

INFLUENCE OF PRENATAL NUTRITION AND
SUPPLEMENTATION ON BIRTH OUTCOMES AND
NEGATIVE AFFECTIVITY IN INFANTS

by

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A THESIS

Presented to the Department of Human Physiology
and the Robert D. Clark Honors College
in partial fulfillment of the requirements for the degree of
Bachelor of Science

June 2021

An Abstract of the Thesis of

Hailey Volk for the degree of Bachelor of Science
in the Department of Human Physiology to be taken June 2021

Title: Influence of Prenatal Nutrition and Supplementation on Birth Outcomes and
Negative Affectivity in Infants

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Excessive inflammation during pregnancy can exert powerful effects on the developing fetus by altering embryonic, fetal, and placental growth and development, predisposing the fetus to adverse birth outcomes and long-term health complications. An anti-inflammatory diet and proper prenatal supplementation could be a promising avenue to combat the inflammatory state pregnancy induces, particularly in obese women. However, there is a lack of data linking maternal environmental mediators of inflammation, such as diet, to birth outcomes and behavior in offspring. We examined the association among prenatal nutrition during the 22nd and 37th week of gestation and birth outcomes, as well as negative affect in infants 1 month after birth, in a cohort of 55 mother-child pairs. We found pro-inflammatory diets in the 3rd trimester and throughout the duration of participants pregnancy, as measured by the Dietary Inflammatory Index (DII), were associated with higher APGAR scores at 5-minutes. Maternal under-supplementation of DHA and EPA and increased iodine supplementation during the 2nd trimester was associated with higher APGAR scores at 5 minutes, while increased iodine supplementation in the 3rd trimester was associated with lower APGAR scores at 5 minutes. Further, increased folic acid supplementation in the

3rd trimester was associated with higher levels of infant negative affectivity 1-month post-partum. This data will add to the broader literature surrounding prenatal diet and birth outcomes and expand it by including supplemental and post-natal behavioral considerations.

Acknowledgements

I would like to thank my thesis committee for their support and expertise throughout this process. Thank you to my primary advisor, Dr. Jennifer Ablow, for sharing her knowledge and challenging me to think critically about my research. Thank you to Dr. Elinor Sullivan, my second reader, for supporting my interests within the study and providing comprehensive critiques that helped me fully examine my topic and the possibilities of this project. Thank you to Dr. Melissa Graboyes, my Clark Honors College representative, for framing my research in a broader context and her thought provoking questions. It was an honor to be advised and mentored by such an amazing group of strong and outspoken women that are involved in novel research and inspire me to continue pursuing my goals.

I would also like to express gratitude to all the staff, volunteers, and participants involved in the Prenatal Environment and Child Health (PEACH) study at Oregon Health Sciences University (OHSU) for their unwavering support, kindness, and opportunity to pursue my passions within this project. I will be forever thankful for all the mothers and children that participated in this study and are expanding the scientific knowledge surrounding pregnancy and early childhood. I would also like to thank the National Institute of Health (R01MH117177) for the funding awarded to Dr. Elinor Sullivan and Dr. Joel Nigg that made this research possible.

Lastly, I would like to thank my family and friends for their support and encouragement throughout this entire thesis process and my undergraduate career.

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Glossary

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<i>Term</i>	<i>Definition</i>
<i>Adiposity</i>	Amount of bodily fat.
<i>Air displacement plethysmography</i>	A technique for measuring body composition that utilizes the volume and pressure of displaced air by the body when it is placed in a plethysmograph machine.
<i>APGAR Score</i>	Appearance, pulse, grimace, activity, and respiration assessment occurring 1 and 5 minutes immediately after birth. Provides a convenient and accepted method for reporting the status of newborns and can indicate the response to resuscitation if needed. Each category is rated from 0-2 and a score between 7-10 at 5 minutes are indicative of good health at birth.
<i>Cytokines</i>	Regulators of response to infection, immune responses, inflammation, and trauma in the body that can promote healing (anti-inflammatory) or make disease worse (pro-inflammatory).
<i>Dietary inflammatory index (DII)</i>	A method of assessing the inflammatory potential of an individual diets. Has been associated with concentrations of circulating cytokines (i.e. more pro-inflammatory has higher levels of pro-inflammatory cytokines).
<i>Gestation</i>	Period between conception to birth. A normal gestational period lasts between 38 and 42 weeks.
<i>Macrophage</i>	A type of white blood cell that surrounds and kills microorganisms, removes dead cells, and stimulates the action of

	other immune cells through the release of chemical signals. Can have either pro-inflammatory or anti-inflammatory effects in the body but tend to express pro-inflammatory phenotypes in people with obesity.
<i>Mediterranean diet</i>	Diets higher in fruits, vegetables, fish, poultry and whole grain bread that are associated with lower levels of circulating pro-inflammatory cytokines
<i>Negative affectivity</i>	A broad behavioral term that can be summarized as feelings of emotional distress and is often defined by feelings of anxiety, sadness, fear, guilt, etc.
<i>Neural tube defects (NTD's)</i>	Severe birth defects of the brain and spine that occurs when the neural tube does not close properly. The 2 most common are spina bifida and anencephaly (a brain defect).
<i>Neutrophils</i>	A type of white blood cell that form an essential part of the innate immune system and help fight infection.
<i>Perinatal</i>	Pertaining to the period immediately before and after birth, roughly starting between the 20 th and 28 th weeks of gestation and ending 1 to 4 weeks after birth.
<i>Postnatal</i>	Period occurring after birth, often used in reference to the newborn.
<i>Preeclampsia</i>	A potentially dangerous pregnancy complication that is characterized by high blood pressure and signs of damage to organ systems that usually begin after the 20 th week of

pregnancy. When untreated, preeclampsia can be fatal and create harmful conditions for both the mother and fetus.

Size for gestational age Categorical division of newborn size based on birthweight and gestational age. Small for gestational age (SGA) are infants below the 10th percentile in weight for their gestational age and large for gestational age (LGA) are infants above the 90th percentile in weight for their gestational age.

Western diet Pro-inflammatory diet commonly consumed in the United States that is defined by a diet that is rich in red meat, high in fat, and high in simple carbohydrates. The western diet is associated with higher levels of circulating pro-inflammatory cytokines in normal populations.

Introduction

According to accumulating evidence and the developmental origin of health and disease hypothesis, during pregnancy variation in nutrition, maternal body size, and exposure to toxins can exert powerful effects on the developing fetus that impacts the child's lifelong health (Langley-Evans & McMullen, 2010). These factors alter embryonic, fetal, and placental growth and development, predisposing the fetus to more adverse birth outcomes and long term health complications like cardiovascular disease, obesity, type 2 diabetes, and cancer (McCullough et al., 2017). The interplay of various maternal environmental exposures during gestation posits a public health importance for moderating potential long-term health and development consequences during the embryonic and postnatal period. A potential avenue to improve fetal outcomes and development is through the reduction of maternal inflammation during gestation.

This project aims to evaluate the extent to which poor maternal nutrition, independently or in combination with increased maternal adiposity, predicts birth outcomes for both the mother and the infant. Additionally, I will examine how changes in the *in-utero* environment induced by poor nutrition, independently or in combination with increased maternal adiposity, programs altered infant behavior, in particular negative affectivity, 1-month after birth. Within these two aims, I hypothesize that fetal developmental exposure to a pro-inflammatory diet and increased adiposity during gestation, increases *in-utero* exposure to inflammation, leading to more adverse birth outcomes (e.g. delivery type, gestational age, birth weight, etc.). Additionally, fetal exposure to improper prenatal supplementation and increased adiposity causes nutritional deficiencies during gestation, leading to more adverse birth outcomes. I also

hypothesize that fetal exposure to a pro-inflammatory diet and increased maternal adiposity during gestation leads to early indices of neurodevelopmental problems, such as higher levels of negative affect and regulatory problems in infants 1-month post-partum. Also, improper supplementation and increased maternal adiposity during gestation leads to early indices of neurodevelopmental problems, such as higher levels of negative affect and regulatory problems in infants 1-month post-partum.

Background

Obesity and Pregnancy

Metabolic and physiologic changes that occur during pregnancy induces the body into an inflamed state and has the potential to compound inflammation that also occurs due to obesity. Inflammation occurs as an attempt by the body to repair itself following an infection or injury that alters bodily homeostasis (Segovia et al., 2014). The body uses immune cells (macrophages, B-cells, T-cells) to repair itself and coordinates these cells using cytokines, a type of inflammatory signaling molecule (Libby, 2008). Cytokines can be either pro- or anti-inflammatory and commonly studied pro-inflammatory cytokines are interleukin (IL)-6, IL-8, tumor necrosis factor (TNF)- α , and C-reactive protein (CRP) (Dinarello, 2000). Inflammation is important for protecting the body against pathogens and injury; however, it can also contribute to the progression and continuation of many chronic diseases. Obesity is an example of this through the induction of chronic, low-grade inflammation in the body (Schmatz et al., 2010). As body mass index (BMI) increases, the amount of fat, or adipose tissue, in the body increases accordingly. It is known that there is increased concentrations of pro-inflammatory cytokines (IL-6, IL-8, TNF- α , and CRP) in people with obesity, indicating that inflammation is above normal in this population (van der Burg et al., 2016). The underlying mechanism is that as the amount of adipose tissue increases, adipocytes (fat cells) release an increased number of pro-inflammatory cytokines and growth factors, called adipokines, that systemically affect the endocrine environment by inducing the body to release macrophages and begin tissue necrosis (Schmatz et al., 2010). This process becomes cyclic where more pro-inflammatory mediators are

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released, perpetuating a chronic state of inflammation in the body (Schmatz et al., 2010).

Due to the prevalence of obesity among pregnant women, it is essential to understand how the chronic inflammation of obesity couples with the already pro-inflammatory state of pregnancy and other environmental factors. In the United States, obesity (body mass index of 30 and above) is the most common adverse condition among pregnant women and occurs in almost a third of pregnancies (Catalano & Shankar, 2017). In obese women, fetal anomalies and deviations in fetal growth rates are more common, suggesting the maternal adiposity (percent fat) during gestation can affect development throughout pregnancy (King, 2006). Maternal inflammation creates a modified *in-utero* environment, increasing the risk of experiencing costly complications like miscarriages, gestational hypertension, preeclampsia, caesarean section, longer postpartum hospital stays, and gestational diabetes during pregnancy and, in turn, placing the child at risk for future health and development complications (Catalano & Ehrenberg, 2006).

Obesity and pregnancy contribute to levels of inflammation individually; however, the mechanism of this is still unknown. It is believed that during pregnancy, the maternal immune system is altered significantly, increasing the concentrations of macrophages, neutrophils, and serum cytokines (Schmatz et al., 2010). This increased maternal immune response is believed to be an evolutionary mechanism that assists in aborting unhealthy pregnancies or allowing healthy ones to progress (Schmatz et al., 2010). As the pregnancy progresses and the placenta grows and develops, higher concentrations of immunosuppressant factors are seen (progesterone and IL-10) to

ensure that the maternal immune system does not see the placenta as a foreign invader (Redman et al., 1999). The immune system response must maintain a delicate balance between an enhanced immune response and immunosuppressive factors to ensure a healthy maternal-placental interaction throughout gestation (Schmatz et al., 2010). This delicate balance is altered by the increased inflammatory response to increased maternal adiposity and can create harmful conditions for both the mother and fetus (Figure 1). Higher concentrations of pro-inflammatory cytokines has been associated with multiple pregnancy complications such as: premature fetal membrane rupture, premature labor, hypertension, and preeclampsia (Pieczyńska et al., 2020). For example, preeclampsia, a serious pregnancy complication that is characterized by hypertension, coagulation abnormalities, and a decrease of uteroplacental blood flow, is more common among obese women during gestation (Redman et al., 1999). It is believed that this occurs due to increased levels of CRP, a pro-inflammatory cytokine, and other circulating cytokines that interfere with placentation, the formation and implantation of the placenta in the uterus, predisposing the body to preeclampsia (Schmatz et al., 2010). As pregnancy already induces a state of inflammation in the body, the interplay between obesity, pregnancy, diet, and the developing fetus could give insight on how to reduce costly complications and adverse developmental outcomes.

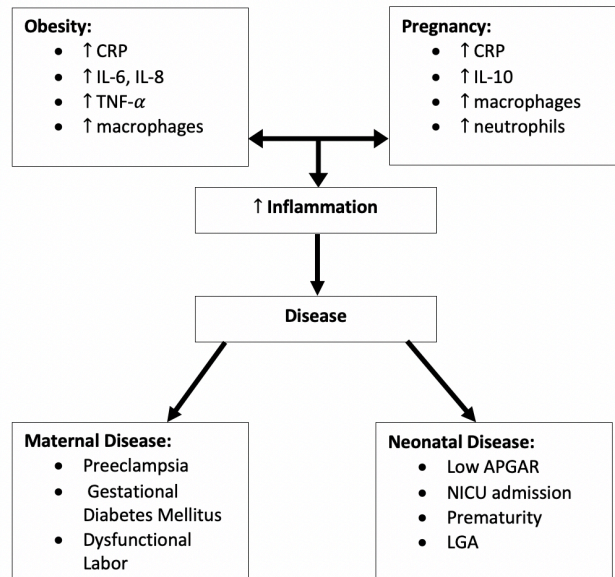


Figure 1: Associations among obesity, pregnancy, inflammation, and disease. Adapted from Schmatz et al. (2010).

Both pregnancy and obesity cause an increased immune response, as shown by increasing levels of pro-inflammatory cytokines and immune cells. These effects are additive and result in an overall higher inflammatory state for the obese mother that can lead to more adverse birth outcomes for both the mother (e.g. preeclampsia, gestational diabetes mellitus, etc.) and the child (e.g. low APGAR, NICU admission, etc.).

Diet

Maternal diet during gestation could impact fetal development via inflammatory pathways. The link between diet and inflammation is well established in non-pregnant populations and the ability for environmental dietary sources of inflammation to couple with the inflammatory state that pregnancy induces, suggests that this link persists in this population as well (Esmailzadeh et al., 2006). In the United States, the “Western-

style” diet that is consumed is rich in red meat, high in fat, and high in simple carbohydrates, all of which may contribute to the increasing concentration of inflammatory cytokines (Pieczyńska et al., 2020). This Western diet has been associated with higher levels of circulating concentrations of CRP and IL-6 in nonpregnant adults, but this link has yet to be explored in pregnant populations (Sen et al., 2016). Diets higher in fruits, vegetables, fish, poultry and whole grain bread are classified as a “Mediterranean diet” and typically those who consume these diets have a lower concentration of CRP (Lopez-Garcia et al., 2004). Diets high in specific nutrients like omega-3 fatty acids, fiber, vitamin E, vitamin C, β -carotene, and magnesium have all been associated with lower levels of inflammation, obesity, and cardiovascular disease (Sen et al., 2016). The role that diet and nutrition have on inflammatory markers in nonpregnant individuals suggests that the same relationship could exist among maternal diet, pregnancy, and birth outcomes due to the ability of the *in-utero* environment to contribute substantially to the growth and development of the fetus.

A common way to assess the inflammatory potential of an individual’s diet is using the Dietary Inflammatory Index (DII), which assesses a person’s diet in terms of pro- and anti-inflammatory foods that are eaten and generates an overall inflammatory score (Shivappa et al., 2014). The DII is known to be an accurate predictor of the level of multiple inflammatory markers, including CRP, IL-10, TNF-alpha, and IL-6 (Pieczyńska et al., 2020). Current research has found that the DII has been associated with levels of CRP in pregnant populations, but the concentrations of other cytokines has not yet been established (McCullough et al., 2017). By utilizing the DII, we can examine the impact of the Western diet on pro-inflammatory cytokine concentrations in

pregnant women and identify the impact of dietary choices on growth and development during gestation and beyond.

Supplementation

Many metabolic changes occur in pregnancy like increased metabolic rate, lower insulin synthesis, increased lipolysis, and increased blood volume to help nourish and grow the fetus (Lowensohn et al., 2016). Most women take prenatal supplements to help fill this new metabolic demand. These supplements are high in nutrients and vitamins to create an *in-utero* environment that maximizes fetal development and maternal adaptation to a state of pregnancy (Christian et al., 2015). The recommended levels of various supplements and nutrients is well known, however, the interaction between supplementation and the diet consumed during gestation is not well understood in terms of birth outcomes.

Even though supplementation during pregnancy is common practice, the composition of prenatal vitamins is often variable. The National Health and Nutrition Examination Survey (NHANES) is a national representative, cross-sectional survey of US citizens conducted by the CDC to determine nutritional information, releasing data in 2 year cycles (NHANES, 2021). This survey gives insight to the average nutritional behaviors of pregnant women in the US, specifically their consumption of various micronutrients from both dietary and supplement sources. According to a study conducted in 2013 using NHANES data, 75% of pregnant women reported using prenatal supplements (Branum et al., 2013). However, this high percentage can be deceiving due to the variable nutrient content and nutritional composition in dietary supplements, many of which are not standardized. It is essential that pregnant women

receive adequate micronutrient intake during pregnancy so harmful conditions due to under or over-supplementation are prevented. Based on an examination of NHANES data from 2001-2014, 10% of pregnant women were at risk for inadequate intake of thiamin, vitamin B₁₂, and vitamin B₆, while only 8% were meeting recommended amounts of choline intake (Bailey et al., 2019). It was also found that 33.4% pregnant women exceeded the upper limit for folic acid consumption, due to over-supplementation (Bailey et al., 2019). The robust set of data from NHANES surveys show a clear misuse of supplementation among most pregnant women, calling for a reevaluation of prenatal supplementation in the US that takes both contemporary nutritional composition and dietary supplementation in account during this critical developmental period.

From fetal development to early childhood, exposures occurring *in-utero* and during the postnatal period, have a significant effect on lifelong health and wellbeing (Procter & Campbell, 2014). This emerging perspective emphasizes how critical creating an optimal *in-utero* environment is, not only for the prevention of adverse birth outcomes, but also for promotion of mental and physical health for the entirety of the offspring's life. My project will add to the expanding literature of the effects of micronutrient deficits on the regulatory abilities of infants 1-month post-partum by examining the nutritional and supplement intake throughout the pregnancy of the mother. The developmental importance of the micronutrients I examine for my project are summarized in Table 1. The subset of micronutrients that I have selected have well known impacts on the neurodevelopment of the fetus and can be found in most commercial prenatal supplements.

Table 1: Recommended micronutrient supplementation during pregnancy from the American College of Obstetrics (ACOG) and American Pregnancy Association, including the developmental importance of the nutrient (Nutrition During Pregnancy, n.d.; “Pregnancy Vitamins and Nutrients,” 2012; Hovdenak & Haram, 2012; Greenberg et al., 2008).

<i>Micronutrient</i>	<i>Dosage</i>	<i>Developmental Impact</i>
Iodine	220 mcg	Essential for healthy brain development
Choline	450 mg	Important for development of fetus’s brain and spinal cord
B6	1.9 mg	Maintains nervous system
B12	2.6 mcg	Helps prevent birth defects of the brain and spine
Folic Acid	400-800 mcg*	Supports general growth and development of fetus and placenta
Thiamine (B-1)	1.4 mg	Essential for metabolic and cellular function, especially within the developing brain

DHA	200 mg	Essential for neurodevelopment
DHA+EPA	300 mg	Combination increases utilization of DHA

*For folic acid supplementation, any amount between 400 to 800 mcg is typically safe for pregnancy.

Iodine

During pregnancy, Iodine is essential for the production of thyroid hormones and is found in most prenatal vitamins (Zhou et al., 2013). Thyroid hormones are important for ensuring proper growth and development of the fetus and severe deficiency is the cause of congenital hypothyroidism, stunting both growth and mental ability (Melse-Boonstra et al., 2012). This deficiency can disrupt fetal neurogenesis, neuronal migration, synaptogenesis, and myelination, all of which are essential for proper brain development and function that can impact the fetus long after birth (Vohr et al., 2017). Iodine plays a significant role in fetal brain development and is commonly under consumed globally, yet its effects have not yet been adequately addressed. It is currently estimated that iodine deficiency ranks third on the list of causes that reduces children’s developmental potential, specifically their neurological development, illustrating how significant this micronutrient is during embryonic development (Walker et al., 2007). Even though iodine deficiency has commonly affected developing countries, it is on the rise in the United States, United Kingdom, and Australia, countries where iodine deficiency had previously under control (Zimmermann, 2009). In these countries, many foods are fortified with iodine (i.e. salt and dairy) as a means to increase dietary intake and reduce deficiencies (Zimmermann, 2009). However, the

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rise of commercially processed foods that commonly contain non-iodized salt and efforts to reduce salt intake due to concerns about cardiovascular health have contributed to the rising levels of iodine deficiency in the United States (Pearce, 2015).

Choline

Another important micronutrient to consume during pregnancy is choline, responsible for structural integrity and signaling functions of cell membranes (Rajarethnem et al., 2017). It also plays a large role in neurotransmission, neural tube closure, hippocampal development, signaling to neurons and liver cells, and overall metabolism (Rajarethnem et al., 2017). Because of choline's significant role in many bodily processes, it is vital that it is consumed during gestation in order to support fetal brain development. In animal models, it has been found that perinatal supplementation of choline enhances spatial and temporal cognition across the rodents lifespan and its supplementation during pregnancy causes a significant increase in the number of hippocampal neurons (Rajarethnem et al., 2017). Despite the known benefits of choline supplementation during gestation for fetal brain development, it is estimated that only 8.5% of women in the US meet the adequate intake of choline during pregnancy (King et al., 2019). The pervasive under supplementation in human pregnant populations calls for the investigation of its impacts on the fetal brain and development throughout childhood.

Vitamin B6

Pyridoxine, commonly known as vitamin B6, is another micronutrient essential for fetal development given its role in protein metabolism in the developing central nervous system (CNS) of the fetus (Hovdenak & Haram, 2012). In the United

States, B6 is commonly found in many foods like fortified cereals, potatoes, bananas, meat, poultry, and fish (Dror & Allen, 2012). For the Western diet, it is estimated that approximately 75% of B6 is bioavailable, meaning that only 75% of the dietary B6 we consume can be actively used by our bodies (Dror & Allen, 2012). During pregnancy, however, B6 consumption from diet alone is no longer sufficient and supplementation is required, having a bioavailability of approximately 90% (Dror & Allen, 2012). In animal models, offspring that experienced B6 deficiency during the prenatal period experienced higher rates of lower body weight, skeletal defects, convulsions, and impaired motor development (Aycock & Kirksey, 1976). In humans, fetal concentrations of B6 are higher than B6 concentrations in the mother from the second trimester until birth and this sequestration suggests its importance in fetal development (Shane & Contractor, 1975). After birth, B6 is further supplemented to the developing infant via breast milk and continues to increase throughout the duration of lactation, further demonstrating the importance of this micronutrient prenatally and perinatally (Sakurai et al., 2005).

Vitamin B12

Another relevant micronutrient during gestation is cobalamin, B12, which is naturally occurring in most animal products (Dror & Allen, 2012). During gestation, maternal metabolism prioritizes B12 availability for the fetus and it is concentrated in the placenta and is transferred to the fetus down a concentration gradient instead of becoming stored in the maternal liver (Dror & Allen, 2012). Multiple case studies have demonstrated the effect of severe vitamin B12 deficiency on the fetal brain, commonly seen in middle and low-income countries, causing

neurological deficiencies impacting the CNS, like impaired motor development, demyelination, and, in severe cases, brain atrophy (Black, 2008; Chandyo et al., 2017). In addition, maternal deficiency has been linked to increased risk of common pregnancy complications like low birth weight, spontaneous abortion, neural tube defects (NTDs), and more (Chandyo et al., 2017). In cases of moderate deficiency, little is known about the consequences of maternal deficiency on fetal brain myelination, neurodevelopment, and cognitive function of infants postnatally (Chandyo et al., 2017). In the United States and other developed countries where severe B12 deficiencies are more unlikely, it is critical to understand how moderate B12 deficiencies impact development of the fetus and into infancy.

Folic Acid

One of the most commonly discussed micronutrients during pregnancy is folic acid, a nutrient that is crucial for proper early brain development and prevention of NTDs (Gao et al., 2016). In North America, most of our diet contains folic acid through food fortification and we consume an average daily amount of 138 micrograms (Chitayat et al., 2016). However, as previously mentioned, during pregnancy metabolic demand for many nutrients increase to levels that cannot be met by diet alone and supplementation needs to occur. For folic acid specifically, the high recommended value stems from fetal demands and it is actively transported into the placenta, concentrating it in the fetal brain (Gao et al., 2016). It has been found that women who are taking prenatal multivitamins that contain folic acid are associated with a decreased risk of several congenital anomalies, beyond just NTDs (Ingrid Goh et al., 2006). This includes protecting the fetus and infant from impaired neurodevelopment, including

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autism spectrum disorders (ASD) in children and an overall improvement in cognitive, intellectual, and motor function (Gao et al., 2016). Folic acid has the ability to foster neurodevelopment in the fetus and can continue to benefit neurodevelopment of the infant into childhood, indicating how essential proper supplementation of this micronutrient is.

Thiamine

Another important B vitamin during pregnancy is thiamine, an essential micronutrient for metabolic and cellular function, especially within the developing brain (Hovdenak & Haram, 2012). Thiamine is actively transported, via placental transport systems, and becomes concentrated in fetal blood at higher levels than maternal blood (Hovdenak & Haram, 2012). In animal models, thiamine deficiency causes several brain impairments, including cerebellar damage and memory impairment (Oliveira et al., 2007). The mechanisms of this damage are unknown, but it is postulated that thiamine deficiency during the prenatal and postnatal period could create disturbances of cellular differentiation, axonal growth, and synapses formation that could induce cellular deficits due to poor development of axons and synapses that are dependent on thiamine availability in the brain (Bâ et al., 2005). The effects of thiamine deficiencies on brain development during gestation have been well studied in animal models and these consequences can also be seen in human populations (Whitfield et al., 2018). The risks and consequences of thiamine deficiencies are highest in the first year of life due to exclusive breastfeeding where thiamine content is low in breastmilk and infants are unable to obtain adequate amounts of this micronutrient (Whitfield et al., 2018). Infant thiamine concentrations are further depleted due to the increased metabolic activity in

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the first few months of life, causing life-long developmental motor, language, cognitive delays (Fattal-Valevski et al., 2009).

Omega-3 Fatty Acids

One of the most well-known micronutrients supplemented during pregnancy is docosahexaenoic acid (DHA), an omega-3 fatty acid that is critical for fetal neurodevelopment (Rogers et al., 2013). Typically, omega-3's are consumed naturally from seafood; however, most pregnant women cannot get enough DHA from their diet due to recommended restriction of seafood consumption during pregnancy because of possible mercury exposure (Greenberg et al., 2008). The mechanism of how the fetus utilizes DHA is relatively well known. During the 3rd trimester, DHA is preferentially transported to the fetus, coinciding with the later stages of brain and retinal maturation (Rogers et al., 2013). When fetal DHA levels are inadequate, the body will begin to utilize omega-6 fatty acids as a replacement, which is associated with impaired or reduced visual and neurological function in animal models (Mulder et al., 2018). The utilization of DHA in the body can be mediated by eicosapentaenoic acid (EPA), another omega-3 fatty acid (Greenberg et al., 2008). During pregnancy, EPA can help selectively transport DHA into fetal circulation and decreases the amount of circulating pro-inflammatory cytokines (Greenberg et al., 2008; Mozurkewich et al., 2018). Pregnant women who are not consuming EPA along with DHA may limit the transport and uptake of DHA into fetal cells, making it extremely difficult to supply the fetus with the needed concentration of DHA to help support neurodevelopment (Greenberg et al., 2008). The impacts of improper DHA supplementation during gestation have long-lasting effects on neurodevelopment and it has been associated with

deficits in psychomotor development, reading skills, visual acuity, problem solving, and attention in children (Rajarethnem et al., 2017). The lasting consequences of DHA under supplementation during gestation and commonly low amounts of omega-3 consumption that is characteristic of the Western diet, calls for further studies to determine the mechanisms of DHA utilization during gestation and postnatally in human populations and the lasting effects of improper supplementation.

Negative Affectivity

When considering how nutritional intake and consumption during gestation relates to birth outcomes and early development of the infant, there is little literature exploring this relationship in human models. As previously stated, the role of specific supplements in neurodevelopment are well known, but their consequences on developmental outcomes of the fetus and infant are not well known, especially in cases of moderate deficiencies that are seen in the United States. In non-human primate models, adult female macaques fed a Western-style diet throughout their pregnancy, similar to the American contemporary diet, had post-partum effects on the newborn's developing regulatory system (Thompson et al., 2018). Specifically, negative behaviors of anxiety and fear (negative affectivity) in infants were associated with increased maternal adiposity and consumption of an inflammatory diet (Thompson et al., 2018). The proposed mechanism of this is that increased adipocyte mass causes the release of pro-inflammatory cytokines, increasing the overall *in-utero* inflammatory profile, altering fetal neurodevelopment in ways that influences long-term behavior and mood (Bilbo & Tsang, 2010). It has been demonstrated that a Western-style diet in non-human primate models has the capacity to induce long-term dysregulation in offspring

via increased reactive anxiety and defensive behaviors in response to fear and stress (Thompson et al., 2018). The potential capacity for the effect of a pro-inflammatory Western diet, increased adiposity, and improper supplementation to couple together to create adverse birth outcomes and impaired neurodevelopment could have potential long-lasting consequences for offspring in human models.

This study aims to evaluate the influence of prenatal nutrition and supplementation, independently or in combination with increased maternal adiposity, on birth outcomes and levels of negative affectivity in 1-month old infants. I hypothesize that fetal exposure to a pro-inflammatory diet and increased adiposity during gestation will lead to more adverse birth outcomes (i.e. higher rates of cesarean deliveries, lower birth weights, smaller for gestational age, etc.) due to an increased *in-utero* exposure to inflammation. I also hypothesize that improper supplementation and increased adiposity will lead to more adverse birth outcomes through the creation of nutritional deficiencies of key micronutrients during gestation. Additionally, I hypothesize that fetal exposure to a pro-inflammatory diet, improper supplementation, and increased adiposity during gestation leads to early indices of neurodevelopmental problems, such as higher levels of negative affect and regulatory problems in infants 1-month post-partum.

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Methods

Participants

300 pregnant women and their offspring were recruited for this study from Oregon Health & Science University's (OHSU) prenatal clinics in the Portland metropolitan area, as well as other clinics in the greater Portland area who responded to PEACH Study recruitment materials. For my project, I have selected 55 participants from the larger sample due to their completion of all of the arms I am examining. Women recruited from OHSU clinics had their electronic medical records reviewed by study staff to determine if they were eligible for participation in the PEACH study. Participants that were recruited from OHSU clinics and non-OHSU sites were screened for eligibility by telephone. Upon consent, non-OHSU participants were asked to sign a HIPPA Authorization for Obtaining Existing Records Outside of OHSU form, giving PEACH Study Staff the ability to access and review the participant's non-OHSU medical records. This study was approved by OHSU's Institutional Review Board (#18579).

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Recruitment

For women giving birth at OHSU, the names of pregnant patients were obtained from the OHSU electronic health record software called Epic. These records were reviewed for eligibility requirements and the recruiter called, and/or emailed eligible patients to invite them to participate in the study. When a woman indicated interest in participation, a further screening followed via telephone, or at the clinic if preferred, to describe the study in greater detail, determine final eligibility, and confirm interest.

Eligibility

Pregnant women aged 18-40, in good health, aside from increased BMI, were eligible for the study. Because BMI will be treated as a continuous risk factor for inflammation on development, we did not specifically recruit obese or overweight individuals. Instead, we monitored the sample to ensure that rates of obese or overweight individuals were consistent with national averages. Participants were pre-screened and excluded if their pregnancies were deemed high risk due to multiple gestation, medical conditions (type 1 or 2 diabetes, mellitus, cancer, hypertension, kidney disease, and epilepsy), the presence of infection (HIV, hepatitis C), three miscarriages, or if the fetus had documented congenital anomalies or genetic conditions such as Down syndrome at chart review prior to recruitment outreach. At screening contact, expectant women were screened and excluded if they reported: smoking (> 5 cigarettes during pregnancy), drinking alcohol (>10 drinks during pregnancy), marijuana use (currently legal in Oregon), and reported illicit drug use. Participants could also be excluded if they were enrolled in another study that involves an intervention. During the pregnancy follow-up period, women who develop gestational diabetes, preeclampsia, or test positive on a urine drug screen at either prenatal visit were excluded. Children born at less than 35 weeks were also excluded. Once recruited and enrolled into the study, all participants were studied at OHSU.

Consent Process

If a woman qualified and agreed to participate, study staff asked her to sign the consent and authorization form, at either the screening visit or at her next prenatal visit. The participant was then scheduled for her first study visit, typically within a 2-week

window of 22 weeks of pregnancy. The participant could also request to sign and return the consent and authorization form by mail and complete the informed consent over the phone. The consent process explained that participation remained voluntary, that families can withdraw at any point in the study without penalty, participation had no effect on eligibility for medical or clinical care, research information will be confidential, and that they would be informed regarding the limits of confidentiality. Confidentiality was limited due to a partial HIPPA Waiver that was submitted in order to collect protected health information during the phone screen such as: date of birth, sex, names, address, phone numbers, email address, medication status, primary household income, history of previous diagnoses and previous head injuries. This information was collected after receiving verbal consent over the phone and participants hold the right to not give the study their verbal consent and may refuse to answer any of the phone screen questions. All risks and benefits were clearly explained, and time was taken to clarify any questions that participants may have had.

Compensation for Participation

Participants were compensated for every measure they complete and could receive up to \$550 for participating in the study.

Prenatal Visit Schedule and Participant Activity

Prenatal assessments occurred around the 22nd week of gestation and again around the 37th week of gestation, coinciding with their second and third trimesters of pregnancy. Visits occurred at OHSU and were scheduled according to the woman's convenience. A summary of the measures that I will be using for my project can be found in Figure 2.

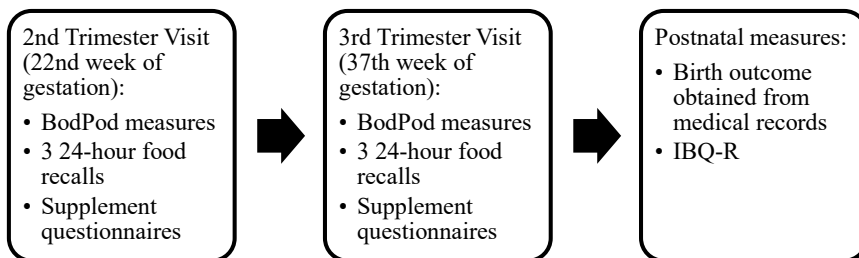


Figure 2: Summary of timing study measurements completed at participant visits and postnatally.

Maternal Prenatal Measures

Maternal Body Composition (pre-pregnancy, 22 and 37 weeks gestation)

Maternal body composition was measured at multiple time points. First, maternal pre-pregnancy weight was obtained from participants' electronic medical records. During pregnancy, participants' weight was measured again at 22 and 37 weeks of gestation. Weight was measured with a digital scale (Scale-Tronix, Model 5002), height with a Harpenden stadiometer, and skinfold thickness at the bicep, tricep, subscapular, and suprailiac areas was measured with a Lange caliper. Percent body fat, fat mass and lean mass was directly measured using air displacement plethysmography using a BodPod. The BodPod uses the Siri equation to calculate fat mass (FM) based on the weight and density measurements taken from the BodPod (van Raaij et al., 1989). An algorithm was developed by van Raaij, et al (1989) to account for alterations in fat mass hydration and density that occur during gestation and was used to alter the FM measurements from the BodPod. All equations can be found in Table 2 (van Raaij et al., 1989).

Table 2: Equations used to calculate maternal FM(kg)¹.

Siri's	$W/100 \times (\frac{495}{D} - 450)$
Adjustment for 24 weeks gestation	$W/100 \times (\frac{504.3}{D} - 460.4)$
Adjustment for 36 weeks gestation	$W/100 \times (\frac{516.3}{D} - 473.7)$

¹W, weight (kg); D, body density (kg/m³).

Maternal Prenatal Nutrition (22, 37 weeks gestation)

At each time point, three unannounced 24-hour food recall phone interviews were conducted by trained nutritionists regarding food intake the day prior. The three interviews occur within two weeks of each time point (one weekend and two weekdays). All interviewers completed a training program and met qualification standards established in the OCTRI Bionutrition Unit using the Nutrition Data System for Research (NDSR) software (Nutrition Coordinating Center (NCC), University of Minnesota). The software facilitates the collection of recalls in a standardized fashion in a multiple-pass interview approach. The three recall days were averaged together for each time point for analysis. A Dietary Supplement questionnaire was completed to identify use of dietary supplements and non-prescription antacids.

Dietary Inflammatory Index (DII)

The DII was developed by researchers at the University of South Carolina to assess the inflammatory aspects of a diet and has been described in depth elsewhere. Briefly, the DII was developed by reviewing scientific literature published through 2010

for associations between food components, or “parameters”, and specific inflammatory markers. Each article was given a score between 1 and -1 based on the effect of the food parameter on inflammation to create a database of 45 parameters. A score of +1 indicates a maximally pro-inflammatory relationship, 0 indicates no significant relationship, and -1 indicates a maximally anti-inflammatory relationship.

Based on the data gathered from the 24 hour food recalls conducted at the 22nd and 37th week gestation visits, 30 food parameters (alcohol, vitamin B-12, vitamin B-6, β -carotene, caffeine, carbohydrate, cholesterol, energy, total fat, fiber, folic acid, iron, magnesium, mono-unsaturated fat, niacin, omega-3 fatty acids, omega-6 fatty acids, protein, poly-unsaturated fat, riboflavin, saturated fat, selenium, thiamin, trans-fat, vitamin A, vitamin C, vitamin D, vitamin E, and zinc) were selected and used for calculations. The individual dietary data of the participants was transformed into a z-score by using the parameter specific global mean and standard deviation provided by the DII database. Since dietary data is often “right skewed”, the z-score was converted to a centered percentile score to minimize this effect. The centered percentile score was then multiplied by the respective inflammatory effect score to obtain the food parameter specific DII score. The individual food parameter scores were summed to calculate an overall DII score for every participant in the study at each of the visits and their scores for both trimesters were developed to generate an overall pregnancy average DII score.

Measures After the Child is Born

Medical records were reviewed to determine delivery health measures including delivery type, child gestational age, birth weight, gender, and perinatal complications. Size for gestational age was determined using a widely-used continuous reference

measures of birth weight for gestational age that were created using data from millions of US births to generate size percentiles (Oken et al., 2003). We obtained the Appearance, Pulse, Grimace, Activity, Respiration (APGAR) score to assess newborn health. 1 month after their baby's birth, participants completed questionnaires asking about their infant's behavior. Specifically, the Revised Infant Behavior Questionnaire (IBQ-R) was used to assess levels of negative affect in the infant. The IBQ-R is a widely used questionnaire that assess infant temperament along 14 different scales, such as smiling and laughter, activity level, fear, soothability, and cuddliness (Gartstein & Rothbart, 2003). Temperament is defined as the individual differences in reactivity (i.e. arousability of motor, emotional, and attentional response) and self-regulation (processes that regulate reactivity). To evaluate dysregulation in the infant, I examined the IBQ-R mean scores for negative affectivity and the categories used to calculate negative affectivity scores (Figure 3). Negative affectivity is calculated using total scores from the assessment of fear, distress to limitations, falling reactivity, and sadness, the definitions of which can be found in Figure 3.

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Commented [JA14R13]: I think at least make clear what scales comprise your measure of dysregulation, with a short definition of each. You can find the short definitions here: <https://research.bowdoin.edu/rothbart-temperament-questionnaires/instrument-descriptions/the-infant-behavior-questionnaire/>

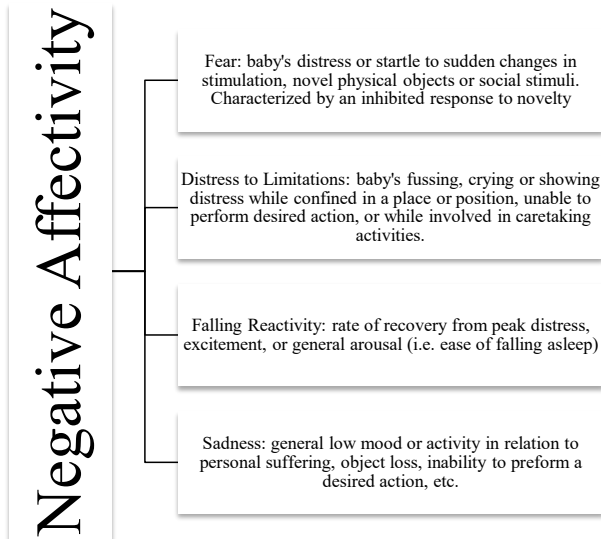


Figure 3: IBQ-R calculation of negative affectivity in infants.

After the IBQ-R is completed, negative affectivity is calculated using the means from the evaluations of fear, distress to limitations, falling reactivity, and sadness.

Definitions provided from Enlow et al. (2016).

Data Analysis

SPSS version 27 software was used for all data analysis. Prior to analysis, categorical variables were coded in order to better analyze the data sets. An example of the coding of categorical variables can be found in Table 3. For all categorical variables that were compared to quantitative data, one-way ANOVAs were used with an alpha of 0.05 and confidence interval (CI) of 95%. If significance was found, a Tukey's honestly significant difference (HSD) post hoc test was used to determine where the statistically significant difference occurred due to the multiple categories of the independent variables. When both the independent and dependent variables were categorical, a Chi² test was ran to determine significance. For the data sets where both variables were

quantitative, a linear regression model was used with an alpha of 0.05 and confidence interval (CI) of 95%.

Table 3. Example of how categorical variables were coded.

Categorical Variable	Coding
Infant Sex	0=female 1=male

Results

Overall, the average DII during pregnancy in this sample was -0.92 ± 1.34 with a range of -3.45 to 2.08 . There was no statistically significant relationship between BMI and FM during the 2nd and 3rd trimester and DII score ($p > 0.05$). Maternal participant characteristics and demographics can be found in Table 4. A higher level of maternal education was associated with lower DII scores (more anti-inflammatory) for the 2nd trimester ($F(4,50) = 3.225$, $p = 0.02$) and their pregnancy average score ($F(4,50) = 2.641$, $p = 0.044$).

Table 4: Maternal participant characteristics according to pregnancy average DII in quartiles.

	Total (n = 55)	DII Quartile (Pregnancy Average)			
		Q1 (n = 14)	Q2 (n = 13)	Q3 (n = 14)	Q4 (n = 14)
Pregnancy average DII	-0.92 ± 1.34	-2.45 ± 0.50	-1.49 ± 0.32	-0.70 ± 0.32	0.91 ± 0.72
DII range	$-3.45, 2.08$				
Age at enrollment	32.79 ± 4.15	34.24 ± 2.84	30.95 ± 3.61	33.14 ± 4.31	32.70 ± 5.22
2nd trimester BMI category, kg/m²					
18.5 to <25	21 (38%)	5 (36%)	6 (46%)	4 (28%)	6 (43%)
25 to <30	25 (45%)	5 (36%)	6 (46%)	5 (36%)	7 (50%)
≥ 30	9 (16%)	4 (28%)	1 (8%)	5 (36%)	1 (7%)
3rd trimester BMI category, kg/m²					
18.5 to <25	11 (20%)	2 (14%)	5 (38%)	1 (7%)	3 (21%)
25 to <30	31 (56%)	7 (50%)	7 (54%)	8 (57%)	9 (64%)
≥ 30	13 (24%)	5 (36%)	1 (8%)	5 (36%)	2 (15%)
Highest Level of Maternal Education					
Some College	5 (9.1%)	0	1 (7.7%)	1 (7.1%)	3 (21%)
Associates Degree	3 (5.5%)	1 (7.1%)	0	0	2 (14%)
Bachelor's Degree	26 (47%)	9 (64%)	7 (54%)	6 (43%)	4 (28%)
Graduate Degree	20 (36%)	4 (28%)	5 (38%)	7 (50%)	4 (28%)
Doctorate	1 (1.8%)	0	0	0	1 (7.1%)
Marital Status					
Never Married	7 (13%)	2 (14%)	1 (7.7%)	2 (14%)	2 (14%)
Married	48 (87%)	12 (86%)	12 (92%)	12 (86%)	12 (86%)
Household Income					
<\$25,000	1 (1.8%)	0	0	1 (7.1%)	0
\$25,000-\$49,999	3 (5.5%)	1 (7.1%)	0	0	2 (14%)
\$50,000-\$100,000	19 (34%)	6 (43%)	7 (54%)	2 (14%)	4 (28%)
>\$100,000	32 (58%)	7 (50%)	6 (46%)	11 (78%)	8 (57%)
Maternal Ethnicity					
Hispanic	2 (4%)	0	0	0	2 (14%)

Non-Hispanic Maternal Race	53 (96%)	14 (100%)	13 (100%)	14 (100%)	12 (86%)
American Indian/ Alaska Native	3 (5.1%)	1 (7.1%)	0	1(7.1%)	1 (6.6%)
Asian/East Indian	3 (5.1%)	0	2 (14%)	1 (7.1%)	0
Black/African American	1 (1.7%)	1 (7.1%)	0	0	0
White/Middle Eastern	51 (88%)	12 (86%)	13 (86%)	12 (86%)	14 (93%)
Method of delivery					
Vaginal	35 (64%)	7 (50%)	11 (85%)	7 (50%)	10 (71%)
Cesarean	20 (36%)	7 (50%)	2 (15%)	7 (50%)	4 (29%)

Data presented are means \pm standard deviations (SDs) or n (%). Q, quartile. A BMI of 18.5 to less than 25 is considered normal, 25 to less than 30 is overweight, and 30 or greater is considered obese. Data is presented in quartiles only for visualization and analysis was conducted using the total ($n = 55$) sample.

Infant characteristic and demographics can be found in table 5. There was a statistically significant relationship between birth length and infant sex ($F(1,53) = 4.388, p=0.041$), where males had significantly larger birth lengths (51.67 ± 1.92) than females (50.52 ± 2.03).

Table 5: Infant characteristics and demographics according to pregnancy average DII quartiles.

	Total ($n = 55$)	DII Quartile (Pregnancy Average)			
		Q1 ($n = 14$)	Q2 ($n = 13$)	Q3 ($n = 14$)	Q4 ($n = 14$)
Child Ethnicity					
Hispanic	2 (4%)	0	1 (8%)	0	2 (14%)
Non-Hispanic	53 (96%)	14 (100%)	12 (92%)	14 (100%)	12 (86%)
Child Race					
American Indian/ Alaska Native	2 (3.3%)	0	0	1 (7%)	1 (5.8%)
Asian/East Indian	6 (10%)	1 (7%)	3 (20%)	1 (7%)	1 (5.8%)
Black/African American	2 (3.3%)	1 (7%)	0	0	1 (5.8%)
White/Middle Eastern	50 (83%)	12 (86%)	12 (80%)	12 (86%)	14 (82%)
Birthweight (kg)	3.44 ± 0.42	3.44 ± 0.43	3.38 ± 0.34	3.39 ± 0.54	3.53 ± 0.39
Discharge weight (kg)	3.26 ± 0.40	3.24 ± 0.40	3.22 ± 0.37	3.21 ± 0.49	3.38 ± 0.36
GA, wk	39.6 ± 1.1	39.86 ± 0.83	39.55 ± 1.12	39.59 ± 1.38	39.41 ± 1.06
BW/GA category					
SGA	3 (5.5%)	1 (7%)	1 (8%)	1 (7%)	0
AGA	49 (89%)	12 (86%)	12 (92%)	12 (86%)	13 (93%)
LGA	3 (5.5%)	1 (7%)	0	1 (7%)	1 (7%)
Birth length (cm)	50.98 ± 2.05	51.44 ± 1.63	50.92 ± 1.68	50.31 ± 2.38	51.24 ± 2.38

Head circumference (cm)	34.62 ± 1.56	34.31 ± 0.84	34.55 ± 2.46	34.43 ± 1.57	35.16 ± 0.94
Infant Sex					
Female	33 (60%)	9 (64%)	9 (69%)	8 (57%)	7 (50%)
Male	22 (40%)	5 (36%)	4 (31%)	6 (43%)	7 (50%)
APGAR at 1 minute	7.93 ± 1.67	7.07 ± 5.23	8.31 ± 0.63	8.21 ± 1.05	8.14 ± 1.61
APGAR at 5 minutes	8.87 ± 0.64	8.71 ± 1.14	8.85 ± 0.38	8.86 ± 0.36	9.07 ± 0.27
1-month IBQ Scores					
NA	3.01 ± 0.59	3.25 ± 0.57	2.82 ± 0.55	3.12 ± 0.55	2.84 ± 0.65
Sadness mean	3.04 ± 1.1	3.45 ± 1.22	2.69 ± 0.76	3.24 ± 1.16	2.74 ± 0.93
Distress to limitations mean	3.9 ± 0.92	4.16 ± 0.74	3.92 ± 0.98	3.94 ± 1.05	3.58 ± 0.88
Fear mean	1.96 ± 0.74	1.99 ± 0.72	1.74 ± 0.38	1.99 ± 0.86	2.09 ± 0.91
Falling reactivity mean	4.85 ± 0.97	4.60 ± 0.89	5.08 ± 0.88	4.68 ± 1.11	5.04 ± 0.97

Data presented are means ± standard deviations (SDs) or *n* (%). GA, gestational age;

BW/GA, birthweight for gestational age; SGA, small for gestational age; AGA, average

for gestational age; LGA, large gestational age; NA, negative affectivity; Q, quartile.

Data is presented in quartiles only for visualization and analysis was conducted using the total (*n* = 55) sample.

DII and Birth Outcomes

There was a statistically significant association between 3rd trimester DII and 5-minute APGAR score ($\beta=0.327$, $r=0.327$, $p=0.015$), where an increase in DII score (more pro-inflammatory) was associated with a higher APGAR score. This association was also seen between pregnancy average DII and 5-minute APGAR score ($\beta=0.276$, $r=0.276$, $p=0.041$). There were no statistically significant association between the DII and any other birth outcomes examined (Table 6).

Table 6: Significant results from linear regression for DII scores and birth outcomes.

DII Score	Birth Outcome	β Coefficient	Standard Error (SE)	P-value
3T DII	APGAR at 5-min	0.327	0.069	0.015
Pregnancy Average DII	APGAR at 5-min	0.276	0.063	0.041

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Supplement Usage

The supplement usage characteristics of our sample for both the 2nd and 3rd trimesters can be found in Table 7. In our sample, there were 55 different prenatal supplements and multivitamins taken over the duration of participants pregnancy. In the 2nd trimester an average of 2.97 ± 1.73 different supplements were taken and in the 3rd trimester an average of 3.09 ± 1.51 supplements were taken, both with a range of 1 to 8. Four participants were taking two different prenatal vitamins at the same time in the 2nd trimester.

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Table 7: Supplement characteristics during the 2nd and 3rd trimester.

Micronutrient	2nd Trimester (n = 55)	3rd Trimester (n = 55)
DHA + EPA		
Under	41 (75%)	29 (53%)
Over	14 (25%)	15 (27%)
DHA		
Under	28 (51%)	29 (53%)
Over	15 (27%)	15 (27%)
Proper	12 (22%)	11 (20%)
Choline		
Under	55 (100%)	55 (100%)
Thiamine		
Under	14 (25%)	17 (31%)
Over	31 (56%)	26 (47%)
Proper	10 (18%)	12 (22%)
Vitamin B₆		
Under	4 (7.3%)	6 (11%)
Over	43 (78%)	38 (69%)
Proper	8 (15%)	11 (20%)
Folic Acid		
Under	28 (51%)	33 (60%)
Over	5 (9.1%)	4 (7.3%)
Proper	22 (40%)	18 (33%)
Vitamin B₁₂		
Under	2 (3.6%)	3 (5.5%)
Over	53 (96%)	52 (95%)
Iodine		
Under	40 (73%)	38 (69%)
Over	7 (13%)	7 (13%)
Proper	8 (15%)	10 (18%)

Prenatal		
Yes	54 (98%)	54 (98%)
No	1 (2%)	1 (2%)

Values are reported in *n* (%).

Supplement Usage and Birth Outcomes

There was a statistically significant association between 2nd trimester DHA and EPA supplementation and the APGAR score at 5 minutes ($F(1,53) = 7.091, p=0.01$). Specifically, under supplementation of DHA and EPA is associated with a higher mean APGAR score (9 ± 0.316) than over supplementation (8.5 ± 1.09). This association was also observed between 3rd trimester DHA and EPA supplementation and the APGAR score at 5 minutes ($F(1,53) = 4.429, p=0.04$).

There was also a statistically significant association between 2nd trimester iodine supplementation and the APGAR score at 5 minutes ($F(2,52) = 3.740, p=0.03$). Specifically, over-supplementation is associated with higher APGAR scores (8.95 ± 0.389), while under-supplementation is associated with lower APGAR scores (8.29 ± 1.496). Post hoc testing demonstrated that the means from the under-supplementation group was statistically significantly different from the over supplementation group ($p=0.028$). Similarly, this association was also seen between the 3rd trimester iodine supplementation and 5-minute APGAR scores ($F(2,52) = 3.748, p=0.03$). However, over-supplementation is associated with lower APGAR scores (8.29 ± 1.496), while under-supplementation is associated with higher APGAR scores (8.95 ± 0.399). Post hoc testing demonstrated that the means from the under-supplementation group were statistically significantly different from the over supplementation group ($p=0.029$).

There were no statistically significant results between the other micronutrients and birth outcomes that were examined ($p>0.05$).

DII and IBQ-R

There were no significant associations between the DII and the IBQ-R categories examined ($p > 0.05$).

Supplement Usage and IBQ-R

There was a statistically significant association between 3rd trimester folic acid supplementation and NA means ($F(2,52) = 4.871, p = 0.012$) (Figure 4). Specifically, under supplementation is associated with lower NA means (2.82 ± 0.515) than proper supplementation (3.27 ± 0.62). Post hoc testing demonstrated that the means from the under-supplementation group were statistically significantly different from the proper supplementation group ($p = 0.024$), but not the over supplementation group ($p = 0.114$).

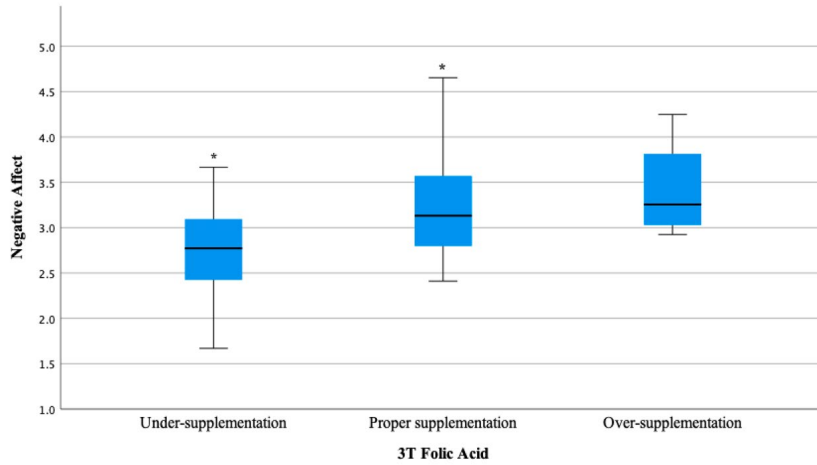


Figure 4: Levels of negative affect (NA) in 1-month infants and folic acid supplementation during the 3rd trimester.

Proper supplementation of folic acid (between 400-800 mcg/day) was significantly associated with greater levels of NA in infants, while under-supplementation was significantly associated with lower levels of NA. * indicates the statistically significant relationships within the levels of folic acid supplementation.

There were no other significant relationships between the micronutrients examined and IBQ-R scores at the 1-month time point ($p > 0.05$).

Discussion

The goal of the current study was to examine whether poor maternal nutrition, in combination with or independent of adiposity, predict more adverse birth outcomes and higher levels of negative affect and regulatory problems in infants 1-month post-partum. Though the effects of maternal adiposity and dietary inflammatory potential have been examined in relation to birth outcomes in previous studies, this study's inclusion of supplement usage and infant regulatory behavior will expand the current literature surrounding maternal factors that influence child outcomes and development. Results from my analysis suggest that certain micronutrient supplementation is associated with higher APGAR scores at 5-minutes. Specifically, decreased DHA and EPA supplementation in the 2nd and 3rd trimesters, increased iodine supplementation in the second trimester, and decreased Iodine supplementation in the 3rd trimester is all associated with higher APGAR scores at 5-minutes. Additionally, an increase in 3rd trimester and pregnancy average DII scores (i.e. more pro-inflammatory) is associated with higher APGAR scores at 5-minutes.

Even though the APGAR score is a widely used instrument for reporting newborn health, it does have limitations and numerous factors that can influence scoring. Assessing the physiological health of an infant at one point in time includes subjective observation and factors like maternal sedation, gestational age, and interobserver variability can influence the scores given (*The Apgar Score*, n.d.). An APGAR score between 7-10 at 5 minutes is indicative of good health at birth and our sample's score ranged from 5-10 with an average of 8.87. Due to our sample being concentrated between normal ranges, it is unlikely that proper supplementation or

reduction of dietary inflammation will drastically improve APGAR scores at 5-minutes. The APGAR score is still a meaningful method for assessing newborn health, but specifically for preterm and term infants with very low scores (0-3), which was not seen in our sample (Casey et al., 2001). It is known that newborns with increased gestational age and size is associated with higher APGAR scores; however, these were not predictive in our sample (Behnke et al., 1987).

Interestingly, we did not see any associations between the other birth outcomes examined and maternal diet and adiposity. As the prior research regarding the DII's influence on birth outcomes is relatively limited, the association between the DII and maternal and birth outcomes is an evolving field of research. Previous studies have found that the consumption of a pro-inflammatory diet is associated with elevated rates of caesarean delivery in women with BMI's > 25 kg/m² and decreased fetal growth (McCullough et al., 2017; Sen et al., 2016). Even though we were not able to replicate these findings in our sample, our novel finding suggesting that higher 5-minute APGAR scores were associated with more pro-inflammatory diets warrants further research with a larger and more diverse sample to determine if this association is still significant.

It is well known that an increase in maternal adiposity is associated with more adverse birth outcomes for both the mother and child (King, 2006). However, our study found no association between BMI or measured fat mass in the 2nd and 3rd trimesters and birth outcomes. Data has shown that increased adiposity causes chronic, low-grade inflammation due to the secretion of pro-inflammatory cytokines in adipose tissues that create harmful conditions to the fetus and pre-dispose mothers with obesity to more birth complications (Catalano & Shankar, 2017). This contrasting result may be

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attributed to the small sample size of this study resulting in a lower statistical power that limited our ability to detect this association in our sample.

Finally, my analyses suggest that increased folic acid supplementation is associated with higher levels of negative affect in infants 1-month post-partum. During pregnancy, folic acid is supplemented to support general growth and development of the fetus and is preventative against NTD's. The association between supplementation and infant temperament could demonstrate that infants that received proper supplementation may have greater engagement and sensitivity to their environment than those who did not receive proper supplementation. Even though use of the IBQ-R for infants as young as 2 weeks of age has shown to have reliability, convergent validity, and relative stability, it is most commonly used to assess infants starting 2 months of age into early childhood (Gartstein & Rothbart, 2003). Starting at 2 months old, caregivers are able to more reliably observe temperament, especially positive emotionality (Rothbart, 1989). Since we used the IBQ-R to assess infant temperament prior to this time period, we could have gotten higher ratings of negative emotionality (i.e. negative affect, fear, etc.) due to decreased expression of positive emotionality, as it is rarely expressed in the newborn period.

One of the most interesting results of this study is the extreme amount of variability and improper supplementation among our sample. There was 55 different prenatal supplements and multivitamins taken over the duration of participants pregnancies. Of these supplements, the most common was Prenatal Multi Plus DHA by Nature Made, which improperly supplements on half of micronutrients examined in this study (Table 8). In fact, none of the supplements used by participants properly

supplemented all of the micronutrients, even the prescription prenatal vitamins. Additionally, none of the supplements met the recommended choline supplementation. Baby and Me 2 by MegaFood and Full Circle Prenatal were the prenatal supplements that had the choline dosage closest to the recommended value, but remained 150 mg below adequate supplementation.

Table 8: Comparison of recommended micronutrient supplementation to Prenatal Multi Plus DHA by Nature Made ingredients.

	Recommended	Prenatal Multi Plus DHA
Iodine	220 mcg	150 mcg
Choline	450 mg	0 mg
B6	1.9 mg	1.9 mg
B12	2.6 mcg	5.2 mcg
Folic Acid	400-800 mcg	800 mcg
Thiamine (B-1)	1.4 mg	1.4 mg
DHA	200 mg	200 mg
DHA+EPA	300 mg	260 mg

As the American diet is changing substantially towards more processed, less nutrient-rich food, it is vital that women are properly supplementing these nutrients, as they will likely not obtain these nutrients from their diet alone. The supplement industry is still widely unregulated, leaving the consumer to decipher supplement labels and trust that these companies are using high quality ingredients that are bioavailable. However, as this study shows, this is extremely problematic. The recommended supplementation of essential nutrients for proper growth and development of the fetus during gestation is

widely known, yet out of the 55 participants in this study, not one was able to completely properly supplement all of the micronutrients examined. As research has shown, improper supplementation during this time period can lead to permanent developmental and growth consequences. Consumers should be assured that every prenatal can adequately meet this nutritional demand at an affordable price.

Limitations

This study is limited by a relatively small, homogenous sample that restricted our ability to draw conclusions with high statistical power. Part of our exclusion criteria eliminates participants with higher risk pregnancies, like those with preeclampsia, that **limits the variability of our sample**. Also, the use of nutrition recalls and supplement questionnaires are inherently error prone as they rely on participant's memory in an uncontrolled environment. Additionally, we were unable to correlate the DII with circulating pro-inflammatory cytokine concentrations. It is still relatively unknown how accurate the DII is in pregnant populations, but it is well established in normal adult populations. Being able to correlate reported dietary inflammatory potential with biological markers would strengthen the use of the DII as an assessment tool in pregnant populations. Lastly, the Covid-19 pandemic has introduced several limitations to human subject's research. As this data is part of a larger, ongoing research study, the participants used in this project were limited due to the inability to have in-person visits that provide data for body composition measurements.

Commented [JA19]: This is making me wonder – do you ever explain why your sample was reduced to 55 from the larger 300?

Conclusion

This study provides an assessment of dietary inflammation and supplementation in relation to birth outcomes and emotional dysregulation of 1-month old infants, with consideration of maternal adiposity. Our results suggests that diet and supplementation may not only impact birth outcomes, but also infant temperament. We have used the DII and supplementation as a means to assess emotional dysregulation in infants in a novel way, finding an association between folic acid supplementation and negative affectivity in infants. Also, we have illustrated the immense variability and inadequacy of prenatal vitamins and the need to regulate the supplement industry to ensure that they are meeting well known nutritional recommendations during a time of crucial fetal development and growth.

Future studies may seek to understand how the DII is associated with circulating pro-inflammatory cytokines in pregnant populations and seek the mechanisms linking inflammation to birth outcomes and infant behavior. Also, studies should use the IBQ-R longitudinally to assess child and infant behavior in relation to DII scores and supplementation in pregnancy to see how poor nutrition impacts child growth and development over time. Lastly, the influence of poor maternal nutrition should be examined in a large, more diverse sample to examine associations with higher statistical power.

Appendix A

Supplement Usage Questionnaire

Pre/Post-Natal Study Supplement Questionnaire
ID# _____

Today's Date: _____

To be asked at 22 and 37 weeks: *During your pregnancy, are/were you taking any of the following supplements?*

To be asked at 1, 6, 12, 24, and 32-36 months postpartum: *Are you currently taking any of the following supplements?*

circle	Type	Name(s) of Supplement (Fish Oil, Vit. B-12, etc)	Brand Name (Good Earth, etc)	Dose (1000mcg, 50 mg, 10g, etc)	Times per day (1x, 2x, etc)	Date Started (month/day/year)
Y N	1. Pre-Natal Vitamins					
Y N	2. Other Multivitamin (e.g. One-A-Day or Centrum) Supplements containing oils or fatty acids					
Y N	3. (e.g. Omega 3, fish or flaxseed oil)					
Y N	4. Vitamin D or other single vitamin (e.g. vitamin D, E, or folic acid)					
Y N	5. Zinc or other mineral (e.g. zinc, calcium, iron, or potassium)					
Y N	6. Other dietary supplements (e.g. glucosamine chondroitin, coenzyme Q-10 or garlic extract)					
Y N	7. Amino acid supplements (e.g. lysine)					
Y N	8. Suppl. containing 1 or more herbal or botanical ingredients (e.g. Echinacea or St. John's Wort)					
Y N	9. Fiber supplements (e.g. Metamucil or Fibercon)					
Y N	10. Over-the-counter antacids (e.g. Tums, Rolaids or Mylanta)					
Y N	11. Other supplements					

Office Use Only: EPRC: _____

Infant Behavior Questionnaire-Revised

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Maria A. Gartstein
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Subject No. _____ Date of Baby's Birth _____
month. day
year
Today's Date _____ Age of Child _____
mos. weeks
Sex of Child _____

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)	(2)	(3)	(4)	(5)	(6)	(7)	(X)
Never	Very Rarely	Less Than Half the Time	About Half the Time	More Than Half the Time	Almost Always	Always	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.

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?

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?

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?

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?

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?

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?

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?

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?

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 sooth

Appendix B

Prenatal Supplement Inventory

Prenatal Name	Prenatal Brand
Prenatal	365
Multivitamin Gummies	Everyday Value
Prenatal Plus	AVkare
Flintstones Complete	Bayer
Prenatal 1 One a Day	Bayer
Prenatal Gummies One a Day	Bayer
Mama Bird	Best Nest Wellness
Prenatal Multiple with DHA and D3	Carlson
CitraNatal DHA	CitraNatal
Prenatal Vitamin	
Prenatal Multi	Garden of Life

Prenatal	Garden of
Multi	Life
Gummies	
Prenatal	Garden of
Once Daily	Life
Raw	Garden of
Prenatal	Life
Pregna Vite	Genestra
Prenatal	Integrative
Forte	Therapeutics
Baby and	MegaFood
Me	
Baby and	MegaFood
Me 2	
One Daily	MegaFood
Iron Free	
Prenatal	Morningpep
Prenatal	Nature
	Made
Prenatal	Nature
Gummies	Made
Prenatal	Nature
Multi	Made
Prenatal	Nature
Multi +	Made
DHA	
Healthy	Nature's
Mom	Bounty
Alive	Nature's
Prenatal	Way
Multivitamin	

with Plant	
DHA	
Prenatal	Nature's Way Alive
Prenatal	Nature's
Multi-	Way Alive
Vitamin	
Perfect	New
Prenatal	Chapter
Multi-	
Vitamin	
Prenatal	Now
Gels + DHA	
Niva Plus	Prescription
Prenatal +	Prescription
DHA Combo	
Pack	
Prenatal	Prescription
Plus	
Multivitamin	
PrePlus	Prescription
Prenatal	
Vol Plus	Prescription
Prenatal	Rainbow
One	Light
Trimester 2	Rainbow
Prenatal	Light
Multivitamin	
Prenatal	Salaam
DHA + Folic	Nutritional's
Acid	

Optimal	Seeking
Prenatal	Health
Prenatal	Signature
Multivitamin	Care
Gummies	
Prenatal	Simple
Once Daily	Truth
Prenatal	Smarty
Complete	Pants
Prenatal and	Solimo
DHA	
Adult	Spring
Gummy	Valley
Prenatal	
Prenatal	Sundance
Women's	
Essentials +	
Folic Acid	
Prenatal	Target
Multivitamin	
Gummies	
Prenatal	Target
Vitamin	
Theranatal	Theralogix
Complete	
Basic	Thorne
Prenatal	
Gummy	Trader Joes
Multivitamin	
Prenatal	Trader Joes
Once Daily	

Essential	VitaFusion
Multivitamin	
Prenatal	
PreNatal	VitaFusion
Prenatal	VitaFusion
Essential	
Multi	
Prenatal	VitaFusion
Gummy	
Vitamins	
Prenatal	Walgreens
Multivitamin	
+ DHA and	
Ginger	
Prenatal	Winco

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