker, 2013; Perry & Calkins, 2018). These include sucking their thumbs, motor actions such as turning away, and involuntary indications of distress through emotions like crying, which are common behaviours in infants at approximately 3-months old (Ekas, Lickenbrock, & Braungart-Rieker, 2013; Perry & Calkins, 2018). Between the ages of 3 and 7-9 months, infants experience significant growth enabling them to become conscious of their emotional states, facilitating their ability to deliberately alter them (Ekas, Lickenbrock, & Braungart-Rieker, 2013). For instance,

an infant may attempt to modify their emotional state with a toy (Ekas, Lickenbrock, & Braungart-Rieker, 2013).

Additionally, at approximately 6-months old, infants start to manage emotional stimulation on their own accord, developing the capacity to transfer their attention, which enables them to adjust their focus away from stressful catalysts (Ekas, Lickenbrock, & Braungart-Rieker, 2013; Perry & Calkins, 2018). Repetition of this behaviour helps the infant learn that they have the capacity to manage their emotions (Ekas, Lickenbrock, & Braungart-Rieker, 2013). When regulating emotions from a behavioural perspective, the goal is to diminish or down-regulate adverse emotions (Ekas, Lickenbrock, & Braungart-Rieker, 2013). For example, both self-soothing and attentional distraction are known as regulatory actions because their goal is to diminish adverse feelings by relocating attention elsewhere from the stressful situation or by engaging in a calming behaviour like sucking the thumb (Ekas, Lickenbrock, & Braungart-Rieker, 2013). For a behaviour to be thought of as regulatory, it is essential that adverse emotions are consequently modified (Ekas, Lickenbrock, & Braungart-Rieker, 2013).

Emotion regulation is not only affected by the ability to self-manage, but it is also influenced by external sources throughout development (Thompson, 1994). From a social perspective, outside resources for emotion management, such as parental care, is typical during the first year of life (Ekas, Lickenbrock, & Braungart-Rieker, 2013). Throughout this period, it is common for infants to shift from relying primarily on caregiver influences for emotion management towards the introduction of self-regulatory actions that enable the infant to control their emotions (Ekas, Lickenbrock, & Braungart-Rieker, 2013; Perry & Calkins, 2018). Nevertheless, caregivers continue to play a role to intervene with emotion regulation throughout

infancy by observing, monitoring, and modifying emotional states to ensure behaviour aligns with cultural norms related to emotions (Thompson, 1994).

As an important period for investigation, infancy is characterized by an increased number of motor, cognitive, and behavioural skills that enable an enhanced capacity for emotion regulation (Ekas, Lickenbrock, & Braungart-Rieker, 2013). Overall, emotion regulation plays an important self-regulatory role throughout infancy and adverse exposures during the prenatal period can result in the inadequate development of neuroregulatory systems, which may predispose the infant to future psychopathological, behavioural, learning, and cognitive issues (Thompson, 1994; Van Den Bergh, 2011).

1.1.9.2 Psychopathology and infant emotion regulation

Fostering optimal emotion regulation in early infancy is fundamental as it has been determined that an inability to self-regulate is predictive of unemployment rates in adulthood (Daly et al., 2015). Unemployment has been correlated with increased susceptibility to stress, poor eating, sleeping, and hygiene habits, as well as alcohol abuse (Daly et al., 2015). Even after controlling for differences in class, intelligence, family, and health related issues, the association between self-regulation and unemployment remained (Daly et al., 2015). Furthermore, dysregulation of emotions has been implicated in the majority of psychopathological issues (Beauchaine, 2015). For example, a lack of regulated dysphoria, panic, or anxiety is common in internalizing conditions (Beauchaine, 2015). Similarly, externalizing issues are often a result of difficulty regulating anger and irritation (Beauchaine, 2015).

Ensuring the development of optimal emotion regulation is further justified as it has been demonstrated that impeding the behavioural actions of an infant can lead to atypical structural changes in the vmPFC as development occurs (Beauchaine, 2015). In addition, an upsurge in levels of cortisol, often due to stress in early life can structurally impact the link between the

amygdala and the vmPFC, resulting in functional implications for regulating emotions later in life (Beauchaine, 2015). Finally, abuse in infancy can also result in issues related to connections between the amygdala and hippocampus with the subgenual cingulate cortex, poorly impacting function in later years (Beauchaine, 2015). It is therefore clear that external events beginning in early infancy can play a significant role to increase the vulnerability of adolescents to issues related to regulating emotions (Beauchaine, 2015). To lay the foundation for optimal emotion regulation both prenatally and in the early postnatal period, it is essential to measure emotion regulation from a physiological, observed, and informant-based perspective (Adrian, Zeman, & Veits, 2011; Van Den Bergh, 2011).

1.1.10 How is emotion regulation measured?

The following section will outline two physiological and one informant-based measure commonly used to measure emotion regulation in infants.

1.1.10.1 Electroencephalography

Electroencephalography (EEG) is one physiological marker used to objectively evaluate the contributions of corticolimbic brain function to emotion regulation (Coan & Allen, 2004a). While many measures exist to evaluate neurobiological outcomes, such as positron emission tomography (PET) and functional magnetic resonance imaging (fMRI), EEG has been established as the most optimal method to collect neurophysiological data from infants (Potapova, 2019). EEG is non-invasive, straightforward in administration, and it is generally considered a sensitive measure of the cortical activity in infants (Potapova, 2019). EEG is adaptable and enables data collection in infants without the need for limited movement, in several different settings, and while awake (Potapova, 2019). It is therefore considered an optimal tool to measure infant emotion regulation.

To interpret and understand individual variations in emotion regulation, it is important to examine patterns of resting alpha brain activity in the anterior cerebral hemisphere using EEG (Bryan et al., 2004; Coan & Allen, 2004a). Asymmetrical frontal cortical activity is measured by comparing left and right hemisphere alpha power activity levels, between 6-9 Hz for infants (Harmon-Jones & Gable, 2018). Research that has merged EEG, hemodynamic, and behavioural task measures suggest that alpha power has an inverse relationship with brain activity (Harmon-Jones & Gable, 2018). A difference score is commonly used in EEG frontal asymmetry research, supported by lesion and amytal studies that suggest asymmetry can be explained as one hemisphere suppressing the opposite (Rutherford & Lindell, 2011; Harmon-Jones & Gable, 2018). In clinical studies that have investigated how unilateral brain lesions can result in a variety of emotional expressions depending on the brain hemisphere affected, frontal hemisphere asymmetry has been linked to emotion regulatory capacity (Rutherford & Lindell, 2011). For example, when patients suffered a lesion to the right hemisphere, they experienced euphoric emotions, while greater negative emotions were observed in patients with left hemisphere lesions (Rutherford & Lindell, 2011).

Similarly, the Wada test has been used to investigate asymmetry, where a derivative of a barbiturate, amytal, was injected into an internal carotid artery, thus inhibiting brain hemisphere activity (Harmon-Jones & Gable, 2018). When amytal was injected towards the left hemisphere, it was deactivated, increasing activity of the right uninhibited hemisphere, resulting in the expression of depressed emotions (Harmon-Jones & Gable, 2018). While, euphoric emotions and expression of bliss were observed with injections to the right hemisphere, leading to its deactivation and a subsequent increase in activity of the left hemisphere (Harmon-Jones & Gable, 2018). These results are likely attributable to contralateral forces, whereby the inhibition

of one hemisphere leads to the release of the other (Harmon-Jones & Gable, 2018). This evidence suggests that resting frontal EEG activity represents a unique individual pattern of emotion regulation that may reveal important differences underlying neuronal recruitment systems (Coan & Allen, 2004a).

EEG results are generally referred to as relative left or relative right frontal activity by subtracting left frontal alpha power from right frontal alpha power (Harmon-Jones & Gable, 2018). If the result is more positive, it will be referred to as relative left activity, whereas a more negative result will indicate relative right activity (Harmon-Jones & Gable, 2018). This approach to evaluating emotion regulation is grounded in the belief that emotions are driven by approach or avoidance motivations, where approach is mainly correlated with positive emotions and avoidance is linked with negative emotions (Rutherford & Lindell, 2011). According to Davidson (1982), greater relative left frontal activity measured in an infant correlates with approach behaviours including cheerful vocalization and joyous expressions (Fox & Davidson, 1986, 87, 88). Avoidance motivations such as behaviours indicating withdrawal like gaze aversion correlates with greater relative right frontal activity in infants (Davidson, 1982; Fox & Davidson, 1986, 1987, 1988). As a measure of infant cognitive development, EEG asymmetry is informative, non-invasive, and widely available (Coan & Allen, 2004a). It can be considered a preliminary psychophysiological indicator of an inherent inclination towards more negative emotional responses in novel and moderately stressful situations (Fox, 1991)

1.1.10.2 Heart Rate Variability

Heart rate variability (HRV) can also be used to index emotion regulation in infants by measuring the activity of the parasympathetic division of the autonomic nervous system (ANS) (Thayer, Yamamoto, & Brosschot, 2010). The ANS plays a role in the regulation of physiological systems, such as the heart, endocrine glands, and smooth muscle (Ernst, 2017).

The main function of the ANS is to maintain homeostasis within the body, which is controlled almost entirely by autonomic reflexes (Ernst, 2017). The two components of the ANS are the sympathetic nervous system (SNS) and the parasympathetic nervous system (PNS) (Ernst, 2017). These systems oppose one another as the SNS functions to mobilize and regulate actions related to stress, whereas the PNS is responsible for the relaxation response (Ernst, 2017). This dynamic balance in the cardiovascular system leads to the variation between intervals of successive heart beats, known as HRV (Xhyheri et al., 2012).

HRV can be defined as the modification in time intervals between sequential heartbeats and it provides an indication of parasympathetic nervous system activity (Laborde, Mosley, & Thayer, 2017). The heart is innervated by both the PNS and SNS (Thayer et al., 2012). Internal cardiac mechanisms and the collaborative activity of the sympathetic and parasympathetic (vagus) nerves at the sinoatrial (SA) node, work to establish heart rate (Thayer et al., 2009). A reduction in heart rate is correlated with a rise in parasympathetic activity and an increase in heart rate is linked to a relative surge in sympathetic activity (Thayer et al., 2012). To determine variability in the timing of the heartbeat, the inter-beat interval, or the series of time intervals between beats, is measured (Thayer et al., 2012). Decreasing the inter-beat interval can be achieved with a relative increase in sympathetic activity, resulting in more brief time periods between heart beats (Thayer et al., 2012). A relative surge in parasympathetic activity initiates a longer time between heart beats, thus increasing the inter-beat interval (Thayer et al., 2012). Therefore, a decrease in HRV values usually reflects high levels of activity in the sympathetic division of the ANS, which may be coupled with low parasympathetic activity (Xhyheri et al., 2012). Conversely, increased HRV values suggest a shift in the equilibrium between the SNS and PNS, moving towards heightened vagal activity (Xhyheri et al., 2012).

The PNS is believed to play a significant role in the generation and regulation of emotions (Perry & Calkins, 2018). The primary nerve of the parasympathetic system is the myelinated vagus (10th cranial nerve) (Laborde et al., 2017; Perry & Calkins, 2018). Functioning to provide inputs to the heart, the vagus nerve generates dynamic modifications in the activity of the cardiac system (Perry & Calkins, 2018). This enables the body to successfully shift between reacting to external stimuli and maintaining metabolic demands (Perry & Calkins, 2018). When an individual is faced with an emotionally demanding circumstance, the ability of the vagus nerve to regulate the heart can be measured (Perry & Calkins, 2018). In the event of a non-emotionally stimulating situation, the effect of the SNS on the activity of the cardiac system will be inhibited by the vagus nerve, due to an increase in parasympathetic activity (Perry & Calkins, 2018). This will result in restoration and relaxation (Perry & Calkins, 2018). Conversely, the vagus nerve will be suppressed in the event of an emotionally demanding circumstance (Perry & Calkins, 2018). This will result in an upsurge of the SNS, producing an increased heart rate and capacity to concentrate, enabling the implementation of effective strategies to manage emotion (Perry & Calkins, 2018). The reduction in activity of the vagus nerve indicates the removal of the influence of the PNS, which can be measured to determine the physiological response of an individual to a stressful event (Perry & Calkins, 2018). Therefore, with regards to HRV and emotion regulation, more optimal regulation is reflected with an increase in HRV and less optimal regulation is demonstrated by a decrease in HRV (Shaffer & Ginsberg, 2017).

1.1.10.3 Measures of HRV: Root Mean Square of Successive Difference

To measure HRV and determine the amount of variance in the inter-beat interval, a time-domain analysis can be conducted (Minarini, 2020). The most commonly used measure in the time domain to evaluate changes in HRV mediated by the vagus nerve is the root mean square of

successive difference (RMSSD) between normal heart beats (Minarini, 2020). RMSSD indicates activity of the parasympathetic system and it reflects the capacity of an infant to self-regulate (May et al., 2016; Minarini, 2020). As a measure of HRV, when RMSSD scores are greater at rest, this suggests that the PNS is active, reflecting appropriate self-regulation (May, 2016; Minarini, 2020). Therefore, in response to a stressor, it would be expected that RMSSD scores would increase, reflecting an appropriate balance in autonomic regulation between the PNS and SNS and thus increased HRV (Shaffer & Ginsberg, 2017). Conversely, a decrease in RMSSD as a result of a stressor would indicate a decrease in HRV, indicating less optimal infant emotion regulation (Shaffer & Ginsberg, 2017).

To distinguish between parasympathetic and sympathetic inputs, determining the power at a variety of frequencies (i.e. very low frequency, low frequency, and high frequency) is beneficial, which can be carried out with a frequency domain analysis (May et al., 2016). Measuring a time domain analysis, represented by RMSSD, and a frequency domain analysis, represented by high frequency power, are highly related as they both reflect activity of the PNS (Minarini, 2020).

1.1.10.4 Measures of HRV: Respiratory Sinus Arrhythmia

Vagal tone refers to parasympathetic activity and the cardiac vagal tone illustrates the association between the heart and the brainstem, demonstrating the involvement of the vagus nerve in cardiac functioning (Laborde et al., 2017; Porges, et al., 1996). Vagal tone is generally referred to as respiratory sinus arrhythmia (RSA), which is a measure of HRV that occurs at the frequency of breathing (Buss, Goldsmith, & Davidson, 2005; Perry & Calkins, 2018; Williams et al., 2015). The vagal tone plays two important roles in cardiac function: the first occurs during conditions characterized by low environmental needs, such as sleep, and the second takes place when there is a high demand in the environment (Porges et al., 1996). In the first case, the vagal

tone promotes physiological homeostasis, prioritizing restoration, relaxation, and growth (Porges et al., 1996). To manage cardiac and metabolic output, the vagus will assume the role of a "brake" in the second case, decreasing heart rate by impeding sympathetic effects and thus increasing vagal output (Porges et al., 1996). The vagal brake can be described as a gradient that impedes the sinoatrial node, also known as the cardiac pacemaker, via efferent vagal fibers (Porges et al., 1996).

As a measure of HRV, cardiac vagal tone or RSA, has been correlated with research related to psychophysiological outcomes such as self-regulation (Laborde et al., 2017). It is regarded as an inherent measure of the ability to regulate emotions and RSA measured at rest is a significant benchmark to determine typical levels of arousal (Perry & Calkins, 2018). RSA is used to quantify and index emotion regulation as it has been found to be linked to the types of regulatory behaviours that infants display (Calkins & Keane, 2004). Higher resting RSA values have been determined to indicate appropriate activity of the parasympathetic system as well as increased flexibility and capacity to adapt, illustrated by an increase in HRV (Buss, Goldsmith, Davidson, 2005; Graziano & Derefinko, 2013). It was additionally determined that RSA illustrates a reliable indicator of prefrontal cortex operation, related to regulation (Beauchaine, 2015).

According to the Polyvagal theory, changes in RSA that occur dynamically indicate an attempt to adaptively regulate emotions (Brooker & Buss, 2010). Consequently, a more optimal capacity to physiologically manage emotions is represented by an increase in RSA from a resting state to an emotionally demanding task, which is due to an increase in activity of the vagus nerve and an increase in HRV (Perry & Calkins, 2018). A decrease in RSA demonstrates the contrary

case, where emotions are maintained rather than adapted, indicating a less optimal capacity for self-regulation and a decrease in HRV (Brooker & Buss, 2010).

1.1.10.5 Measures of HRV: Ratio between high frequency and low frequency power

As a measure within the frequency domain, the ratio between the low frequency (LF) power and high frequency (HF) power is considered an evaluation of the balance between sympathetic and parasympathetic activity (May et al., 2014). As a frequency domain measure, an LF/HF ratio that is low illustrates a higher degree of parasympathetic activity and increased HRV, often observed during energy conservation resting states (Shaffer & Ginsberg, 2017). Conversely, an LF/HF ratio that is high at rest demonstrates a greater degree of activity in the sympathetic system, and a decrease in HRV (Shaffer & Ginsberg, 2017). Across a stressful task, a decrease in the LF/HF ratio would indicate increased parasympathetic activity, increased HRV and as a result, a more optimal capacity to regulate emotions (Shaffer & Ginsberg, 2017). An increase in the LF/HF ratio would demonstrate increased sympathetic activity and a decrease in HRV, illustrating poor emotion regulation (Shaffer & Ginsberg, 2017). While RMSSD, RSA, and the LF/HF ratio are important measures of HRV and the autonomic regulation of the heart, they also demonstrate the level of integration of the CNS and the peripheral nervous system (May et al., 2014). To illustrate this link, the relationship between EEG and HRV will be explored.

1.1.10.6 The relationship between EEG and HRV

The link between HRV and EEG is explained by the common brain regions, the prefrontal cortex and the amygdala, that is shared between these systems (Mather & Thayer, 2018). While the CNS, a neural physiological system and the ANS, a peripheral physiological system are commonly perceived as distinct bodily systems, they function dynamically as feedback systems

(Perry & Calkins, 2018). The CNS and the cardiac system function in a reciprocal fashion, where an intricate network of efferent and afferent pathways are linked with additional physiological and anatomical processes related to the nervous system (Perry & Calkins, 2018). These networks together with the PNS and SNS contribute to the relationship between the brain and the heart (Perry & Calkins, 2018).

Through the vagus nerve and the central autonomic network, the neurovisceral integration model (NIM) suggests a link between the heart and the prefrontal cortex (Laborde et al., 2017). Based on the NIM, a higher vagal tone indicates more optimal emotion regulation and vice versa (Laborde et al., 2017). Additionally, the NIM posits that the pre-frontal cortex plays an inhibitory role on brain structures below the cortex (i.e. the amygdala) (Williams et al., 2015). This enables the individual to have an adaptive response to environmental demands, characterized by the successful organization of behavioural and emotional reactions (Williams et al., 2015). Increased inhibition of active brain areas at rest, therefore, indicates more optimal emotion regulation and the regulatory role of these neural structures extends to monitor the ANS (Williams et al., 2015). As illustrated by the influence of the PNS on the heart and other peripheral organs dominating over the SNS, it is evident that parasympathetic control mediated by the vagus nerve is responsible for physiological regulation that subsequently impacts psychological regulation (Williams et al., 2015). According to the NIM theory, the evaluation of vagally mediated HRV indicates the flexibility of the brain to regulate peripheral systems (Williams et al., 2015).

To further demonstrate the relationship between the prefrontal cortex and the ANS, Ahern et al. (2001) found that during an intracarotid sodium amobarbitual test, leading to prefrontal cortex inactivation, there was a decline in HRV. This may indicate that the prefrontal cortex plays an important role in cardiac function (Hansen et al., 2004). The structural

connection linking the physiology of the heart to the psychology of emotion regulation is thought to be the dynamic inhibition of neural circuitry (Williams et al., 2015). Therefore, together the maturing cardiac autonomic nervous system, indexed by HRV reflected through RMSSD, RSA, and LF/HF, and the CNS, indicated through EEG measured by FAA, may be the earliest targets for intervention to ensure the development of an optimal capacity for emotion regulation (May et al., 2014; Van Den Bergh, 2011).

1.1.10.7 Infant-Behavioural Questionnaire-Revised (IBQ-R)

The third measure of infant emotion regulation is informant-based and grounded in the Rothbart & Derryberry (1981) theory of infant temperament. They define temperament as "constitutionally based individual differences in reactivity and self-regulation" (Gartstein, Bell, & Calkins, 2014). The biological and behavioural response to environmental changes are believed to represent the reactivity dimension of temperament (Stifter & Jain, 1996). Whereas, the methods and strategies employed to moderate the biological and behavioural response represents the regulation dimension (Stifter & Jain, 1996). Temperament has been related to processes used to settle emotional conflict through attentional control (Morasch & Bell, 2012). Based on this, infants who have developed the capacity for more mature attentional control demonstrate optimal regulation of emotions (Morasch & Bell, 2012). Furthermore, attentional control in infancy as indexed by temperament, has been related to physiological measures of regulation such as autonomic control measured by RSA, RMSSD, and the LF/HF ratio (Morasch & Bell, 2012).

As a measure reported by caregivers, three higher order constructs of temperament have been derived from the IBQ-R (Gartstein et al., 2014). These include Negative Emotionality, Positive Affectivity/ Surgency, and Regulatory Capacity/ Orienting (see Table 1 for a summary of these scales and their sub-scales) (Gartstein et al., 2014). Each of these constructs have

demonstrated adequate psychometric properties such as convergent validity with physiological assessments (Potapova, 2019). Negative affectivity is one of the first constructs of temperament to develop and it has been correlated with distress in toddlerhood and neurotic behaviour in adulthood (Gartstein et al., 2014). A measure of temperament is essential as a proxy for emotion regulation because the way in which temperament develops has been related to psychopathological issues in infancy and childhood (Gartstein et al., 2014). For instance, increased levels of negative affectivity in infancy has been linked with maternal reports of depression and anxiety at 7 years old (Gartstein et al., 2014).

Table 1. Scale and sub-scale definitions for the Infant Behaviour Questionnaire-Revised (IBQR)

Positive Affectivity/Surgency	
Activity Level	Gross motor activity, including movement of arms and legs, squirming and locomotor activity. ("When put into the bath water, how often did the baby splash or kick?"; similar in length/content to the original IBQ scale)
Approach	Rapid approach, excitement, and positive anticipation of pleasurable activities. ("When given a new toy, how often did the baby get very excited about getting it?")
High Intensity Pleasure	Pleasure or enjoyment related to high stimulus intensity, rate, complexity, novelty, and incongruity. ("During a peek-a-boo game, how often did the baby smile?")
Perceptual	Detection of slight, low intensity stimuli from the external environment.
Sensitivity	("How often did the baby notice fabrics with scratchy texture (e.g., wool)?")
Smile and Laughter	Smiling or laughter during general caretaking and play. ("How often during the last week did the baby smile or laugh when given a toy?"; shorter and different in content from the original IBQ scale)
Vocal Reactivity	Amount of vocalization exhibited by the baby in daily activities. ("When being dressed undressed during the last week, how often did the baby coo or vocalize?")
Negative Emotionality	
Distress to Limitations	Fussing, crying or showing distress while a) in a confining place or position; b) in caretaking activities; c) unable to perform a desired action. ("When placed on his/her back, how often did the baby fuss or protest?"; shorter, but similar in content to the original IBQ scale)
Fear	Startle or distress to sudden changes in stimulation, novel physical objects or social stimuli; inhibited approach to novelty. ("How often during the last weed did the baby startle to a sudden or loud noise?"; different in content from the original IBQ)
Sadness	Lowered mood and activity related to personal suffering, physical state, object loss, or inability to perform a desired action; general low mood. ("Did the baby seem sad when the caregiver was gone for an unusually long period of time?")
Falling Reactivity/ Rate of Recovery from Distress	Rate of recovery from peak distress, excitement, or general arousal; ease of falling asleep. ("When frustrated with something, how often did the baby calm down within 5 minutes?")
Orienting/Regulatory Capacity	
Cuddliness	Expression of enjoyment and molding of the body to being held by a caregiver. (When rocked or hugged, during the last week, how often did the baby seem to enjoy him/herself?")
Duration of Orienting	Attention to and/or interaction with a single object for extended periods of time. ("How often during the last week did the baby stare at a mobile, crib bumper or picture for 5 minutes or longer?"; similar in length/content to the original IBQ scale)
Low Intensity Pleasure	Amount of pleasure or enjoyment related to low stimulus intensity, rate, complexity, novelty and incongruity. ("When playing quietly with one of is/her favorite toys, how often did the baby show pleasure?")
Soothability	Reduction of fussing, crying, or distress when soothing techniques are used by the caregiver. ("When patting or gently rubbing some part of the baby's body, how often did s/he soothe immediately?"; similar in length/content to the original IBQ scale)

(Gartstein, Bell, & Calkin, 2014)

The negative affectivity scale is made up of four sub-scales (sadness, distress to limitations, fear, and falling reactivity) (Gartstein & Rothbart, 2003). The sadness sub-scale is defined as decreased activity and mood as a result of stress, distress to limitations is described as crying, distress, and fussiness that occurs when an infant cannot achieve a goal, fear is understood as avoiding new situations or exhibiting stress in response to them, and finally falling reactivity includes the rate at which an infant recovers from the height of agitation or distress (Gartstein & Rothbart, 2003).

The IBQ-R is a valuable instrument to measure infant temperament as it was created to represent the biological and behavioural constructs that comprise the psychobiological model of temperament (Rothbart & Derryberry, 1981). The revised version was produced in 2003, to build on the goal of the original questionnaire by determining how the elements of temperament related to regulation affect foundational neurobehavioural systems (see Appendix A1) (Rothbart, 2011). The negative affectivity scale of the IBQ-R was chosen to be examined in further detail as it reflects the individual diversity that can be observed in the vulnerability of the infant to encounter negative emotions (Rouse & Goodman, 2014).

Furthermore, consistently recognized at approximately three months old, negative affectivity is a reliable measure and it remains stable over time (Gartstein & Rothbart, 2003). Overall, the stability of the negative affectivity scale over time, its emergence early in development, and its relationship to psychopathology risk later in life makes it an important measure of emotion regulation (Rouse & Goodman, 2014). Since negative affectivity reveals the susceptibility of an individual to negative emotions, occurs early in development, and has been correlated with future psychopathology risk, there has be a keen interest in determining

predictors of this scale (Rouse & Goodman, 2014). Pregnancy behaviour emerged as a potential predictor due to research relating adverse *in utero* exposures to subsequent changes in neuroregulatory systems (Rouse & Goodman, 2014). These neuroregulatory systems are linked to the emotional and behavioural dimensions of temperament, thus relating the IBQ-R, pregnancy behaviour, and future emotion regulatory outcomes (Rouse & Goodman, 2014).

1.1.11 Summary and Rationale

From this detailed review of the literature, it is evident that early neurodevelopment has important implications for mental health outcomes across the lifespan. According to DOHaD and the extended DOBHaD, the prenatal period may be the earliest point of intervention, where exposure to healthy lifestyle changes (nutrition and exercise) demonstrates clinically important effects (Gluckman et al., 2008; Van Den Bergh, 2010). Evidence highlights that positive experiences during the early years results in optimal neurodevelopment, while the influence of difficult early conditions can give rise to cognitive issues with extensive consequences (Shonkoff, 2010). Investing in lifestyle intervention programs during the prenatal and postnatal period may provide the most value for early childhood development as it is challenging to reverse the negative effects of delaying intervention in those at high risk (Shonkoff, 2010).

It is clear that further research is required to investigate how infants whose mothers participated in a lifestyle intervention during pregnancy (nutrition and exercise) regulate emotion in response to an emotionally demanding task. Therefore, an investigation was conducted based on the following research question: how do infants whose mothers participated in a nutrition and exercise intervention during pregnancy regulate emotions in response to a stressor such as a toy removal task? If it is determined that these infants demonstrate optimal emotion regulation, this may portend significant implications for the DOHBaD, demonstrating the value of investing in strategically timed lifestyle interventions for early infant and childhood development.

Chapter 2：Purpose and Hypotheses

2 Purpose

The primary purpose of the current pilot study was to determine emotion regulation of infants (as represented by physiological measures: FAA, RMSSD, RSA, and LF/HF and an informant-based measure: negative affectivity scale of IBQ-R) at one-year of age, whose mothers participated in a Nutrition and Exercise Lifestyle Intervention Program (NELIP) during pregnancy. The secondary purpose was to determine whether the negative affectivity scale was a predictor of infant emotion regulation in response to a stressor.

2.1 Hypotheses

It was hypothesized that infants whose mothers participated in the NELIP during pregnancy will have optimal emotion regulation (positive relative left FAA, high resting RMSSD and RSA values, an increase in RMSSD and RSA values and a decrease in the LF/HF ratio after a stressor (toy removal task), indicating appropriate PNS dominance, as well as a low score on the negative affectivity scale of IBQ-R) at one-year of age. Additionally, as a validated measure of infant temperament, the negative affectivity scale of the IBQ-R was hypothesized to predict emotion regulatory capacity at one-year of age.

Chapter 3 : Methods

3 Introduction

The current pilot study is a 12-month follow-up sub-study of a larger intervention trial approved by the Health Sciences Research Ethics Board at Western University (108080; see Appendix B2). The trial was registered on clinicaltrials.gov (NCT02804061) and consort guidelines were followed. Written informed consent as well as medical clearance was obtained from all participants for both the larger trial and the sub-study follow up (see Appendix C3).

3.1 Participants

Infants whose mothers participated in the Nutrition and Exercise Lifestyle Intervention Program (NELIP) during pregnancy were followed up at 12-months of age.

3.1.1 Inclusion and Exclusion Criteria

Pregnant women with a singleton pregnancy, less than 18 weeks gestation, 18 years of age or older, and non-smokers were eligible to participate in the larger trial. Women were excluded if they had contraindications to exercise or a known chronic disease. Infants born to women in the larger trial were included in the current pilot follow-up study.

3.2 Larger Trial Study Design

3.2.1 Nutrition and Exercise Lifestyle Intervention Program (NELIP)

After establishing that women met the inclusion criteria and signed written informed consent was obtained, all women who participated in the NELIP received a nutrition and exercise intervention during pregnancy. Women were asked to complete a Weight and Health History questionnaire regarding general health and demographics (see Appendix D4).

The NELIP from the larger trial is described elsewhere (Mottola et al., 2010). Briefly, women were given a specialized meal plan adapted from the gestational diabetic diet. The goals of the meal plan included consuming three well-composed meals and 3-4 snacks per day,

achieving a total energy intake of 1800-2200 kcal/day, with 200-250 g/day of complex carbohydrates (Mottola et al., 2010). Face-to-face nutrition counselling based on 24-hour food logs took place once per week.

In addition, women were required to come into the lab each week for a 25-minute walk, which increased in duration by 2 minutes weekly up to a maximum of 40 minutes. To achieve a total minimum of three walks per week, women were required to complete two additional walks at home (Mottola et al., 2010). At birth, gestational age, infant sex, and mode of delivery were recorded from medical records. Women and their babies returned to the lab at one-year postpartum for a follow-up visit. Infant weight was measured to the nearest kg wearing only a clean diaper. Infant length and head circumference were measured to the nearest cm using a plastic tape measure.

3.2.2 NELIP+ Brain Current Sub-Study

At 6 months post-delivery, women were asked if they wanted to participate in the NELIP+ Brain sub-study that occurred at 12 months postpartum. For interested participants, a separate letter of information and consent form also approved by the Research Ethics Board at Western University was administered and informed written consent to the sub-study was obtained (see Appendix C3). This sub-study added approximately 30 minutes to the already scheduled 12-month follow-up visit.

3.2.3 NELIP+ Brain Emotion Regulation Measurements

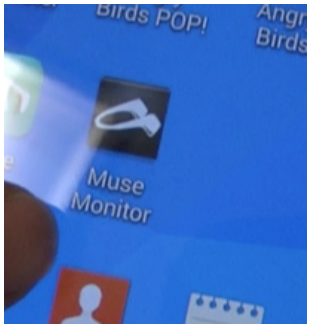
To determine the emotion regulation of infants whose mothers participated in the NELIP, physiological and informant-based measures were conducted. The first physiological measure was to evaluate the electrical activity of the prefrontal cortex using the MUSE band (Muse, RRID:SCR_014418, Toronto, Canada). The MUSE band monitors brain activity (Wolfson,

2014) and sensors AF7 and AF8 were positioned at the left and right regions of the forehead respectively to measure EEG signals in the prefrontal cortex (Hashemi et al., 2016).

The infant sized MUSE band (see Figure 5) was placed on the forehead with stickers on the cheeks, just in front of the ears. The MUSE band was then connected via Bluetooth to the Muse Monitor app, downloaded on an iPod touch (5th generation, Shenzhen, China) (see Figure 6). To verify that the band was connected to the app, the horseshoe shaped gauge located in the left-hand corner of the data collection screen was checked to confirm that all sensors were filled in with their appropriate colour indicating each sensor was activated.

The second physiological measure, HRV, was evaluated simultaneously using an ECG. A laptop (PC) with Biolab data acquisition software was connected to a wireless router via an ethernet cord to establish an uninterrupted internet connection. The Biolab data acquisition software was opened on the PC. Disposable snap silver chloride electrode stickers were attached to the opposite end of electrode leads and inserted into their appropriate bio units on the data acquisition mobile unit (Bio2-infant) (see Figure 7). Two electrodes were put on back of the infant, one on the right upper scapula and the other diagonally on their lower back (see Figure 8). See Figure 9 for a summary of equipment used to acquire HRV data. To connect the mobile unit to the Biolab software, Wifi mode was selected. The “start” button was selected to record the data. Appropriate time markers were pre-set to indicate important events during the protocol. These included: the end of the resting condition, the start of the toy removal task (when the toy was taken away), and when it was returned to mark the end of the test.

Figure 5. MUSE band



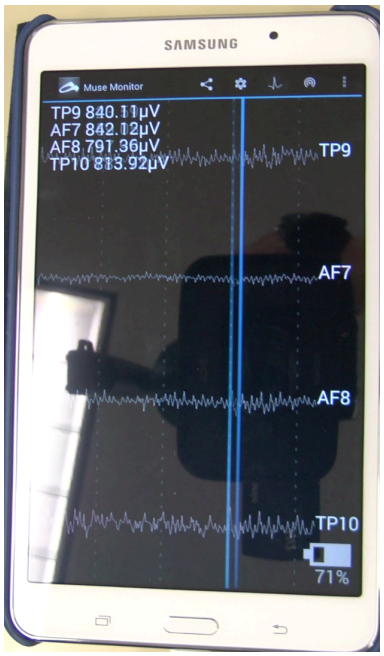


Figure 6. Muse Monitor App connected to smart device to display data acquisition screen

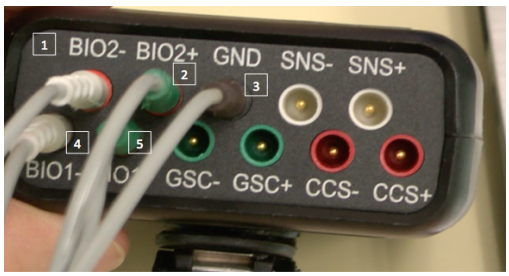


Figure 7. Mobile unit with electrode leads plugged into appropriate units

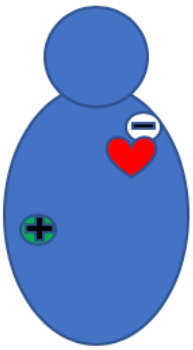


Figure 8. Electrocardiogram lead placement on infant

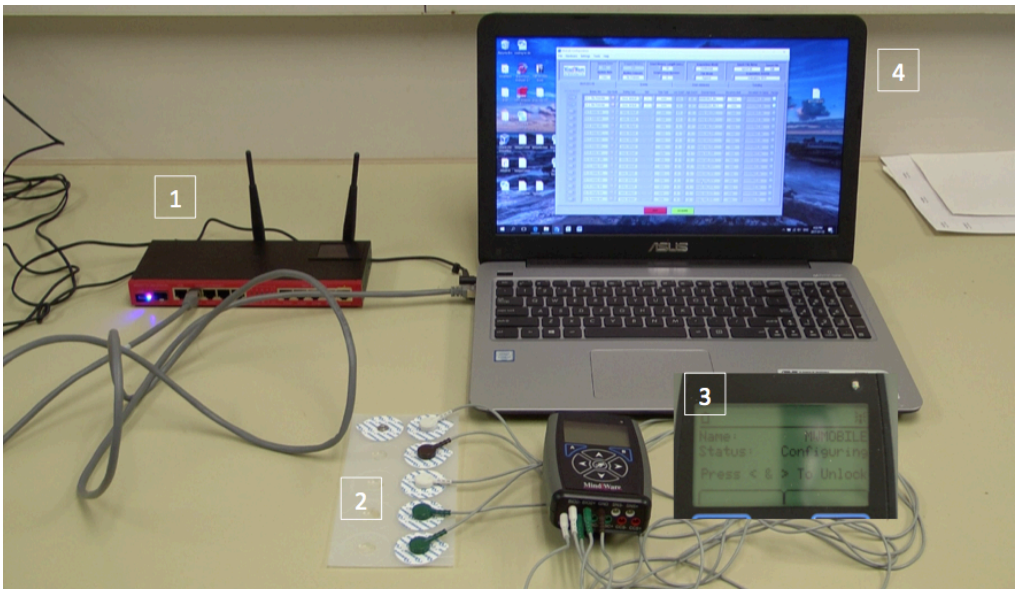


Figure 9. 1) wireless router 2) electrode leads 3) mobile unit 4) data acquisition laptop

1. **Resting-** Once both sets of physiological measures, the MUSE band for EEG and the electrodes for ECG and their respective equipment were set up, a 6-minute baseline resting state began. Mother and baby were asked to sit quietly either watching a video or reading a book while EEG and ECG measures were recorded simultaneously. The mother was instructed to try and keep the infant as relaxed as possible. See Figure 10 for a summary of the protocol.
2. **Toy Removal Task-** Next, a 6-minute toy removal task was conducted while EEG and ECG measures were continuously collected from the infant. The measure of stress recorded by EEG and HRV reflects an average over the entire toy removal protocol. The toy removal task was carried out in four parts: 1) For the first 2 minutes, the mother and infant play together with a toy of interest, similar to how they would play with a toy at home; 2) After two minutes, the mother was signaled to remove the toy and place it out of reach of the infant but still within sight (time marker was inserted). The mother was instructed to assume a neutral facial expression and avoid interacting with the baby for the two-minute duration; 3) Next, the mother was prompted to return the toy to the infant to resume play, but she was directed to maintain a neutral facial expression and avoid engaging with the infant for one-minute (time marker inserted) and 4) The mother and infant resumed play for the last minute of the task.
3. The MUSE band and ECG electrodes were removed and the mother then completed the IBQ-R, an informant-based measure, which took approximately 10 minutes. The IBQ-R was administered face to face with each mother who was asked to recall the last one or two weeks, depending on the question, to evaluate the frequency that the infant participated in a specified daily behaviour. Responses ranged from 1, representing

'never' to 7, indicating 'always' with a series of graded responses in between (Rothbart, Posner, & Kieras, 2008). Mothers verbally indicated responses to a research assistant who recorded each answer.

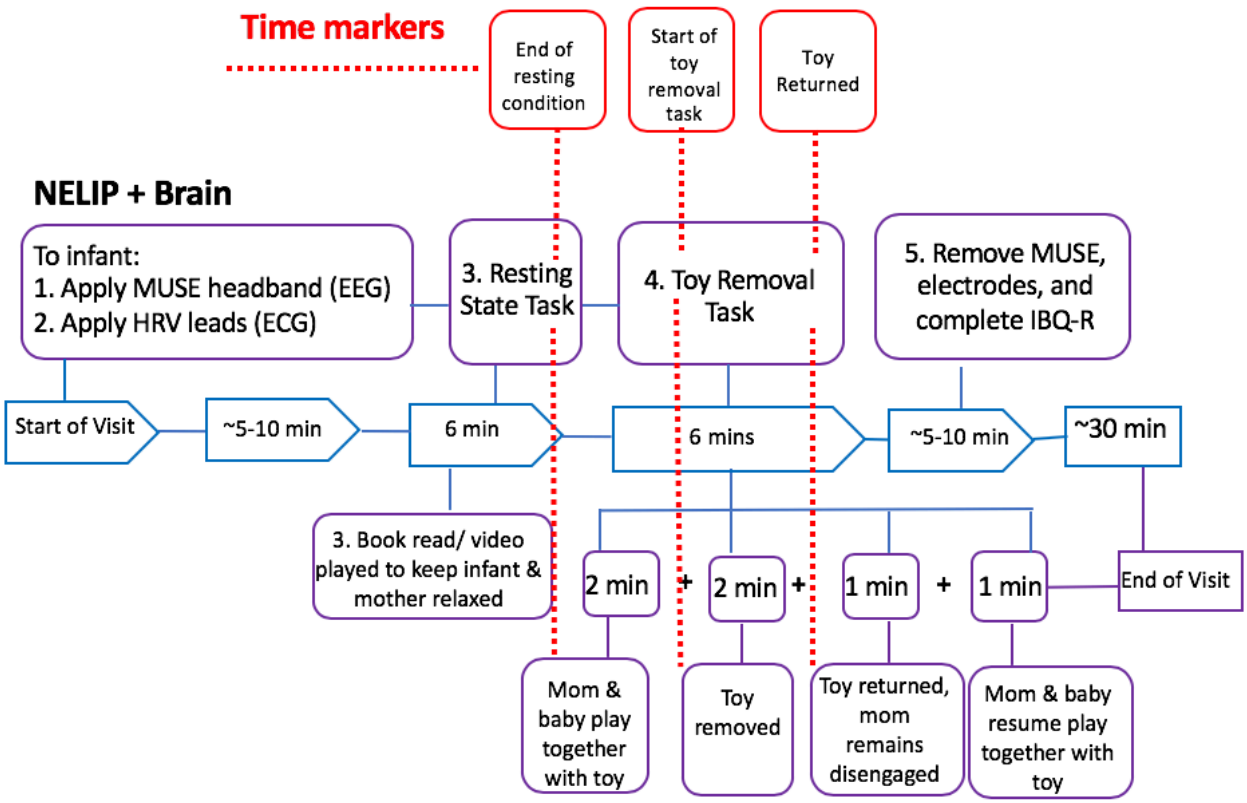


Figure 10. At one-year post-delivery, the MUSE headband (to measure brain activity; EEG) and the electrode leads (to measure heart rate variability; HRV) are placed on the infant. Data are recorded for 6 minutes while the infant and mother read a book or watch a video (resting state). During the toy removal task that lasts 6 minutes, mom and baby play with a toy (for 2 minutes), the toy is removed for 2 minutes and the toy is returned to the infant (end of toy removal task.).

3.2.4 Outcome Measures

Patterns of Frontal Alpha Asymmetry (FAA)- To determine individual differences in emotion regulation, infant patterns of FAA were assessed. If a negative FAA value was determined, this indicated greater relative right frontal EEG activity, which has been correlated with a predisposition to experiencing negative emotions and difficulty regulating those emotions (Allen & Kline, 2004; Coan & Allen, 2004b). A positive FAA value reflected greater relative left frontal asymmetry, indicating the experience of more positive emotions in response to environmental stressors (Allen & Kline, 2004; Coan & Allen, 2004b). To calculate FAA, a frequency domain analysis was performed using EEGLAB v 14.0.0 software, which was run via MATLAB_R2019a software (see Appendix E5 for the complete MATLAB protocol).

HRV- To measure electrical activity of the heart and determine which division of the ANS (SNS or PNS) was dominant, both time and frequency domain analyses were conducted. The time domain measure included the RMSSD and a Fast Fourier Transform was used for frequency domain measures. From this, power in the LF (0.040-0.200 Hz) and HF (0.200-1.200Hz) bands were obtained. The RSA was described in the HF band. The ratio between the LF and HF power band was evaluated as well to determine the balance between SNS and PNS activity (see Appendix F6 for a complete Mindware protocol).

IBQ-R- For the purpose of the current pilot study, the negative affectivity scale (determines anxiety, despondency, and distress) was prioritized as an important measure of emotion regulation as it included the 'distress to limitations,' 'fear,' 'sadness,' and 'falling reactivity' sub-scales. For each of these subscales, a higher score was related to a more negative emotion regulatory capacity. See Table 1 (Chapter 1) for detailed definitions of each scale and sub-scale.

Once the data were collected, these were entered into an Excel spreadsheet and checked by two independent reviewers. To score the IBQ-R data, each numerical response item for a

particular temperament scale was added together and divided by the number of items that received a numerical response in that section. If an item was skipped or the "does not apply" box was checked, no numerical score was assigned. If an item was marked with an 'R,' this indicated that it was a reverse item and therefore an inverted scoring scale was applied, where a score of 7 became 1, 6 became 2, and so on (Rothbart, Posner, & Kieras, 2008).

3.2.5 Sample Size Calculation

To our knowledge, this is the first pilot study where the primary outcome of interest is emotion regulation in infants whose mothers participated in a nutrition and exercise intervention during pregnancy. Therefore, a sample size calculation *a priori* was not completed. Observed effect sizes are reported for all outcomes and they were calculated referring to Cohen's criteria (1988, 1992): Cohen's *d* for a Paired t-test (small=0.20, medium=0.50, large=0.80); Pearson's *r* for Wilcoxon-signed ranks test and multiple regression (small=0.1, medium=0.3, large=0.5).

3.2.6 Statistical Analysis

Descriptive statistics were used for demographics variables, and the results were presented as means ± standard deviation (SD). Normality of the sample distribution was tested using the Shapiro Wilk's test for the primary and secondary outcomes. RSA, RMSSD, and the LF/HF ratio were the variables with a non-normal distribution. A Paired t-test was used to compare the mean FAA value of infants during a baseline resting state task to a stressful toy removal task. A nonparametric Wilcoxon-signed rank test was used to compare the mean RSA, RMSSD, and LF/HF ratio of infants during a resting state task to a stressful toy removal task. A multiple regression was conducted to determine if any of the factors that make up the 'Negative Affectivity' scale of the IBQ-R ('Distress to Limitations,' 'Fear,' 'Sadness,' and 'Falling Reactivity') predicted infant emotion regulation at 12 months of age (Rothbart, Posner, & Kieras, 2008). Statistical Package for Social Sciences software (version 23.0 for Windows; SPSS Inc.,

Chicago, IL, USA) and Excel™ were used to calculate all statistical comparisons. The level of significance was set at $\alpha < 0.05$.

Chapter 4 : Results

4 Study Recruitment

Of the 29 women who met the inclusion criteria, 22 provided informed consent for the sub-study and seven were not enrolled for the following reasons: did not consent to be enrolled in the sub-study (n=5), moved away from London, ON before the 12 months postpartum visit (n=1) and was unable to complete visit at 12 months due to the beginning of a global pandemic (n=1) (COVID-19).

Out of the 22 infants tested, 13 of them had useable EEG data, 18 had useable RMSSD and LF/HF data, and 19 had useable RSA data. Of the EEG data, 3 files did not record properly resulting in data too short to analyze and 6 infants were too upset to begin the toy removal task. With regards to the ECG measured by RSA, 1 infant was too upset to begin the task and 2 files were not analyzable due to technical difficulties with the acquisition software. For RMSSD and the LF/HF data, 1 additional file was not analyzable due to technical difficulties with the acquisition software. IBQ-R data were collected from all 22 mothers who agreed to participate in the study.

4.1 Demographics

Maternal demographics are presented in Table 2 and infant characteristics are presented in Table 3.

Table 2. Maternal characteristics (n=22)

Maternal Age (years)	32.68 ± 4.00
Pre-pregnancy BMI (kg/m^2)	26.47 ± 5.76
Maternal Education (*n, %)*	
College	2; 9.10
Bachelors	9; 40.90
Masters	10; 45.50
Professional	1; 4.50
Maternal Ethnicity (*n, %)*	
Caucasian	21; 95.50
Hispanic	1; 4.50
GA at delivery (weeks)	39.22 ± 1.21
Mode of Delivery (*n, %)*	
Vaginal	18; 81.80
C-section	4; 18.20

BMI= Body Mass Index
GA= Gestational Age
All data are presented as mean ± sd unless otherwise indicated

Table 3. Infant characteristics (n=22)

Infant age (months)	12.31 ± 0.41
Infants Sex (*n, %)*	
Male	11; 50.00
Female	11; 50.00
Infant Weight (kg)	9.50 ± 0.85
Infant Length (cm)	71.89 ± 3.02
Infant Head Circumference (cm)	46.38 ± 1.37

All data are presented as mean ± sd

4.2 Emotion Regulation Outcomes

4.2.1 EEG

EEG data were collected from 13 infants during the protocol, which included 6 minutes of resting and a 6-minute toy removal task. At rest, the mean infant FAA value was 0.08 ± 0.66, which reflects greater relative left frontal asymmetry. This finding illustrates that on average at rest, infants exhibited an optimal capacity to regulate emotions. The mean infant FAA value as a result of the toy removal task was 0.27 ± 0.72, with no significant difference from the resting state, $t(12)= -1.47$, $p=0.167$. The fact that the FAA values remained positive, demonstrated regulation in response to a stressor and optimal emotion regulatory capacity. Effect sizes were found to be small for the FAA between rest and the toy removal task (see Table 4).

Table 4. Infant FAA Scores (n=13)

		Effect Size	**p-value**
FAA Resting	0.08 ± 0.66	0.12	0.167
FAA Toy Removal Task	0.27 ± 0.72	0.37	

All data are presented as mean ± sd unless otherwise indicated
FAA= frontal alpha asymmetry

4.2.2 HRV

4.2.2.1 RMSSD

RMSSD data were collected from 18 infants during the 6-minute resting state task and the 6-minute toy removal task. At rest, the mean infant RMSSD value was 54.98 ± 54.16 msec and after the toy removal task was complete, the mean infant RMSSD was 88.77 ± 48.71 msec. A Wilcoxon Signed-Ranks Test demonstrated that the infant RMSSD value as a result of the toy removal task was significantly higher than the infant RMSSD value at rest Z= -2.90, p<0.004. Effect size was large (r=0.48). This result illustrates that these infants are appropriately responding to the stressor, indicating optimal emotion regulation, increased HRV, and a balance in autonomic regulation.

4.2.2.2 RSA

RSA data was collected from 19 infants. The mean infant RSA value at rest was 5.90 ± 1.71, which falls within the expected RSA range (2.0-6.0) for infants at rest (Shader et al., 2018). After the toy removal task was complete, the mean RSA value for infants was 7.30 ± 1.63, increasing from the pre-task value. A Wilcoxon Signed-Ranks Test indicated that the infant RSA value post toy removal task was significantly higher than the infant RSA value at rest Z= -3.22, p< 0.001,

with a large effect size (r=0.52). This finding demonstrates that these infants adaptively regulated their emotions, establishing an optimal capacity for self-regulation in response to stress.

4.2.2.3 LF/HF Ratio

Data regarding the LF/HF ratio were collected from 18 infants. The mean infant LF/HF ratio at rest was 3.51 ± 3.30. The mean LF/HF ratio for infants as a result of the toy removal task was 2.10 ± 1.8, decreasing from the pre-task value. A Wilcoxon Signed-Ranks Test indicated that the infant LF/HF ratio after the toy removal task was significantly lower than the infant LF/HF ratio at rest Z= -2.77 p<0.006, with a large effect size (r=0.46). This indicates that these infants have a more optimal capacity to regulate emotions with an appropriate balance between the SNS and PNS.

4.3 IBQ-R

IBQ-R data were collected from 22 women. The mean score that mothers reported for the negative affectivity scale was 3.73 ± 0.51, indicating that overall their infants positively regulated their emotions in response to anxiety and distress. To determine whether the sub-scales of the negative affectivity scale predict infant emotion regulation, a multiple regression was conducted for each measure of emotion regulation (EEG; n=13, RMSSD; n=18, RSA; n=19, and LF/HF ratio; n=18) from resting to after the toy removal task.

4.3.1 IBQ-R and EEG

The regression demonstrated that the negative affectivity scale as a whole, did not explain a significant amount of the variance in infant emotion regulation measured using EEG (F= (1,11)2.74, p=0.13, R^2= 0.20, $R^2_{Adjusted}$= 0.13). The negative affectivity scale overall (*Beta*=0.45, t(11)=1.65 p=0.13) was found to be a non-significant predictor of infant emotion regulation as measured by EEG, with a positive but non-significant correlation (r(11)=0.45, p=0.13).

In addition, it was found that distress to limitations, fear, sadness, and falling reactivity subscales did not explain a significant amount of the variance in infant emotion regulation measured using EEG (F= (4,8)1.45, p=0.30, R^2= 0.42, $R^2_{Adjusted}$= 0.13). The analysis shows that the distress to limitations sub-scale (*Beta*=0.15, t(8)=0.36, p=0.73), the fear sub-scale (*Beta*=0.14, t(8)=0.52, p=0.62), the sadness sub-scale (*Beta*=0.49, t(8)=1.24, p=0.25), and the falling reactivity sub-scale (*Beta*=-0.37, t(8)=-1.21, p=0.26) did not significantly predict infant emotion regulation measured by EEG. The distress to limitations (r(8)=0.36, p=0.23) and fear (r(8)=0.17, p=0.59) subscales were found to be positively but non-significantly correlated with infant emotion regulation measured by EEG. The sadness sub-scale (r(8)=0.55, p=0.05) was found to be positively and significantly correlated with infant emotion regulation. The distress to limitations sub-scale demonstrated a moderate relationship, fear showed a small correlation, and finally the sadness sub-scale had a large correlation. The falling reactivity sub-scale (r(8)=-0.20, p=0.51) was negatively correlated with infant emotion regulation.

4.3.2 IBQ-R and RMSSD

The regression for infant emotion regulation evaluated by the RMSSD demonstrated that the negative affectivity scale overall did not explain a significant amount of the variance (F= (1,16)0.09, p=0.80, R^2= 0.00, $R^2_{Adjusted}$= -0.06). The negative affectivity scale overall (*Beta*=-0.07, t(16)=-0.27 p=0.80) did not predict infant emotion regulation as measured by RMSSD, with a negative non-significant correlation (r(16)=-0.07, p=0.80).

In addition, it was determined that distress to limitations, fear, sadness, and falling reactivity subscales did not explain a significant amount of the variance in infant emotion regulation measured using RMSSD (F= (4,13)1.35, p=0.30, R^2= 0.29, $R^2_{Adjusted}$= 0.07). The analysis demonstrated that the distress to limitations sub-scale (*Beta*=-0.55, t(13)=-1.74, p=0.10), the fear

sub-scale (*Beta*=-0.27, *t*(13)=-1.10, *p*=0.30), the sadness sub-scale (*Beta*=0.40, *t*(13)=1.17 *p*=0.26), and the falling reactivity sub-scale (*Beta*=0.30, *t*(13*)*=1.19, *p*=0.25) did not predict infant emotion regulation measured by RMSSD. The sadness (*r*(13)=0.05, *p*=0.84) and falling reactivity (*r*(13)=0.27, *p*=0.28), sub-scales were found to be positively but non-significantly correlated with infant emotion regulation. Sadness demonstrated a small correlation and falling reactivity demonstrated a medium correlation. Negative, non-significant, correlations were found between the distress to limitations (*r*(13)=-0.30, *p*=0.30) and fear (*r*(13)=-0.19, *p*=0.46) sub-scales with infant emotion regulation determined by RMSSD. The distress to limitations correlation was medium and the fear correlation was small.

4.3.3 IBQ-R and RSA

For infant emotion regulation measured using RSA, the negative affectivity scale overall did not explain a significant amount of the variance (*F*= (1,17)0.23, *p*=0.64, R^2= 0.01, $R^2_{Adjusted}$= -0.04). The negative affectivity scale overall (*Beta*=0.12, *t*(17)=0.48 *p*=0.64) did not predict infant emotion regulation as measured by RSA. The negative affectivity scale (*r*(17)=0.12, *p*=0.64) was positively but non-significantly correlated with infant emotion regulation measured by RSA.

Furthermore, it was found that that distress to limitations, fear, sadness, and falling reactivity subscales did not explain a significant amount of the variance in infant emotion regulation measured using RSA (*F*= (4,14)1.15, *p*=0.37, R^2= 0.25, $R^2_{Adjusted}$= 0.03). Based on the analysis, the distress to limitations sub-scale (*Beta*=-0.50, *t*(14)=-1.57, *p*=0.14), the fear sub-scale (*Beta*=-0.18, *t*(14)=-0.74, *p*=0.47), the sadness sub-scale (*Beta*=0.52, *t*(14)=1.50 *p*=0.15), and the falling reactivity sub-scale (*Beta*=0.14, *t*(14)=0.54, *p*=0.60) did not significantly predict infant emotion regulation measured by RSA. The sadness (*r*(14)=0.23, *p*=0.34) and falling reactivity

(r(14)=0.28, p=0.24) sub-scales were found to be positively but non-significantly correlated with infant emotion regulation measured by RSA. Both sadness and falling reactivity demonstrated small correlations. Additionally, negative, non-significant, and small correlations were found between the distress to limitations (r(14)=-0.15, p=0.53) and fear (r(14)=-0.05, p=0.84) sub-scale with infant emotion regulation determined by RSA.

4.3.4 IBQ-R and LF/HF Ratio

The regression for infant emotion regulation evaluated by the LF/HF ratio demonstrated that the negative affectivity scale overall did not explain a significant amount of the variance (F= (1,16)1.64, p=0.22, R^2= 0.09, $R^2_{Adjusted}$= 0.04). The negative affectivity scale overall ($Beta$=-0.30, t(16)=-1.28 p=0.22) did not predict infant emotion regulation as measured by the LF/HF ratio. The negative affectivity scale (r(16)=-0.30, p=0.22) was negatively and non-significantly correlated with infant emotion regulation measured by the LF/HF ratio.

The distress to limitations, fear, sadness, and falling reactivity subscales did not explain a significant amount of the variance in infant emotion regulation measured using the LF/HF ratio (F= (4,13)0.86, p=0.51, R^2= 0.32, $R^2_{Adjusted}$= -0.33). Furthermore, the analysis shows that the distress to limitations sub-scale ($Beta$=-0.53, t(13)=-1.58, p=0.14), the fear sub-scale ($Beta$=-0.10, t(13)=-0.38, p=0.71), the sadness sub-scale ($Beta$=0.27, t(13)=0.75, p=0.47), and the falling reactivity sub-scale ($Beta$=-0.16, t(13)=-0.61, p=0.55) did not predict infant emotion regulation measured by the LF/HF ratio.

The distress to limitations (r(13)=-0.40, p=0.10), fear (r(13)=-0.13, p=0.61), sadness, (r(13)=-0.17, p=0.49), and falling reactivity (r(13)=-0.20, p=0.42) sub-scales were found to be negatively and non-significantly correlated with infant emotion regulation measured by the

LF/HF ratio. Distress to limitations demonstrated a large effect, while fear, sadness, and falling reactivity were small.

Chapter 5 : Discussion

5 How did infants regulate emotions in response to stress?

The primary purpose of this pilot study was to determine the emotion regulatory capacity of infants at one-year of age, whose mothers participated in a nutrition and exercise intervention during pregnancy. Infants whose mothers participated in the NELIP while pregnant showed positive relative left frontal asymmetry during both the resting state and toy removal task, and across both time points, indicating these infants may regulate emotions in response to stress in an optimal manner. Similarly, measures of HRV demonstrated an increase in variability from rest to post stressor with the appropriate arm of the autonomic nervous system active during each condition. This aligns with EEG findings, demonstrating optimal and appropriate infant emotion regulation. Furthermore, the maternal-reported low scores for the negative affectivity scale on the IBQ-R confirmed these findings related to emotion regulation. The negative affectivity sub-scales (distress to limitations, fear, sadness, and fall reactivity) however, were generally found to be poor predictors of infant emotion regulation.

No literature exists currently that investigates emotion regulation in infants whose mothers participated in nutrition and exercise interventions while pregnant. However, the relationship between emotion regulation and left frontal EEG asymmetry has been documented previously (Davidson & Fox, 1989; Gartstein et al., 2014; Kee & Bell, 2006; Potapova, 2019; Smith et al., 2016). Davidson & Fox (1989) measured FAA in 10-month old female infants in response to a stressful maternal separation task, where the mother engaged in approach behaviours followed by leaving the testing room. EEG was recorded from the left and right frontal and parietal scalp regions at four channels (P3, P4, F3, and F4) using a lycra stretchable cap. Asymmetry at rest

was used to separate the infants into two groups; criers and non-criers based on their response to maternal separation (Davidson & Fox, 1989). Findings were comparable to the current study as an increase in left FAA was determined in the group of infants who were coded as non-criers.

Gartstein, Bell, & Calkins (2014) investigated the relationship between infant temperament at 5 months of age using the IBQ-R and FAA evaluated by EEG in response to an arm restraint task that followed a similar method to the current toy removal task. The IBQ-R was used to determine if a relationship existed with frontal EEG asymmetry, but this was not measured until 10-months (Gartstein et al., 2014). FAA scores at rest in the study by Gartstein et al.(2014) were similar (0.03 ± 0.26) to the current pilot study (0.08 ± 0.66), as well as the FAA scores recoded from the arm restraint task (0.05 ± 0.45), compared to the toy removal task (0.27 ± 0.72). In addition, the average scores on the IBQ-R negative affectivity scale were similar between both studies (3.01 ± 0.67, 3.73 ± 0.51, respectively) and the negative affectivity scale was also found to be a poor predictor of EEG outcomes (Gartstein et al., 2014). However, infant sex was found to be a possible mediator (Gartstein et al., 2014). Future work should consider evaluating the IBQ-R in relation to infant FAA stratified by sex.

Kee & Bell (2006) recruited infants at 8-months old and recorded EEG measures, which they correlated with elements of regulation during childhood at both 4 and 8-years of age. At 4-years old, they reported that greater right FAA measured in infancy (8-months) was linked with increased parental reported impulsivity (Kee & Bell, 2006). Similarly, at 8-years old, high surgency scores were correlated with greater right FAA at 8-months (Kee & Bell, 2006). Furthermore, Kee & Bell (2006) found that 4-year old children who demonstrated a capacity to delay gratification in response to Kochanska's Bow task, where they were presented with a colourful gift bag and asked to wait until the experimenter came back with a bow to touch it

(Spinrad, Eisenberg, & Gaertner, 2007), had an increased likelihood of displaying left FAA at 8-years old. This indicates that FAA measures in infancy may inform the longitudinal emotion regulatory capacity of children. Future research should follow up with the infants of the current study into childhood to determine if left FAA measures are maintained.

Similar to the toy removal task employed in the current study, Potapova (2019) used a Still Face Paradigm to measure emotion regulatory capacity via EEG of infants who ranged from 6-12 months of age. Although Potapova (2019) used similar measures as the current pilot study (FAA and IBQ-R), the goal differed as they investigated whether the regulation/orienting scale (IBQ-R) explained infant FAA scores in response to a stressful still face paradigm. Average resting FAA scores (0.07± 0.42) were similar to the current pilot study (0.08± 0.66). Although the FAA scores differed during the still face procedure (0.09± 0.35) and the toy removal task (0.27± 0.72), both scores were positive, indicating greater relative left FAA and more optimal emotion regulatory capacity. It is also possible that the difference in age of infants between the two studies contributed to the discrepancy in task FAA scores as the development of regulation strategies change significantly during the first year of life (Ekas, Lickenbrock, & Braungart-Rieker, 2013). This is important to consider since measures on the negative affectivity scale and EEG asymmetry at 10 months of age predicted effortful control (regulation) at 2.5 to 3 years of age in toddlers that demonstrated right FAA at 10 months (Smith et al., 2016). Examining FAA measures at 12-months of age in the current study may therefore be beneficial in order to design interventions that support the emotional development of children as they grow and develop.

Conradt & Ablow (2010) investigated the behavioural and physiological reaction of infants who were subject to the still face paradigm. Similar to the current pilot study, electrodes were

used to measure HRV during both a resting neutral state and in response to the still face paradigm (Conradt & Ablow, 2010). The IBQ-R was also completed by the mother, where the negative affectivity scale was used to assess infant temperament (Conradt & Ablow, 2010). Results of the study by Conradt & Ablow (2010) were contrary to the current pilot study as they found a significant decrease in infant RSA values, from the play episode, where mothers were instructed to engage with their babies without toys for 2-minutes, indicating a decrease in HRV, to the still-face episode, where mothers were required to stop engaging with their babies and maintain a neutral face for 2-minutes. This decrease in RSA across a stressful task is contrary to the expected increase in HRV seen with an increase in RSA that was present in the current pilot study (Conradt & Ablow, 2010). The discrepancy in results may be due to the fact that mothers from the Conradt & Ablow (2010) study came from low-income households, unlike the mothers in the present study as well as the difference in sample size (n=95; Conradt & Ablow, 2010 versus n=19; present study).

Furthermore, a study by Alkon et al., (2006) carried out a 7-minute protocol administered to infants at 6 and 12 months that comprised three challenges (each lasting 1-minute) followed by two resting periods (2-minutes long) to evaluate HRV measured by RSA at rest and in response to emotional, physical, and social challenges. RSA at rest in 12-month old infants in the study by Alkon et al. (2006) was lower (3.8 ± 0.9), compared to the current pilot study (5.90 ± 1.71), indicating less HRV. In addition, infants in the study by Alkon et al. (2006) study demonstrated a significant decrease in RSA values (3.7 ± 0.9) during the challenging task, compared to the pilot study that showed a significant increase (7.30 ±1.63). This illustrates that the 12-month old infants in the current study had more optimal emotion regulation, demonstrated by an increase in RSA, representing an increase in HRV in response to stress. The different stressful tasks used to

elicit a response from infants in the Alkon et al, (2006) paper compared to the current study may account for the discrepant RSA values. Alkon et al. (2006) had a 7-minute protocol that was designed to provoke infant reactions related to social, emotional, and physical challenges, while the toy removal task used in the current study was used specifically to evaluate the capacity of the infant to delay gratification and self-regulate in response to frustration (Kochanska, Murray, & Harlan, 2000; Tronick & Beeghly, 2011). Additionally, the literature has demonstrated that a higher resting RSA value indicates appropriate activity of the parasympathetic system as well as increased flexibility and capacity to adapt (Buss, Goldsmith, Davidson, 2005; Graziano and Derefinko, 2013). Based on this, it is possible that the higher resting RSA values observed in the current pilot study are indicative of infants with more optimal PNS functioning. This can be further supported by the different populations studied, where Alkon et al., (2006) evaluated infants who were Hispanic from low-income families who primarily worked on farms compared to the current study that investigated infants of predominantly Caucasian and educated mothers.

Similar to Alkon et al., (2006), Calkins & Keane (2004) used a task designed to elicit a multi-faceted response related to empathy, attention, problem-solving and frustration. Consistent with Alkon et al. (2006), findings demonstrated a significant reduction in RSA from baseline, demonstrating a decrease in HRV, which supports less-optimal self-regulation. The discrepancy in RSA observed in the current study may be explained by the nature of the toy removal task, designed only to evaluate a single dimension of self-regulation in response to frustration (Kochanska, Murrary, & Harlan, 2000). Future work may therefore include evaluating RSA in infants at 12 months in response to a multi-dimensional stressor.

According to Suurland et al., (2017) 6-month old infants who are considered high risk due to the psychopathological status of their mother have increased RSA withdrawal indicating poor

PNS regulation and decreased HRV compared to infants who are the same age but deemed low risk. Based on these results that demonstrate the relationship between maternal risk status and the autonomic response of the infant to stress, it is evident that exposure to prenatal adversity can negatively impact infant emotion regulation (Suurland et al., 2017). Therefore, if prenatal and early postnatal experience can increase the vulnerability of the infant to adverse outcomes, future directions should include adding a standard-care control group to the current pilot study to determine whether *in utero* exposure to nutrition and exercise results in more positive autonomic regulatory outcomes for infants.

A positive impact of exercise during pregnancy on cardiac autonomic regulation of the infant at one-month of age has been reported (May et al., 2014). Aerobic exercise during pregnancy (measured at 28, 32, and 36 weeks gestation), increased fetal HRV and this relationship extended to influence infant HRV at one-month (May et al., 2010). Infants whose mothers exercised during pregnancy had significantly higher RMSSD values at rest, indicating more HRV, than infants whose mothers did not exercise while pregnant (May et al., 2014). This is similar to findings related to RMSSD in the current study where despite the age difference (12 months old versus one month old), both infants were born to mothers who were active while pregnant. May et al. (2014) however did not find a significant relationship between maternal exercise and a decrease in the LF/HF ratio. This may reflect the difference in infant age, as one-month old infants may not have the capacity to regulate the balance between the sympathetic and parasympathetic systems. Future work should follow infants from one month of age through to 1 year of age, including a toy removal task to assess the maturation of the two systems.

Due to the biological basis of infant temperament, previous research (LoBue et al., 2011; Schmidt, 2008) has related the informant-based IBQ-R to neurobiological measures, similar to

the current study. LoBue et al., (2011) investigated the relationship between IBQ-R sub-scales and frontal EEG asymmetry in response to video recordings eliciting both positive and negative emotions in 7-9 month old infants. Contrary to the current pilot study, LoBue et al., (2011) found that EEG asymmetry predicted distress to limitations, fear, and falling reactivity over the lateral-frontal region of the brain (LoBue et al., 2011). This discrepancy in results may be due to the difference in EEG measurement tools, where the present study evaluated alpha asymmetry in the AF7 and AF8 regions using the MUSE band, whereas LoBue et al. (2011) used tin electrodes and a stretch-lycra cap to record and evaluate EEG asymmetry at F7 and F8 in addition to F3, F4, P3, and P4 sites. This may have resulted in a more robust measure of EEG asymmetry. In addition, the task to elicit an emotional response differed between studies, where LoBue et al. (2011) examined elicit patterns of prefrontal activity related to avoidance with pictures of snakes and frightening voices. However, the greater relative left EEG asymmetry observed in response to these tasks demonstrated stimuli elicited approach-related responses, which may account for the differences in the ability of the IBQ-R to predict emotion regulation (LoBue et al., 2011).

Similarly, Schmidt (2008) investigated frontal EEG asymmetry in relation to the fear and pleasure scales on the infant behaviour questionnaire in 9-month old infants. A relationship was determined between infants who exhibited greater relative right frontal asymmetry and higher scores on the maternally reported fear scale (Schmidt, 2008). Conversely, infants who demonstrated patterns of greater relative left frontal asymmetry were found to have higher maternal report scores on the pleasure scale (Schmidt, 2008). These findings indicated that patterns of EEG asymmetry predict infant temperament (Schmidt, 2008), which is contrary to the current study. A potential explanation includes the sample size, where Schmidt (2008) had useable EEG data from 30 infants, while the current study only had useable data from 13.

Overall, across studies related to FAA, RMSSD, RSA, LF/HF, IBQ-R and emotion regulation, infants range in age, the tools used for evaluation differ, the stressful task is varied, and different scales of the IBQ-R are prioritized. In addition, research is scarce with regards to lifestyle interventions administered during pregnancy and infant emotion regulation. Despite this, the relationship consistently observed in the literature suggests that a positive left FAA reflects more optimal infant emotion regulation, which aligns with findings of the current study.

5.1 Strengths and Limitations

Strengths of the current pilot study include the non-invasive nature of both physiological measures (EEG and ECG) to obtain emotion regulatory information from one year old infants. Despite the discreet design and ease of use of the MUSE band, it provided frontal EEG activity from only two sites (AF7 and AF8). Infants attempted to remove the MUSE band on several occasions, and it may be possible that EEG data were primarily provided by infants who were less distressed, potentially biasing results. Additionally, due to the nature of the toy removal task, infant gross motor movements to retrieve the toy, may have contributed to the collection of artifacts that decreased the quantity of analyzable data. However, given that conducting psychophysiological research with infants can be challenging and low sample sizes and missing data are common, as is the case with the present study, the straightforward toy removal procedure was advantageous as a potential infant stressor (Buss, Goldsmith, & Davidson, 2005).

A robust measure of infant emotion regulation was obtained with the use of both physiological laboratory assessments and a parental recount of infant temperament, as this helped researchers understand the disposition of the infant in the home environment, across diverse settings and times of day (Rouse & Goodman, 2014). While questionnaires reported by caregivers can introduce bias to the measure of infant temperament due to reduced accuracy of memory and the influence of social desirability, strengths of the IBQ-R include the recall period

limited to 1-2 weeks prior and the fact that caregivers are not required to compare temperament of their infant to others (Rouse & Goodman, 2014).

Limitations of the current study include the one time point of measurement to examine the emotional response of the infant to a challenging task. Autonomic and central nervous system responses to stress continue to develop during the first few years of life, and therefore future directions may include the design of a longitudinal study to assess changes in the emotion regulatory capacity from infancy to the child over time (Suurland et al., 2017). While the investigation of the reaction of the infant to a single lab challenge may mitigate the potential difficulty of psychophysiological research in infants, it is limited as results may be unique to the challenging task or depend on the stressor. Therefore, future work should include physiological evaluations of infants across multiple tasks and in a variety of contexts to avoid this potential bias.

The FAA, RMSSD, RSA, AND LF/HF scores that indicated levels of regulation in response to the toy removal task stressor do not include an evaluation of recovery or return to rest after the toy was given back to the infant. Future work that breaks down the toy removal task into resting, stressor, and recovery measures would be beneficial to further study vagal reactivity.

The use of the negative affectivity scale of the IBQ-R as a predictor of emotion regulation may be investigated more concretely through determination of maternal-infant synchrony in response to a stressful task. Since relationships form in early life, such as those that develop between parents and offspring during infancy and childhood, and significantly impact the development of emotion regulation, a better understanding of the synchrony (by measuring the mother and infant at the same time) between mother and infant may help explain correlations

between maternal reported infant temperament and physiologically measured emotion regulation in both infant and mother (Thompson, 1994).

To strengthen results of the current study, nutrition and exercise effects during pregnancy should be assessed and related to emotion regulatory capacity in the mother and infant as early as 6 months after birth in order to examine longitudinal changes at one year and beyond as the infant systems mature. Comparing these findings to mother-infant pairs from pregnant women who receive standard care during pregnancy would be beneficial to more definitively ascertain the role of nutrition and exercise during pregnancy on infant emotion regulatory outcomes. As well, future studies should continue to use multiple physiological measures of emotion regulation (EEG and ECG) for an accurate depiction of infant emotion regulation and the relationship between mother and infant.

5.2 Summary, Future Directions, and Conclusions

In summary, the current pilot study demonstrated that infants whose mothers participated in a nutrition and exercise intervention during pregnancy have greater left FAA at rest and in response to a stressor, indicating positive emotion regulation. This finding is strengthened by the increase in infant HRV (measured by RMSSD and RSA) and the decrease in the LF/HF ratio observed after the toy removal task, which reflects appropriate autonomic regulation. From the maternal-report measure, infant scores on the negative affectivity scale of the IBQ-R were low, indicating positive emotion regulation. However, overall the sub-scales of negative affectivity were not found to be good predictors of infant emotion regulation as measured by FAA, RMSSD, RSA, or the LF/HF ratio.

Future directions include using the pilot data from the current study to evaluate emotion regulation in infants as early as 6-months whose mothers participated in a nutrition and exercise program during pregnancy and followed into childhood with a longitudinal design to determine

how emotion regulation changes throughout development and maturation. Using multiple physiological measures of infant emotion regulation may enable researchers to discern if one is a more robust measure over the other. A standard care group should be added to determine if infants exposed to nutrition and exercise *in utero* have better emotion regulation through infancy. Finally, evaluating EEG and HRV in mothers and infants simultaneously will provide an understanding of maternal-infant synchrony, which may help explain findings related to maternal reports of infant temperament and emotion regulation.

Based on the results of the current study, there is potential positive value for infants who were exposed to a nutrition and exercise intervention *in utero.* With an increased sample size, and the addition of a recovery measure to the toy removal task, this pilot study shows promise for the investment in preventative lifestyle interventions to support a healthy prenatal environment and subsequent neurobiological development. Pilot data should be used to inform the design of future studies and confirm the potentially significant role lifestyle interventions delivered during gestation may play in the development of infant emotion regulation.

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Appendices

Appendix A1: Infant Behaviour Questionnaire- Revised (IBQ-R)

Infant Behavior Questionnaire - Revised

Subject No. _____

Today's Date _____

Sex of Child _____

Date of Baby's Birth _____ ____ _____
month. day year

Age of Child _____ _____
mos. weeks

INSTRUCTIONS:
Please read carefully before starting:

As you read each description of the baby's behavior below, please indicate how often the baby did this during the LAST WEEK (the past seven days) by circling one of the numbers in the left column. These numbers indicate how often you observed the behavior described during the last week.

(1) Never	(2) Very Rarely	(3) Less Than Half the Time	(4) About Half the Time	(5) More Than Half the Time	(6) Almost Always	(7) Always	(X) Does Not Apply

The "Does Not Apply" (X) column is used when you did not see the baby in the situation described during the last week. For example, if the situation mentions the baby having to wait for food or liquids and there was no time during the last week when the baby had to wait, circle the (X) column. "Does Not Apply" is different from "Never" (1). "Never" is used when you saw the baby in the situation but the baby never engaged in the behavior listed during the last week. For example, if the baby did have to wait for food or liquids at least once but never cried loudly while waiting, circle the (1) column.

Please be sure to circle a number for every item.

(1) Never	(2) Very Rarely	(3) Less Than Half the Time	(4) About Half the Time	(5) More Than Half the Time	(6) Almost Always	(7) Always	(X) Does Not Apply

Feeding

During feeding, how often did the baby:
1 2 3 4 5 6 7 X . . . . (1) lie or sit quietly?
1 2 3 4 5 6 7 X . . . . (2) squirm or kick?
1 2 3 4 5 6 7 X . . . . (3) wave arms?
1 2 3 4 5 6 7 X . . . . (4) notice lumpy texture in food (e.g., oatmeal)?

In the last week, while being fed in your lap, how often did the baby:
1 2 3 4 5 6 7 X . . . . (5) seem to enjoy the closeness?
1 2 3 4 5 6 7 X . . . . (6) snuggle even after she was done?
1 2 3 4 5 6 7 X . . . . (7) seem eager to get away as soon as the feeding was over?

How often did your baby make talking sounds:
1 2 3 4 5 6 7 X . . . . (8) while waiting in a high chair for food?
1 2 3 4 5 6 7 X . . . . (9) when s/he was ready for more food?
1 2 3 4 5 6 7 X . . . . (10) when s/he has had enough to eat?

Sleeping

Before falling asleep at night during the last week, how often did the baby:
1 2 3 4 5 6 7 X . . . . (11) show no fussing or crying?

During sleep, how often did the baby:
1 2 3 4 5 6 7 X . . . . (12) toss about in the crib?
1 2 3 4 5 6 7 X . . . . (13) move from the middle to the end of the crib?
1 2 3 4 5 6 7 X . . . . (14) sleep in one position only?

After sleeping, how often did the baby:
1 2 3 4 5 6 7 X . . . . (15) fuss or cry immediately?
1 2 3 4 5 6 7 X . . . . (16) play quietly in the crib?
1 2 3 4 5 6 7 X . . . . (17) cry if someone doesn't come within a few minutes?

How often did the baby:
1 2 3 4 5 6 7 X . . . . (18) seem angry (crying and fussing) when you left her/him in the crib?
1 2 3 4 5 6 7 X . . . . (19) seem contented when left in the crib?
1 2 3 4 5 6 7 X . . . . (20) cry or fuss before going to sleep for naps?

When going to sleep at night, how often did your baby:
1 2 3 4 5 6 7 X . . . . (21) fall asleep within 10 minutes?
1 2 3 4 5 6 7 X . . . . (22) have a hard time settling down to sleep?
1 2 3 4 5 6 7 X . . . . (23) settle down to sleep easily?

3

(1) Never	(2) Very Rarely	(3) Less Than Half the Time	(4) About Half the Time	(5) More Than Half the Time	(6) Almost Always	(7) Always	(X) Does Not Apply

When your baby awoke at night, how often did s/he:
1 2 3 4 5 6 7 X . . . . (24) have a hard time going back to sleep?
1 2 3 4 5 6 7 X . . . . (25) go back to sleep immediately?

When put down for a nap, how often did your baby:
1 2 3 4 5 6 7 X . . . . (26) stay awake for a long time?
1 2 3 4 5 6 7 X . . . . (27) go to sleep immediately?
1 2 3 4 5 6 7 X . . . . (28) settle down quickly?
1 2 3 4 5 6 7 X . . . . (29) have a hard time settling down?

When it was time for bed or a nap and your baby did not want to go, how often did s/he:
1 2 3 4 5 6 7 X . . . . (30) whimper or sob?
1 2 3 4 5 6 7 X . . . . (31) become tearful?

Bathing and Dressing

When being dressed or undressed during the last week, how often did the baby:
1 2 3 4 5 6 7 X . . . . (32) wave her/his arms and kick?
1 2 3 4 5 6 7 X . . . . (33) squirm and/or try to roll away?
1 2 3 4 5 6 7 X . . . . (34) smile or laugh?
1 2 3 4 5 6 7 X . . . . (35) coo or vocalize?

When put into the bath water, how often did the baby:
1 2 3 4 5 6 7 X . . . . (36) smile?
1 2 3 4 5 6 7 X . . . . (37) laugh?
1 2 3 4 5 6 7 X . . . . (38) splash or kick?
1 2 3 4 5 6 7 X . . . . (39) turn body and/or squirm?

When face was washed, how often did the baby:
1 2 3 4 5 6 7 X . . . . (40) smile or laugh?
1 2 3 4 5 6 7 X . . . . (41) fuss or cry?
1 2 3 4 5 6 7 X . . . . (42) coo?

When hair was washed, how often did the baby:
1 2 3 4 5 6 7 X . . . . (43) smile?
1 2 3 4 5 6 7 X . . . . (44) fuss or cry?
1 2 3 4 5 6 7 X . . . . (45) vocalize?

4

(1) Never	(2) Very Rarely	(3) Less Than Half the Time	(4) About Half the Time	(5) More Than Half the Time	(6) Almost Always	(7) Always	(X) Does Not Apply

Play

How often during the last week did the baby:

1 2 3 4 5 6 7 X . . . . (46) look at pictures in books and/or magazines for 2-5 minutes at a time?
1 2 3 4 5 6 7 X . . . . (47) look at pictures in books and/or magazines for 5 minutes or longer at a time?
1 2 3 4 5 6 7 X . . . . (48) stare at a mobile, crib bumper or picture for 5 minutes or longer?
1 2 3 4 5 6 7 X . . . . (49) play with one toy or object for 5-10 minutes?
1 2 3 4 5 6 7 X . . . . (50) play with one toy or object for 10 minutes or longer?
1 2 3 4 5 6 7 X . . . . (51) spend time just looking at playthings?
1 2 3 4 5 6 7 X . . . . (52) repeat the same sounds over and over again?
1 2 3 4 5 6 7 X . . . . (53) laugh aloud in play?
1 2 3 4 5 6 7 X . . . . (54) repeat the same movement with an object for 2 minutes or longer (e.g., putting a block in a cup, kicking or hitting a mobile)?
1 2 3 4 5 6 7 X . . . . (55) pay attention to your reading during most of the story when looking at picture books?
1 2 3 4 5 6 7 X . . . . (56) smile or laugh after accomplishing something (e.g., stacking blocks, etc.)?
1 2 3 4 5 6 7 X . . . . (57) smile or laugh when given a toy?
1 2 3 4 5 6 7 X . . . . (58) smile or laugh when tickled?

How often during the last week did the baby enjoy:

1 2 3 4 5 6 7 X . . . . (59) being sung to?
1 2 3 4 5 6 7 X . . . . (60) being read to?
1 2 3 4 5 6 7 X . . . . (61) hearing the sound of words, as in nursery rhymes?
1 2 3 4 5 6 7 X . . . . (62) looking at picture books?
1 2 3 4 5 6 7 X . . . . (63) gentle rhythmic activities, such as rocking or swaying?
1 2 3 4 5 6 7 X . . . . (64) lying quietly and examining his/her fingers or toes?
1 2 3 4 5 6 7 X . . . . (65) being tickled by you or someone else in your family?
1 2 3 4 5 6 7 X . . . . (66) being involved in rambunctious play?
1 2 3 4 5 6 7 X . . . . (67) watching while you, or another adult, playfully made faces?
1 2 3 4 5 6 7 X . . . . (68) touching or lying next to stuffed animals?
1 2 3 4 5 6 7 X . . . . (69) the feel of soft blankets ?
1 2 3 4 5 6 7 X . . . . (70) being rolled up in a warm blanket?
1 2 3 4 5 6 7 X . . . . (71) listening to a musical toy in a crib?

5

(1) Never	(2) Very Rarely	(3) Less Than Half the Time	(4) About Half the Time	(5) More Than Half the Time	(6) Almost Always	(7) Always	(X) Does Not Apply

When playing quietly with one of her/his favorite toys, how often did your baby:
1 2 3 4 5 6 7 X . . . . (72) show pleasure?
1 2 3 4 5 6 7 X . . . . (73) enjoy lying in the crib for more than 5 minutes?
1 2 3 4 5 6 7 X . . . . (74) enjoy lying in the crib for more than 10 minutes?

When something the baby was playing with had to be removed, how often did s/he:
1 2 3 4 5 6 7 X . . . . (75) cry or show distress for a time?
1 2 3 4 5 6 7 X . . . . (76) seem not bothered?

When tossed around playfully how often did the baby:
1 2 3 4 5 6 7 X . . . . (77) smile?
1 2 3 4 5 6 7 X . . . . (78) laugh?

During a peekaboo game, how often did the baby:
1 2 3 4 5 6 7 X . . . . (79) smile?
1 2 3 4 5 6 7 X . . . . (80) laugh?

How often did your baby enjoy bouncing up and down:
1 2 3 4 5 6 7 X . . . . (81) while on your lap?
1 2 3 4 5 6 7 X . . . . (82) on an object, such as a bed, bouncer chair, or toy?

How often did the infant look up from playing:
1 2 3 4 5 6 7 X . . . . (83) when the telephone rang?
1 2 3 4 5 6 7 X . . . . (84) when s/he heard voices in the next room?

When your baby saw a toy s/he wanted, how often did s/he:
1 2 3 4 5 6 7 X . . . . (85) get very excited about getting it?
1 2 3 4 5 6 7 X . . . . (86) immediately go after it?

When given a new toy, how often did your baby:
1 2 3 4 5 6 7 X . . . . (87) get very excited about getting it?
1 2 3 4 5 6 7 X . . . . (88) immediately go after it?
1 2 3 4 5 6 7 X . . . . (89) seem not to get very excited about it?

Daily Activities

How often during the last week did the baby:
1 2 3 4 5 6 7 X . . . . (90) cry or show distress at a change in parents' appearance, (glasses off, shower cap on, etc.)?
1 2 3 4 5 6 7 X . . . . (91) when in a position to see the television set, look at it for 2 to 5 minutes at a time?

6

(1) Never	(2) Very Rarely	(3) Less Than Half the Time	(4) About Half the Time	(5) More Than Half the Time	(6) Almost Always	(7) Always	(X) Does Not Apply

How often during the last week did the baby:

1 2 3 4 5 6 7 X . . . . (92) when in a position to see the television set, look at it for 5 minutes or longer?
1 2 3 4 5 6 7 X . . . . (93) protest being placed in a confining place (infant seat, play pen, car seat, etc)?
1 2 3 4 5 6 7 X . . . . (94) startle at a sudden change in body position (for example, when moved suddenly)?
1 2 3 4 5 6 7 X . . . . (95) appear to listen to even very quiet sounds?
1 2 3 4 5 6 7 X . . . . (96) attend to sights or sounds when outdoors (for example, wind chimes or water sprinklers)?
1 2 3 4 5 6 7 X . . . . (97) move quickly toward new objects?
1 2 3 4 5 6 7 X . . . . (98) show a strong desire for something s/he wanted?
1 2 3 4 5 6 7 X . . . . (99) startle to a loud or sudden noise?
1 2 3 4 5 6 7 X . . . . (100) look at children playing in the park or on the playground for 5 minutes or longer?
1 2 3 4 5 6 7 X . . . . (101) watch adults performing household activities (e.g., cooking, etc.) for more than 5 minutes?
1 2 3 4 5 6 7 X . . . . (102) squeal or shout when excited?
1 2 3 4 5 6 7 X . . . . (103) imitate the sounds you made?
1 2 3 4 5 6 7 X . . . . (104) seem excited when you or other adults acted in an excited manner around him/her?

When being held, how often did the baby:

1 2 3 4 5 6 7 X . . . . (105) pull away or kick?
1 2 3 4 5 6 7 X . . . . (106) seem to enjoy him/herself?
1 2 3 4 5 6 7 X . . . . (107) mold to your body?
1 2 3 4 5 6 7 X . . . . (108) squirm?

When placed on his/her back, how often did the baby:

1 2 3 4 5 6 7 X . . . . (109) fuss or protest?
1 2 3 4 5 6 7 X . . . . (110) smile or laugh?
1 2 3 4 5 6 7 X . . . . (111) wave arms and kick?
1 2 3 4 5 6 7 X . . . . (112) squirm and/or turn body?

When the baby wanted something, how often did s/he:

1 2 3 4 5 6 7 X . . . . (113) become upset when s/he could not get what s/he wanted?
1 2 3 4 5 6 7 X . . . . (114) have tantrums (crying, screaming, face red, etc.) when s/he did not get what s/he wanted?

When placed in an infant seat or car seat, how often did the baby:

1 2 3 4 5 6 7 X . . . . (115) wave arms and kick?
1 2 3 4 5 6 7 X . . . . (116) squirm and turn body?
1 2 3 4 5 6 7 X . . . . (117) lie or sit quietly?
1 2 3 4 5 6 7 X . . . . (118) show distress at first; then quiet down?

7

(1) Never	(2) Very Rarely	(3) Less Than Half the Time	(4) About Half the Time	(5) More Than Half the Time	(6) Almost Always	(7) Always	(X) Does Not Apply

When frustrated with something, how often did your baby:
1 2 3 4 5 6 7 X . . . . (119) calm down within 5 minutes?

When your baby was upset about something, how often did s/he:
1 2 3 4 5 6 7 X . . . . (120) stay upset for up to 10 minutes or longer?
1 2 3 4 5 6 7 X . . . . (121) stay upset for up to 20 minutes or longer?
1 2 3 4 5 6 7 X . . . . (122) soothe her/himself with other things (such as a stuffed animal, or blanket)?

When rocked or hugged, in the last week, how often did your baby:
1 2 3 4 5 6 7 X . . . . (123) seem to enjoy her/himself?
1 2 3 4 5 6 7 X . . . . (124) seemed eager to get away?
1 2 3 4 5 6 7 X . . . . (125) make protesting noises?

When reuniting after having been away during the last week how often did the baby:
1 2 3 4 5 6 7 X . . . . (126) seem to enjoy being held?
1 2 3 4 5 6 7 X . . . . (127) show interest in being close, but resisted being held?
1 2 3 4 5 6 7 X . . . . (128) show distress at being held?

When being carried, in the last week, how often did your baby:
1 2 3 4 5 6 7 X . . . . (129) seem to enjoy him/herself?
1 2 3 4 5 6 7 X . . . . (130) push against you until put down?

While sitting in your lap:
1 2 3 4 5 6 7 X . . . . (131) how often did your baby seem to enjoy her/himself?
1 2 3 4 5 6 7 X . . . . (132) how often would the baby not be content without moving around?

How often did your baby notice:
1 2 3 4 5 6 7 X . . . . (133) low-pitched noises, air conditioner, heating system, or refrigerator running or starting up?
1 2 3 4 5 6 7 X . . . . (134) sirens from fire trucks or ambulances at a distance?
1 2 3 4 5 6 7 X . . . . (135) a change in room temperature?
1 2 3 4 5 6 7 X . . . . (136) a change in light when a cloud passed over the sun?
1 2 3 4 5 6 7 X . . . . (137) sound of an airplane passing overhead?
1 2 3 4 5 6 7 X . . . . (138) a bird or a squirrel up in a tree?
1 2 3 4 5 6 7 X . . . . (139) fabrics with scratchy texture (e.g., wool)?

When tired, how often was your baby:
1 2 3 4 5 6 7 X . . . . (140) likely to cry?
1 2 3 4 5 6 7 X . . . . (141) show distress?

8

(1) Never	(2) Very Rarely	(3) Less Than Half the Time	(4) About Half the Time	(5) More Than Half the Time	(6) Almost Always	(7) Always	(X) Does Not Apply

At the end of an exciting day, how often did your baby:
1 2 3 4 5 6 7 X . . . . (142) become tearful?
1 2 3 4 5 6 7 X . . . . (143) show distress?

For no apparent reason, how often did your baby:
1 2 3 4 5 6 7 X . . . . (144) appear sad?
1 2 3 4 5 6 7 X . . . . (145) seem unresponsive?

How often did your baby make talking sounds when:
1 2 3 4 5 6 7 X . . . . (146) riding in a car?
1 2 3 4 5 6 7 X . . . . (147) riding in a shopping cart?
1 2 3 4 5 6 7 X . . . . (148) you talked to her/him?

Two Week Time Span

When you returned from having been away and the baby was awake, how often did s/he:
1 2 3 4 5 6 7 X . . . . (149) smile or laugh?

When introduced to an unfamiliar adult, how often did the baby:
1 2 3 4 5 6 7 X . . . . (150) cling to a parent?
1 2 3 4 5 6 7 X . . . . (151) refuse to go to the unfamiliar person?
1 2 3 4 5 6 7 X . . . . (152) hang back from the adult?
1 2 3 4 5 6 7 X . . . . (153) never "warm up" to the unfamiliar adult?

When in the presence of several unfamiliar adults, how often did the baby:
1 2 3 4 5 6 7 X . . . . (154) cling to a parent?
1 2 3 4 5 6 7 X . . . . (155) cry?
1 2 3 4 5 6 7 X . . . . (156) continue to be upset for 10 minutes or longer?

When visiting a new place, how often did the baby:
1 2 3 4 5 6 7 X . . . . (157) show distress for the first few minutes?
1 2 3 4 5 6 7 X . . . . (158) continue to be upset for 10 minutes or more?
1 2 3 4 5 6 7 X . . . . (159) get excited about exploring new surroundings?
1 2 3 4 5 6 7 X . . . . (160) move about actively when s/he is exploring new surroundings?

When your baby was approached by an unfamiliar person when you and s/he were out (for example, shopping), how often did the baby:
1 2 3 4 5 6 7 X . . . . (161) show distress?
1 2 3 4 5 6 7 X . . . . (162) cry?

9

(1) Never	(2) Very Rarely	(3) Less Than Half the Time	(4) About Half the Time	(5) More Than Half the Time	(6) Almost Always	(7) Always	(X) Does Not Apply

When an unfamiliar adult came to your home or apartment, how often did your baby:
1 2 3 4 5 6 7 X . . . . (163) allow her/himself to be picked up without protest?
1 2 3 4 5 6 7 X . . . . (164) cry when the visitor attempted to pick her/him up?

When in a crowd of people, how often did the baby:
1 2 3 4 5 6 7 X . . . . (165) seem to enjoy him/herself?

Did the baby seem sad when:
1 2 3 4 5 6 7 X . . . . (166) caregiver is gone for an unusually long period of time?
1 2 3 4 5 6 7 X . . . . (167) left alone/unattended in a crib or a playpen for an extended period of time?

When you were busy with another activity, and your baby was not able to get your attention, how often did s/he:
1 2 3 4 5 6 7 X . . . . (168) become sad?
1 2 3 4 5 6 7 X . . . . (169) cry?

When your baby saw another baby crying, how often did s/he:
1 2 3 4 5 6 7 X . . . . (170) become tearful?
1 2 3 4 5 6 7 X . . . . (171) show distress?

When familiar relatives/friends came to visit, how often did your baby:
1 2 3 4 5 6 7 X . . . . (172) get excited?
1 2 3 4 5 6 7 X . . . . (173) seem indifferent?

Soothing Techniques

Have you tried any of the following soothing techniques in the last two weeks? If so, how quickly did your baby soothe using each of these techniques? Circle (X) if you did not try the technique during the LAST TWO WEEKS.

When rocking your baby, how often did s/he:
1 2 3 4 5 6 7 X . . . . (174) soothe immediately?
1 2 3 4 5 6 7 X . . . . (175) not soothe immediately, but in the first two minutes?
1 2 3 4 5 6 7 X . . . . (176) take more than 10 minutes to soothe?

When singing or talking to your baby, how often did s/he:
1 2 3 4 5 6 7 X . . . . (177) soothe immediately?
1 2 3 4 5 6 7 X . . . . (178) not soothe immediately, but in the first two minutes?
1 2 3 4 5 6 7 X . . . . (179) take more than 10 minutes to soothe?

10

(1) Never	(2) Very Rarely	(3) Less Than Half the Time	(4) About Half the Time	(5) More Than Half the Time	(6) Almost Always	(7) Always	(X) Does Not Apply

When walking with the baby, how often did s/he:
1 2 3 4 5 6 7 X . . . . (180) soothe immediately?
1 2 3 4 5 6 7 X . . . . (181) not soothe immediately, but in the first two minutes?
1 2 3 4 5 6 7 X . . . . (182) take more than 10 minutes to soothe?

When giving him/her a toy, how often did the baby:
1 2 3 4 5 6 7 X . . . . (183) soothe immediately?
1 2 3 4 5 6 7 X . . . . (184) not soothe immediately, but in the first two minutes?
1 2 3 4 5 6 7 X . . . . (185) take more than 10 minutes to soothe?

When showing the baby something to look at, how often did s/he:
1 2 3 4 5 6 7 X . . . . (186) soothe immediately?
1 2 3 4 5 6 7 X . . . . (187) not soothe immediately, but in the first two minutes?
1 2 3 4 5 6 7 X . . . . (188) take more than 10 minutes to soothe?

When patting or gently rubbing some part of the baby's body, how often did s/he:
1 2 3 4 5 6 7 X . . . . (189) soothe immediately?
1 2 3 4 5 6 7 X . . . . (190) not soothe immediately, but in the first two minutes?
1 2 3 4 5 6 7 X . . . . (191) take more than 10 minutes to soothe?

Appendix B2: Research Ethics Board Approval Notice

Western Research

Date: 25 June 2019

To: Michelle Mottola

Project ID: 108080

Study Title: Strategizing the best approach to prevent early excessive gestational weight gain using a Nutrition and Exercise Lifestyle Intervention Program (NELIP).

Application Type: Continuing Ethics Review (CER) Form

Review Type: Delegated

REB Meeting Date: 02/Jun/2019

Date Approval Issued: 25/Jun/2019

REB Approval Expiry Date: 05/Jul/2020

Dear Michelle Mottola,

The Western University Research Ethics Board has reviewed the application. This study, including all currently approved documents, has been re-approved until the expiry date noted above.

REB members involved in the research project do not participate in the review, discussion or decision.

Western University REB operates in compliance with, and is constituted in accordance with, the requirements of the TriCouncil Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2); the International Conference on Harmonisation Good Clinical Practice Consolidated Guideline (ICH GCP); Part C, Division 5 of the Food and Drug Regulations; Part 4 of the Natural Health Products Regulations; Part 3 of the Medical Devices Regulations and the provisions of the Ontario Personal Health Information Protection Act (PHIPA 2004) and its applicable regulations. The REB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Please do not hesitate to contact us if you have any questions.

Sincerely,

Daniel Wyzynski, Research Ethics Coordinator, on behalf of Dr. Joseph Gilbert, HSREB Chair

Note: This correspondence includes an electronic signature (validation and approval via an online system that is compliant with all regulations).

Appendix C3: Written informed consent

LETTER OF INFORMATION AND CONSENT

Strategizing the best approach to prevent early excessive gestational weight gain using a Nutrition and Exercise Lifestyle Intervention Program (NELIP)

Principal Investigator
Dr. Michelle Mottola, PhD FACSM, Director – Exercise and Pregnancy Lab, School of Kinesiology

Local Study Investigators
1. Dr. Harry Prapavessis, PhD, School of Kinesiology, UWO
2. Karishma Hosein, BScKin, Exercise and Pregnancy Lab, UWO
3. Taniya Nagpal, BHSc, Exercise and Pregnancy Lab, UWO
4. Dr. Barb de Vrijer, MD, Dept. Obstetrics and Gynecology
5. Dr. Karina Kasawara, PhD, Postdoctoral Fellow, University of Campinas, Brazil
6. Stephanie Paplinskie, MA, Exercise and Pregnancy Lab, UWO
7. Dr. Roberta Bgeginski, PhD, Postdoctoral associate, Exercise and Pregnancy Lab, UWO
8. Mollie Manley, BHSc, Exercise and Pregnancy Lab, UWO

Conflict of Interest
There are no conflicts of interest to declare related to this study.

Invitation to Participate in Research
You are being invited to participate in this research study about health in pregnancy because, you are 12 to 18 weeks pregnant and are eligible to participate. Your participation is voluntary, so choosing not to participate will have no negative consequences or effect on the care that you receive at your primary health care clinic or place of delivery.

Why is this study being done?
Although weight gain is expected during pregnancy, excessive weight gain may put mothers at risk of health problems like diabetes and high blood pressure. Excessive gestational weight gain is defined by the 2009 Institute of Medicine weight gain guidelines as > 16 kg if you are normal weight, > 11.5 kg if you are overweight and > 9 kg if you are obese. Babies of women who gain

above these guidelines may also be at risk of being born too large and developing future health problems. We are interested in helping women to gain a healthy amount of weight during pregnancy to prevent problems associated with gaining excessive weight during pregnancy. A total of 230 pregnant women will be participating in this study. The results of this study will allow us to design future programs and guidelines for pregnant women so that mothers may have the healthiest pregnancy possible. Because this is a smaller pilot study, we may use these findings to guide the future direction of a larger study.

The purpose of this study is to evaluate the success of starting a program of healthy eating first followed by starting exercise by 25 weeks of pregnancy, or starting a program of exercise first, followed by starting a healthy eating program by 25 weeks or starting both programs together. We will monitor your weight gain to see which strategy works best at preventing early and total excessive weight gain during pregnancy.

What will happen during the study?
The program will begin between 12 to 18 weeks of pregnancy. If you decide to participate, you will be randomly assigned (like the flip of a coin) to one of the intervention strategies. You will have a 1 in 3 chance of being placed in any group. Neither you, the study staff, nor the study investigators can choose which group you will be in.

Your participation involves the following:

First Visit: Tour of the facility, information session and pre -screening
Before you are randomized into your specific group or strategy, we will have you sign the consent form (attached). Once consent is signed, we will have you complete a medical screening questionnaire (PARmed-X for Pregnancy). All women will receive usual care and advice from their primary health care provider and he/she must sign the PARmed-X form to confirm you have a low-risk pregnancy before your participation in the study begins. Study participation will begin at 12-18 weeks of pregnancy and continue until the birth of your baby, with follow-up when your baby is 2, 6 and 12 months old. You will be asked to complete the Weight and Health History questionnaire about your general health, the Kaiser Physical Activity Survey and the Pregnancy Physical Activity Questionnaire, that will give us information about your activity levels during pregnancy. You will be given a Food Frequency Questionnaire and also asked about what you ate yesterday (24 hour recall) in order to see what your food intake profile looks like. In addition, you will be given a questionnaire about your current level of anxiety and stress. Also at the first visit, you will be asked if you have a smart phone (Android or iPhone). The purpose of this is to see if you want to track your food intake (everything you eat and drink) using a smart phone app. You will be given the option to track your food using either a paper log, email or smart phone application for 3 days in a row, including 1 weekend day (For example, Thursday, Friday and Saturday or Sunday, Monday and Tuesday). We ask that you be as honest as possible and not change your eating habits while you are recording your food intake over these three assigned days. We will use this information to help make a nutrition meal plan that is suited to you. If you do not have a smart phone we will provide you with a 3-day food intake record in paper form that you will fill out in the same way. You will also be given a Fitbit activity tracker that you will wear on your wrist that will track how active you are over these same three days. We will provide you with a personalized user name and password to protect

your privacy online. The Fitbit tracker and your food intake record will allow us to monitor your nutrition and activity before you start the program. We will make an appointment for you to return to the lab the following week to find out which group you have been randomized into. The total time for this first visit will be approximately 60 to 90 minutes.

At the next visit, you will return your Fitbit and we will measure and record your height and your weight. At this time we will also measure your skinfolds. This is a measure of your fat just under the skin at 4 specific sites: at the front and back of your arm, between your shoulder blades, and just above your hip bone. We want to monitor how the fat at these sites will change over the course of your pregnancy. At these sites, your skin and fat underneath will be gently pinched between a caliper or tweezers. The sensation you will feel is just like when you "pinch an inch" on your body and you may feel the calipers as a tickle against your skin. Once this is complete, we will then randomize you into one of three strategies. If you are in the group that receives exercise first or both nutrition and exercise as your initial strategy, you will continue using the Fitbit to track your activity levels for the duration of the program. If you are in the group that receives the nutrition program first or both nutrition and exercise, you will be given a specialized meal plan and you will continue to record your food intake for a 24-hour period once per week using your choice of recording method (paper log, email or smart phone) for the duration of the program.

If you are randomized into having the Nutrition strategy introduced first:

The purpose of the controlled nutrition meal plan is to promote good eating habits, to control excessive weight gain and to help prevent gestational diabetes. This strategy will take into account your 3-day food intake record. It will allow you to have three balanced meals and two to three snacks per day, emphasizing high fiber and low sugar content foods and having healthy portion sizes. Once per week throughout the program, you will be required to record for a 24hour period everything you eat and drink during that time period using either a pen and paper food log, email or smart phone application. This will assist us in adjusting your nutrition program as your pregnancy progresses and to promote good eating habits and prevent excessive weight gain. We will make a weekly scheduled appointment to the lab at your convenience for a "weigh-in" and to discuss any nutrition concerns you may have. These weekly visits will take approximately 30 minutes, and will continue until you reach 24-weeks gestation. At 24-weeks gestation, during your weekly visit, we will give you the Kaiser Physical Activity Survey to complete again, we will repeat your skinfold measurements and record your weight. We will ask you to repeat the 3-day food intake record using your choice of recording method like you did at the beginning of the study. In addition, we will give you a Fitbit tracker to also record your activity levels like you did at the beginning of the study. At your following weekly visit (approximately 25-weeks gestation), you will begin the exercise strategy (please see below) while continuing the nutrition strategy, and will continue to come to the lab for your weekly scheduled "weight-ins," walking and discussion of nutritional concerns.

If you are randomized into having the Exercise (Walking Program) strategy introduced first:

The purpose of the exercise strategy is to promote an active lifestyle, to prevent excessive gestational weight gain and to help prevent gestational diabetes. This strategy will take into

account your previous physical activity habits. You will begin the walking program at a walking pace that is easy for you to maintain without becoming breathless (out of breath) for 25 minutes. We recommend that you complete 3 to 4 total (2 to 3 on your own) exercise sessions per week until delivery. For each subsequent week, the exercise time will increase by 2 mins up to a maximum of 40 mins per walking session, which will be maintained until delivery. We will make a weekly scheduled appointment to the lab at your convenience for a "weigh-in" and for you to walk with us. These weekly visits will take approximately 45 to 60 minutes, and will continue until you reach 24-weeks gestation. At 24 weeks gestation, during your weekly visit, we will give you the Kaiser Physical Activity Survey to complete again, we will repeat your skinfold measurements and record your weight. We will ask you to repeat the 3-day food intake record using your choice of recording method like you did at the beginning of the study. In addition, you will use your Fitbit tracker to also record your activity levels like you did at the beginning of the study. At your following weekly visit (approximately 25 weeks gestation), you will begin the nutrition strategy (please see above) while continuing the exercise strategy, and will continue to come to the lab for your weekly scheduled "weight-ins," walking and discussion of nutritional concerns.

If you are randomized into having both Nutrition and Exercise strategies introduced first:

You will be given both strategies at the same time (see above) and will continue these strategies until delivery. At 24-weeks gestation, during your weekly visit, we will give you the Kaiser Physical Activity Survey to complete again, we will repeat your skinfold measurements and record your weight. We will ask you to repeat the 3-day food intake record using your choice of recording method) like you did at the beginning of the study. In addition, you will use your Fitbit tracker to also record your activity levels like you did at the beginning of the study. At your following weekly visit (approximately 25-weeks gestation), you will continue your nutrition and exercise strategies as you did before.

Regardless of strategy assignment, at 36 to 38 weeks of pregnancy, we will give you the same questionnaire plus one exit questionnaire about your experience in the program, we will measure your skinfolds and record your weight just like we did when you were 24-weeks gestation. At this visit you will be required to return your Fitbit. Regardless of strategy assignment, we ask that you or your partner contact us as soon as possible after the birth of your baby. We will contact you within 6 to 18 hours after you deliver. One of our research staff will visit you and your new baby and, with your help, we will measure the length, head size, chest size and abdomen size of your baby, length of limbs and limb girths, using a cloth tape measure. We will record the birth weight of your baby, any complications which may have occurred during delivery, and the APGAR scores. These are numbers that refer to your baby's colour, breathing and reflexes at 1 minute and 5 minutes after birth. Finally, we will measure 6 skin fold sites on your baby using a special infant skinfold caliper. The sites that we will measure are: the front and back of the arm, between the shoulder blades, the front of one thigh, the front of the belly by the belly-button, and just above the hip bone. There are no known risks with this procedure. We will also ask you what your last known body weight was before delivery.

You and your baby will return to the lab at 2, 6 and 12 months post-delivery for follow-up. You will complete the same questionnaires that you filled in from your last pregnancy visit along with two additional questionnaires about breastfeeding and solid foods. In addition, we will ask

you what you ate and drank in the last 24 hours before your visit. We will measure your infant's length, weight and head, chest, abdomen, hip, arm, mid-thigh and calf circumference using a cloth tape measure like we did at birth. We will measure the same 6 skinfold sites on your infant as we measured at birth. The front and back of the arm, between the shoulder blades, the front of the thigh, the front of the belly by the belly-button and just above the hip bone. You will be weighed and we will also measure your waist (at the area of your belly-button) and hips (at the widest part of your hips) using a soft cloth tape and repeat the skinfold measurements that we did when you were pregnant. The total time for each of these visits will be approximately 60 to 90 minutes.

At your 6 month post-delivery visit we will offer you an option of adding a sub-study to your 12 month visit. This sub-study is totally optional and will add an additional 30 minutes to your scheduled 12 month visit. If you are interested we will provide you with a separate letter of information and consent form for the separate sub-study at 6 months postpartum before we schedule you for the following 12 month visit. If you do not wish to participate in the sub-study you would continue as you would now with the originally scheduled 12 month visit.

Voluntary Participation

Participation in this study is voluntary. You may refuse to participate, refuse to answer any questions or to withdraw from the study at any time with no effect on your future care.

Withdrawal from Study

You may change your mind about participating in the study and withdraw (stop taking part in the study) at any time. If you do withdraw, we will still use your information that has been collected up to that point. If, during the course of the study, your physician determines that continuation of the study would worsen your health, or the health of your baby, you will be advised to discontinue the study. When you discontinue, we will still use your information that has been collected up to that point to help answer the research question. No new information will be collected without your permission. **We must insist that you return our Fitbit to us immediately following your decision to withdraw.**

An alternative to the study procedures described above is to not participate in the study and just continue on as you do now. There is no guarantee of personal benefit from participating in the study. If you withdraw from the study prior to completion we will contact you by phone to record your final weight before delivery and birth information (birth weight, length, head circumference, APGAR scores and any problems with labour and birth).

Are there any risks to participating in this study?

The risks involved with participating in this study are minimal. When you first begin the exercise walking program, you may experience some soreness in your muscles, but this will go away within a few days.

Are there any benefits to participating in this study?

Participating in this study may help you to learn more about health in pregnancy – specifically, exercise and nutrition – and may prevent excessive gestational weight gain.

How will your information be kept confidential?
Your confidentiality will be respected. The information collected from you will be used for this current research project only. Your record will be kept locked in a cabinet in a secure office. Your name, address, telephone number and email address will be collected in order to contact you. You will be given a unique identification number and any personal or health information collected from you will not be personally identifiable in any way. Your records will be kept in a secure and confidential location for a minimum of 15 years and then destroyed.

Your unique Fitbit username will not include any personal identifiers. Only members of the research team will know your username and password.

When the results of this study are published, reported or presented to other health care professionals and researchers, your name (or the names of any other participant) will not be associated with any specific result without your consent to the disclosure.

All information collected for this study (including personal health information) will be kept confidential and will not be shared with anyone outside the study unless required by law. Absolute confidentiality, however, cannot be guaranteed, as representatives of the University of Western Ontario Health Sciences Research Ethics Board may require access to your study related records or may need to follow-up with you to monitor the conduct of this research.

Will there be any cost to me?
No. Your participation in this research will not involve any additional costs to you or your health care insurer, and you will not be compensated for your participation in the study. We will arrange for you to park free of charge at UWO.

What are your rights as a participant?
If you are harmed as a direct result of taking part in this study, all necessary medical treatment will be made available to you at no cost.

You do not waive any legal rights by signing the consent form. You will be given a copy of this letter of information and consent form once it is signed.

Questions about the Study
If you have any questions about this study or your treatment, please contact the principal study investigator, Dr. Michelle Mottola (Department of Anatomy and Cell Biology, Schulich School of Medicine and Dentistry; School of Kinesiology, Faculty of Health Sciences) of the University of Western Ontario

If you have any questions about your rights as a research participant or the conduct of this study, you may contact The Office of Research Ethics

Consent form

Strategizing the best approach to prevent early excessive gestational weight gain using a Nutrition and Exercise Lifestyle Intervention Program (NELIP)

I have read the letter of information. This study has been explained to me and any questions I had have been answered. I know that I may leave the study at any time. I agree for myself and my child to participate.

Please check the appropriate box below and initial:
____ I agree to be contacted for future research studies
____ I do NOT agree to be contacted for future research studies

_____ Your	_____	_____
Name (PLEASE PRINT)	Your Signature	Date (DD-MM-YYYY)

My signature means that I have explained the study to the participant named above. I have answered all questions.

_____	_____
Name of Person obtaining consent	Signature

Western UNIVERSITY · CANADA

McMaster University Health Sciences

Letter of information

Sub-Study Title: NELIP+Brain: Assessing emotion regulation in the infants of women receiving three different Diet+Exercise Interventions in pregnancy.

Local Principal Investigator: Michelle F. Mottola PhD FACSM
Student Investigator: Mollie Manley; **Postdoctoral Associate**: Roberta Bgeginski, PhD
External Investigators: Ryan J. Van Lieshout MD PhD, John Krzeczkowski, PhD student, McMaster University

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Conflict of Interest
There are no conflicts of interest to declare related to this sub-study.

Why are we doing this sub-study? While it is important for mothers to eat healthy foods and get enough exercise in pregnancy, much remains unknown about how these things affect their babies' brain development. The purpose of the NELIP+Brain (NELIP+B) study is to find out how nutrition and exercise during pregnancy affect brain markers of your infant's abilities to control their emotions (i.e., settle themselves) at 12 months of age. The information from this sub-study will be used to help us understand how these skills develop and how we might prevent or lessen the impact of problems with emotions in children. You and your child are being invited to participate in this sub-study because you were a participant in the original NELIP study, and this sub-study (called NELIP+B) adds approximately 35 more minutes on to the scheduled 12 month follow-up visit. Participation in this add-on sub-study is voluntary and you can withdraw from it at any time.

What will happen during the sub-study? The sub-study will occur during your already scheduled 12 month visit to the Exercise and Pregnancy Laboratory. Participating in the sub-study will add approximately 35 minutes to your already scheduled 1 hour time so total time for both will now be 1 hour and 35 minutes. During the extra 35 minutes for the sub-study you will fill out a questionnaire about your child's emotions and behaviours (about 10 minutes).

During a 5-minute period of quiet rest (when both you and your infant are calm), we will record heart and brain activity from both you and your infant at the same time. Heart activity will be recorded using small disposable stickers (electrodes) placed on your child's back (Please see Figure 1 below) and two will be placed on you (right collar bone and your left lower rib). Brain activity will be assessed using the MUSE® brain sensing headband-which will be placed over the forehead and behind the ears and on both you and your infant with attachments to the ear lobes (Please see Figure 2 below). These bands are wireless (not attached to any cords). We will also measure brain activity using a device called functional near-infrared spectroscopy (fNIRS) that will be placed like a bathing cap over you and your infants head (See Figure 3 below).

Voluntary Participation
Participation in this sub-study is voluntary. You may refuse to participate, refuse to answer any questions or to withdraw from the sub-study at any time with no effect on your future care or your previous participation in the original NELIP study.

Withdrawal from Study

You may change your mind about participating in the sub-study and withdraw (stop taking part in the study) at any time. The data collected up until that point will be kept on file and no further contact will be made with you regarding the sub-study once you have withdrawn.

An alternative to the study procedures described below is to not participate in the sub-study and just continue on as you do now. There is no guarantee of personal benefit from participating in the sub-study.

Figure 1: Example of stickers (electrodes) to measure heart activity placed on an infant's back. Mothers will only have two stickers (electrodes), one on their right collarbone, and the other on their lower left rib. These will measure heart activity of both you and your infant.

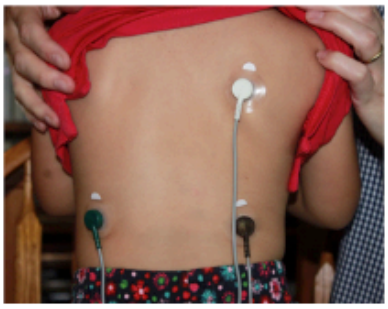

Figure 2: Example of mother and infant wearing the MUSE® brain sensing headband and ear lobe attachments. This measures brain activity of both you and your infant. We are interested in the activity in the front part of the brain because this is where emotion is controlled.

Figure 3: Example of a mother and an infant wearing an fNIRS (functional near-infrared spectroscopy) device. This device measures brain activity using light sensors. The light sensors are placed into a cap, and the cap will fit over the head like a bathing cap. We are interested in the brain activity in the front part of the brain in both mothers and infants.

While wearing the heart activity stickers, the head band, and the fNIRS cap, we will record heart and brain activity during a short task. In this task your child will play with an attractive toy for 2 minutes, then the toy will be removed from their reach for 2 minutes before they are allowed to play with it again (another 5 minutes). We will digitally record you and your child during the completion of the above tasks to record your child's reaction. If you would like to see these recordings we can show them to you after the tests. These recordings will be scored immediately after your visit and then destroyed.

How will your information be kept confidential? Any information collected about you or your child will be given a unique identification number and any personal information collected from you will only be linked to this number on a master list that will be kept separate and in a locked cabinet. Paper copies of de-identified information will be kept in a locked filing cabinet in the Exercise and Pregnancy laboratory and electronic data will be stored on a secure server at Western (password protected); both will only be available to research team members. Any information that is to be shared with our colleagues from McMaster will be de-identified, encrypted and password protected. Whenever study information is published or presented at conferences, it will be reported in group format so it will be impossible to identify individual responses. Your records will be kept in a secure and confidential location for a minimum of 7 years and then destroyed.

When the results of this sub-study are published, reported or presented to other health care professionals and researchers, your name (or the names of any other participant) will not be associated with any specific result.

All information collected for this sub-study (including personal health information) will be kept confidential and will not be shared with anyone outside the sub-study unless required by law. Absolute confidentiality, however, cannot be guaranteed, as representatives of the University of Western Ontario Health Sciences Research Ethics Board may require access to your sub-study related records or may need to follow-up with you to monitor the conduct of this research.

Is there any payment for participation? You will not be paid to participate in this sub-study. In appreciation of your time and involvement, you will receive a President's Choice $25 gift certificate.

Will there be any cost to me? No. Your participation in this research will not involve any additional costs to you or your health care insurer. We will provide complementary parking during your visit.

What are the risks/benefits to me? There will be no direct clinical benefits to you or your child in this sub-study, but your participation will be helping us to better understand the link between lifestyle factors during pregnancy and healthy infant development – this will help children in the future. There is also a small possibility that you may feel some distress occurring in response to the testing or the test results or that your child may become tired, distressed or fussy. There are no known risks due to the headband used to measure brain activity and the other electrodes used to measure heart activity. Some children may experience a little discomfort to wearing the headband (like wearing a hat) and with the electrode stickers. There are no known risks to using the fNIRS device. The amount of light that goes into the brain with fNIRS is about the same as the amount of light that goes into the brain when walking outside on a sunny day. The NIRS procedure is non-invasive, painless, and safe. The fNIRS system uses a class 1 laser, which is safe for eye and skin exposure. The laser will not emit enough heat to cause any burning or discomforts. During testing, you can take a break or stop participating at any time.

What if I change my mind about being in the study? Your participation and your child's participation in the sub-study are voluntary and you can decide to stop at any time, even after signing the consent form or part-way through the study. If you do end your participation in the sub-study, there will be no consequences to you or your child but we will ask if we can keep the data that was collected up until that point; this will be your choice.

Questions about the Sub-study?
If you have any questions about this sub-study, please contact the principal study investigator, Dr. Michelle Mottola (Department of Anatomy and Cell Biology, Schulich School of Medicine and Dentistry; School of Kinesiology, Faculty of Health Sciences) of the University of Western Ontario, at

If you have any questions about your rights as a research participant or the conduct of this study, you may contact The Office of Research Ethics

Consent Form

Sub-Study Title: NELIP+Brain: Assessing emotion regulation in the infants of women receiving three different Diet+Exercise Interventions in pregnancy.

I have read the letter of information. This sub-study has been explained to me and any questions I had have been answered.

Your Name (Please print)	Signature	Date (Day/Month/Year)

My signature means that I have explained the study to the participant named above. I have answered all questions.

Name of Person obtaining consent (Please print)	Signature	Date

Appendix D4: Weight and health history questionnaire

Unique Identifier: _____ **Today's date:** _____

Weight & Health History Questionnaire

Please answer the following questions to the best of your ability.
All of the answers gained through this survey will be held in the strictest of confidence.

Section A – Background Information:

1) What is your **age?** _____ (years)

2) What is your **ethnic** background?

☐ Caucasian ☐ Hispanic ☐ Aboriginal (please circle: First Nations, Métis, Inuit)
☐ Asian ☐ African American ☐ Other, please specify _____

3) What is your **height?** _____ feet _____ inches, OR _____ centimeters

4) What **education level** did you complete? Please check all that apply.

☐ Elementary school ☐ High school ☐ College
☐ University (please circle: certificate, bachelor, master, doctorate)
☐ Other, please specify_____

Section B – Current Pregnancy:

5) What has been your **usual adult body weight?** _____ pounds, OR _____ kilograms

6) How much **weight** did you **plan to gain** during this pregnancy?
_____ pounds, OR _____ kilograms

7) How much **weight** did you gain during this pregnancy?
_____ pounds, OR _____ kilograms

8) What was your **body weight one year before this pregnancy?**
_____ pounds, OR _____ kilograms

9) What was your **body weight immediately before this pregnancy?**
_____ pounds, OR _____ kilograms

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10) Were you actively trying to reduce your body weight in the **year before this pregnancy?**

☐ No ☐ If Yes, how much weight did you lose? _____ pounds, OR _____ kilograms

11) What have your **eating habits** been like in the **year before this pregnancy?** Check all that apply.

☐ one meal per day, specify when _____

☐ two meals per day, specify when _____

☐ three meals per day

☐ snack(s) every day, specify when _____

☐ Special diet, please specify name _____

☐ Trying to follow Canada's Food Guide to Healthy Eating

☐ Other nutrition plan, please specify _____

12) What has your pattern of **physical activity** been like in the **year before this pregnancy?**

Type of Physical Activity	**Frequency**	Average **Duration** of your exercise sessions	**Intensity** (low, medium, high)	**Location** (home, outdoors, gym, etc.)
	_____ time(s) per week	_____ minutes		
	_____ time(s) per week	_____ minutes		
	_____ time(s) per week	_____ minutes		
	_____ time(s) per week	_____ minutes		

13) How would you qualify your **current level of stress** on most days?

☐ No stress.

☐ Low stress level.

☐ Moderate stress level.

☐ High stress level. You perceive it as a problem.

14) Was this your **first pregnancy?**

☐ No ☐ Yes

Section C – Previous Pregnancies:

15) Please fill the following chart.

	Age you were	**Body weight** you were immediately **before** pregnancy	**Weight** you **gained during** pregnancy	**Weight retained after** pregnancy (never really lost)
1st pregnancy		_____pounds, OR _____kg	_____pounds, OR _____kg	_____pounds, OR _____kg
2nd pregnancy		_____pounds, OR _____kg	_____pounds, OR _____kg	_____pounds, OR _____kg
3rd pregnancy		_____pounds, OR _____kg	_____pounds, OR _____kg	_____pounds, OR _____kg
4th pregnancy		_____pounds, OR _____kg	_____pounds, OR _____kg	_____pounds, OR _____kg
5th pregnancy		_____pounds, OR _____kg	_____pounds, OR _____kg	_____pounds, OR _____kg

Other pregnancies: _____

16) For each pregnancy, what were the gestational age, gender and approximate birth weight and length?

	Gestational Age	**Gender**	Birth **weight**	Birth **Length**
1st baby	_____ weeks		_____pounds _____ounces, OR _____kg	_____inches, OR _____cm
2nd baby	_____ weeks		_____pounds _____ounces, OR _____kg	_____inches, OR _____cm
3rd baby	_____ weeks		_____pounds _____ounces, OR _____kg	_____inches, OR _____cm
4th baby	_____ weeks		_____pounds _____ounces, OR _____kg	_____inches, OR _____cm
5th baby	_____ weeks		_____pounds _____ounces, OR _____kg	_____inches, OR _____cm

Other babies: _____

17) Please indicate how you fed your baby(ies).

	Breastfeeding started	Duration of breastfeeding only	Age breastfeeding was stopped	Age at introduction of first solid foods
1st baby	____ Yes, ____ No	_____ months	_____ months	_____ months
2nd baby	____ Yes, ____ No	_____ months	_____ months	_____ months
3rd baby	____ Yes, ____ No	_____ months	_____ months	_____ months
4th baby	____ Yes, ____ No	_____ months	_____ months	_____ months
5th baby	____ Yes, ____ No	_____ months	_____ months	_____ months

Other babies: _____

Section D – Weight History:

18) What was **your birth weight?** _____ pounds _____ ounces, OR _____ kilograms

19) What was **your birth length?** _____ inches, OR _____ centimeters

20) How has your **body weight** been **since** you were **19** years of age?

- ☐ stable (always about the same weight, only changing by a couple of pounds when I am not pregnant), please skip to question 28
- ☐ unstable and progressively increasing
- ☐ unstable, because it has been going up and down often
- ☐ unstable, I feel I have been gaining weight with each pregnancy
- ☐ Other, please describe _____

21) By how many pounds or kilograms does your body weight tend to fluctuate (or change) per year?

In average about _____ pounds, OR _____ kilograms per year.

22) What do you think causes your body weight to be unstable? Please explain.

_____

_____

_____

23) Have you ever actively tried to lose weight?

☐ Yes ☐ If No, please skip to question 28

24) How old were you when you first actively tried to lose weight? _____

25) What method did you use when you first actively tried to lose weight?

_____

26) Since you were 19 years old, **how many times** have you been **actively trying to lose weight** and **at what ages**? Please explain.

_____

_____

27) List all the methods you have tried to lose weight.

☐ Vitamin/mineral supplement, please specify _____

☐ Dietary changes or special diets, please specify _____

_____

☐ Physical activity, please specify _____

_____

☐ Pills or herbal products, please describe _____

_____

☐ Prescribed medication, please describe _____

_____

☐ Surgery, please describe _____

_____

☐ Meetings with a health care professional(s), please indicate which professional(s) _____

_____

☐ Other, please describe _____

_____

28) What was the **maximum** weight you ever **lost, how long** did it take you to lose that weight and what **method** did you use?

I lost _____ pounds, OR _____ kilograms in _____ months, using the following method

_____

29) Have you ever consulted a physician about weight issues or for weight management purposes?

☐ No

☐ No, but I would like to.

- ☐ Yes, and it was helpful. Explain _____
- ☐ Yes, but it was not helpful. Explain _____

30) Have you ever consulted a registered dietitian about weight issues or for weight management purposes?

- ☐ No
- ☐ No, but I would like to.
- ☐ Yes, and it was helpful. Explain _____
- ☐ Yes, but it was not helpful. Explain _____

31) If you choose a method to lose weight in the future, what will you be looking for as important characteristics? Check the **three (3) most important factors** for you.

☐ Group meetings	☐ Individual support
☐ Short-term results	☐ Long-term results
☐ Minimum time commitment	☐ Follow-up support
☐ Education	☐ Learning healthier lifestyle choices
☐ Expert advice by registered dietitian	☐ Expert advice by physician
☐ Expert advice by exercise physiologist	☐ Cost
☐ Safety	☐ Help with stress management

☐ Other, please specify _____

Section E – About Your Health:

32) Have you ever been diagnosed with:

Type 1 Diabetes Mellitus	☐ Yes	☐ No
Type 2 Diabetes Mellitus	☐ Yes	☐ No
Gestational Diabetes Mellitus	☐ Yes	☐ No
Pre-diabetes	☐ Yes	☐ No
Gestational hypertension	☐ Yes	☐ No
Polycystic Ovarian Syndrome	☐ Yes	☐ No

33) Do you currently, or have you ever taken medication for diabetes or pre-diabetes:

☐ No ☐ If Yes, please describe _____

Section F – About Your Family:

34) How many **siblings** do you have?

_____ Sister(s) _____ Brother(s) ☐ I do not know

35) How many of your **siblings** are **overweight or obese**?

_____ Sister(s) _____ Brother(s) ☐ I do not know

36) Is there a history of **overweight or obesity** in the rest of your **immediate family**? (Check all that apply)

☐ Your mother ☐ Your father

☐ Grandmother on your mother's side ☐ Grandfather on your mother's side

☐ Grandmother on your father's side ☐ Grandfather on your father's side

☐ None ☐ I do not know ☐ Other(s), please specify:_____

37) How many of your **siblings** have **diabetes**?

_____ Sister(s) _____ Brother(s) ☐ I do not know

38) Is there a history of **diabetes** in the rest of your **immediate family**? (Check all that apply)

☐ Your mother ☐ Your father

☐ Grandmother on your mother's side ☐ Grandfather on your mother's side

☐ Grandmother on your father's side ☐ Grandfather on your father's side

☐ None ☐ I do not know ☐ Other(s), please specify:_____

39) What is the height and weight of the **father** of your child to be?

Height _____ feet _____ inches, OR _____ centimeters

Weight _____ pounds, OR _____ kilograms

40) What is the **ethnic** background of the **father** of your child to be?

☐ Caucasian ☐ Hispanic ☐ Aboriginal (please circle: First Nations, Métis, Inuit)

☐ Asian ☐ African American ☐ Other, please specify _____

41) How many **siblings** does the **father** of your child to be have?

_____ Sister(s) _____ Brother(s) ☐ I do not know

42) How many of the **father's siblings** are **overweight or obese**?

_____ Sister(s) _____ Brother(s) ☐ I do not know

43) How many of the **father's siblings** have **diabetes**?

_____ Sister(s) _____ Brother(s) ☐ I do not know

Appendix E5: Complete MATLAB protocol to determine infant FAA values

The MUSE files saved as .csv were uploaded to a Dropbox folder through the Muse Monitor app where they were imported into EEGLAB software for analysis. The sampling rate of the EEG files were 250 Hz in the alpha frequency band and typically between 6-9 Hz for infants. For each file "import everything" was selected. The channels of interest, raw AF7 and AF8, were selected and the data were filtered with the lower edge of the frequency pass band set as one Hz and the higher edge of the frequency pass band set at 30 Hz.

To pre-process the raw EEG data from the AF7 and AF8 electrodes and decrease the presence of artifacts that may arise due to motor movements and/or eye blinks, visual inspection was performed. The processed EEG data was broken down into 2-second duration epochs, with a 1-second (50%) overlap. In preparation to apply a Fast Fourier Transformation (FFT), a Hanning-window was used. To establish which frequencies underlie the data, each epoch was frequency transformed. The frontal asymmetry was calculated for each epoch and the values were averaged across epochs to produce a global frontal asymmetry score.

$$Frontal\ Asymmetry\ Index = \ln\left(\frac{alpha\ power\ right\ AF8}{alpha\ power\ left\ AF7}\right)$$

ln= natural logarithm

Appendix F6: Complete Mindware protocol to determine infant RMSSD, RSA, HF and LF values

To analyze HRV data (RMSSD, RSA, and LF/HF ratio) the appropriate .mwi file was identified and loaded into the Mindware program. Once uploaded, a channel map window was completed to ensure the channels were matched to the correct signal types. For these data, the channel types selected were ECG, as well as the respiratory cardiac impedance signals (Z0, dZ/dt) as a measure of respiration. Next, the file was broken down into 60-second epochs by adjusting the segment time.

Once the appropriate frequency domain parameters were set, manual edits were made to the R-peaks of each 60-second data segment. This was achieved by selecting ‘analyze’ then ‘edit R’s’, which enabled the insertion markers to indicate successful R-peaks (represented by blue symbols) and the deletion of markers that revealed artefacts (represented by yellow symbols). The segment was edited to ensure that no more than 10% of the total heart beats within a segment included artifacts. If the yellow box at the bottom right of the ‘edit R’ window exceeded this 10% cutoff, then data were discarded. Following this, if more than one third of the segments were deleted because the file could not be cleaned within the 10% rule, then the entire HRV file was discarded.

When all successful R-peaks were identified, the white ‘write’ button at the top of the window was selected. This ensured that the data extracted from the clean and segmented file was saved in an Excel spreadsheet.

Curriculum Vitae

Name: Mollie Manley

Post-secondary Education and Degrees:
The University of Western Ontario
London, Ontario, Canada
2014-2018, Honours BHSc, Health Science

The University of Western Ontario
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2018-2020 MSc, Integrative Bioscience, Kinesiology

Honours and Awards:
The Ontario Graduate Scholarship
2019-2020
Kinesiology Graduate Student Conference Travel Award
2019
The Western Graduate Research Scholarship
2018

Related Work Experience
Teaching Assistant
The University of Western Ontario
2018-2020
Research Volunteer
R. Samuel McLaughlin Exercise and Pregnancy Laboratory
The University of Western Ontario
2016-2017

Publications:
TS Nagpal, H Prapavessis, C Campbell, B de Vrijer, R Bgeginski, K Hosein, S Paplinske, **M Manley**, MF Mottola (2019). "Sequential Introduction of Exercise First Followed by Nutrition Improves Program Adherence During Pregnancy: A Randomized Controlled Trial," International Journal of Behavioral Medicine. https://doi.org/10.1007/s12529-019-09840-0

Scientific Presentations:
M Manley, TS Nagpal, MF Mottola. "An exploratory analysis of dietary patterns in pregnant women," "Canadian National Perinatal Research Meeting," Calgary, Alberta, February 12-15, 2020.

M Manley, MF Mottola. "Assessing Emotion Regulation in Infants of Women Receiving Three Different Diet and Exercise Interventions in Pregnancy: A Research Proposal," "Child Health Symposium," London, Ontario, May 10, 2019.

M Manley, TS Nagpal, MF Mottola. "A Food Frequency Questionnaire to Determine Dietary Patterns in Pregnant Women: A Pilot Study," "6th Canadian Obesity Summit," Ottawa, Ontario, April 23-26, 2019.