Fall 12-14-2018

Association Between Systemic Inflammation and Nutritional Compounds in Maternal-Infant Dyads

Jessica N. Snowden
University of Nebraska Medical Center

ASSOCIATION BETWEEN SYSTEMIC INFLAMMATION AND NUTRITIONAL COMPOUNDS IN MATERNAL-INFANT DYADS

by

Jessica Snowden, M.D.

A THESIS

Presented to the Faculty of

the University of Nebraska Graduate College

in Partial Fulfillment of the Requirements

for the Degree of Master of Science

Medical Sciences Interdepartmental Area Graduate Program

(Clinical & Translational Research)

Under the Supervision of Professor Ann Anderson-Berry, M.D., Ph.D.

University of Nebraska Medical Center

Omaha, Nebraska

December, 2018

Advisory Committee:

Corrine Hanson, Ph.D., R.D. Lani Zimmerman, Ph.D., R.N.

ACKNOWLEDGEMENTS

The work presented here was made possible by many contributors, beginning with my primary advisors Drs. Ann Anderson-Berry and Corrine Hanson, who were instrumental in guiding me to apply my basic science experience to a clinical framework and navigating the complications that human subjects research present as opposed to my prior mouse work. They both deftly navigated the balance of respecting my experience and expertise while challenging me to stretch my boundaries to learn more in the realm of clinical research. I would also like to thank Dr. Chi Chi Zimmerman for her patience in guiding medical school faculty into new research avenues as part of the Clinical Translational Research Mentored Scholars Program, encouraging us to think outside the box and accept that despite our expertise in other areas, we still had a lot to learn. Dr. Tammy Kielian has been and will always be essential to my path a physician scientist, providing a framework of technical and scientific skill to allow me to design and interpret the inflammation work presented here, a nudge to finish grants and papers, and always a guiding hand and cheering section as I progress through my career.

The research results presented here would not be possible without the significant contributions of Matthew Van Ormer and Matthew Beaver, who were instrumental in the collection of the patient samples, clinical data associated with the samples and multiplex cytokine analysis. Additional thanks to Iman M. Ahmad, Ph.D., who performed the superoxide dismutase analysis, and Jiangtao Luo,Ph.D., who provided significant input on the statistical design for this project.

The support and patience of my colleagues in the University of Nebraska Medical Center Pediatric Infectious Disease division and my family as I took on the challenge of graduate school made this work and all of my other accomplishments possible. Thank you to you all.

ASSOCIATION BETWEEN SYSTEMIC INFLAMMATION AND NUTRITIONAL COMPOUNDS IN MATERNAL-INFANT DYADS

Jessica Snowden, MD, MHPTT, MS

University of Nebraska Medical Center, 2018

Advisor: Ann Anderson-Berry, MD, PhD

Many events during pregnancy and early infancy can affect infant brain development. Inflammation during pregnancy, around delivery and during early infancy appears to adversely affect infant brain development. As the brain is rapidly growing and developing from conception through early childhood, it is particularly vulnerable during this time to inflammatory insults, which may be exacerbated or ameliorated by nutritional factors. Inflammatory compounds, as well as many nutritional compounds, can be either pro- or anti-inflammatory. These compounds are of particular importance in preterm infants, who are at risk of deficiency in anti-inflammatory micronutrients typically stored as a result of prenatal maternal diets and thus reliant on post-natal dietary supplementation. Understanding the ways in which nutritional status and inflammation interact with each other has been identified as a key gap to fill in improving our ability to treat and prevent neurodevelopmental impairment as a result of prematurity. We examined the innovative conceptual framework by which nutritional compounds such as alpha- and beta-carotenes, lutein, lycopene and alpha-tocopherol are associated with decreased levels of pro-inflammatory compounds associated with inflammation *in utero* and after delivery. These studies will lay the foundation for long-term studies of neurodevelopment outcomes in these infants, as well as allow us to identify key pathways we might target for dietary or pharmacologic immunomodulation to improve neurologic outcomes in high risk infants.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

List of Abbreviations (in alphabetical order)

CNS: Central nervous system

IL-10: Interleukin-10

IL-1β: Interleukin-1β

IL-2: Interleukin-2

IL-6: Interleukin-6

IL-8: Interleukin-8

NICHD: National Institute for Child Health and Human Development

NICU: Neonatal Intensive Care Unit

RDS: Respiratory distress syndrome

SOD: Superoxide dismutase

TNF-α: Tumor necrosis factor-α

WST: 2-(4-Iodopheny)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt

CHAPTER ONE: INTRODUCTION

Almost 10% of infants in Nebraska are born prematurely, or preterm, before 37 weeks gestation[1]. As healthcare has advanced, the number of these infants that survive the neonatal period has increased; however, the rates of neurodevelopmental impairment that occur in these infants have not decreased[2]. Depending on gestational age and birthweight, 30-60% of preterm infants have neurodevelopmental impairments that persist into later childhood, including visual problems, cerebral palsy, developmental delays and problems with school function[2-6]. Understanding the factors that have the greatest impact on development can lead to treatment and prevention strategies that improve health for children throughout the United States and the world.

Inflammation and Neurodevelopment.

Many events during pregnancy and early infancy can affect infant brain development. The time from conception through early childhood is sometimes referred to as the "1000 days critical window" because of the rapid anatomic and functional changes in the developing brain at this age[7]. There is increasing evidence that inflammation in early development, from intrauterine, peri-partum and early childhood insults, may have lifelong impacts on neurologic function[8,9]. For example, elevated pro-inflammatory compounds in cord blood have been associated with an increased risk of cerebral palsy in several case series[8,9]. Animal studies attempting to better define the impact of specific inflammatory processes on neurologic function have demonstrated that cytokine exposure during infancy increases the risk for changes in memory or neuropsychiatric function later in life following a second immune challenge[10-13]. Additionally, following *E. coli* sepsis, rodents are less responsive to cognitive enrichment strategies than those without a history of infection[10]. Importantly, these effects are only observed in rodents less than 30 days of

age, revealing an early period of vulnerability in the developing brain[10,12]. In addition to risks with pro-inflammatory cytokines, anti-inflammatory cytokines may provide a balancing protective effect. My animal work in adult and infant mice with central nervous system (CNS) infections has identified a role for the potent anti-inflammatory cytokine interleukin-10 (IL-10) in neuroprotection following *S. epidermidis* catheter infection in the brain[14]. As both CNS and peripheral inflammation are associated with neurologic sequelae, it is possible that IL-10 serves a similar protective function in both systemic and CNS infections, as well as other stressors. While IL-10 and other cytokines have been evaluated as possible biomarkers for infection in infants, these studies have not taken into the gestational age of the infant nor the association of these immunologic responses with potentially immunomodulatory nutritional factors[15,16].

Dietary Modulation of Inflammation.

As the brain is rapidly growing and developing from conception through early childhood, it is particularly vulnerable during this time to inflammatory insults, which may be exacerbated or ameliorated by nutritional factors[7]. This is of particular importance in preterm infants, who are at risk of deficiency in anti-inflammatory micronutrients typically stored as a result of prenatal maternal diets and thus reliant on post-natal dietary supplementation[17]. Without antioxidants to balance the effects of oxidative stress and inflammation, infants are at risk of abnormalities in neurologic development, bronchopulmonary dysplasia, and retinopathy of prematurity[18]. Major nutritional antioxidants include alpha- and beta-carotenes, lutein, lycopene, and alpha-tocopherol[19]. Infants must acquire these compounds through dietary sources as humans cannot synthesize these compounds[18,19]. Vitamin E, in particular, occurs in both an alpha- and gamma-tocopherol isoform[19]. While alpha-tocopherol has anti-inflammatory effects, gamma-tocopherol is conversely and potently pro-inflammatory[19]. Thus, the balance of

these two compounds could have important implications for regulating inflammation and thereby affecting neonatal development but have not been previously defined. At a recent National Institute of Child Health and Human Development (NICHD) meeting on "Research Gaps at the Intersection of Child Neurodevelopment, Nutrition, and Inflammation in Low-Resource Settings," understanding of the ways in which nutritional status and inflammation interact with each other and with neurodevelopment was identified as the first key gap to fill in improving our ability to treat and prevent neurodevelopmental impairment as a result of prematurity[20]. To fill this gap, we will evaluate the relationship between pro- and anti-inflammatory cytokines, nutritional pro- and antioxidants in term and preterm infants.

Conceptual Framework

We propose the innovative conceptual framework by which nutritional compounds such as alpha- and beta-carotenes, lutein, lycopene, and alpha-tocopherol reduce pro-inflammatory compounds associated with inflammation *in utero* and after delivery, which may result in improved neurologic outcomes in the future (**Figure 1**). By countering these pro-inflammatory compounds directly through anti-oxidant activities and indirectly by increases in anti-inflammatory IL-10, these nutritional compounds may improve the neurologic outcomes of premature infants. As premature infants are known to have lower levels of these protective nutritional compounds[17], strategies to both improve maternal diet and to provide post-natal supplementation may serve to increase levels of these compounds and improve neurologic outcomes. In this study, we have evaluated maternal and neonatal levels of pro- and anti-inflammatory compounds (such as interleukin-1β, tumor necrosis factor-α, interleukin-6, interleukin-8, interleukin-2, and IL-10) and nutritional compounds (such as lutein, + zeaxanthin, β-cryptoxanthin, *trans*-lycopene, *cis*-lycopene, total lycopene, α-carotene, *trans*-β-carotene,

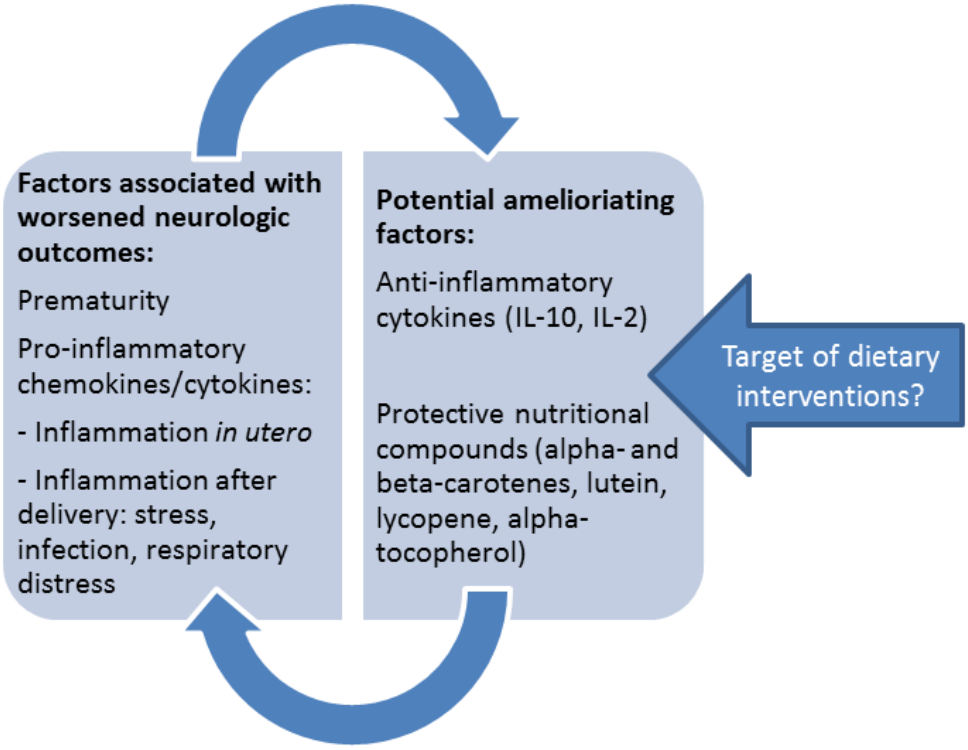

Figure 1: Proposed model of inflammatory modulation and neurologic outcomes

cis-β-carotene, total-β-carotene, retinol, α-tocopherol, and γ-tocopherol) to examine their interactions with each other in term and pre-term infants.

Hypothesis

The hypothesis of this proposal is that nutritional compounds such as alpha- and beta-carotenes, lutein, lycopene, and alpha-tocopherol are associated with lower levels of pro-inflammatory compounds associated with inflammation *in utero* and after delivery, which may result in improved neurologic outcomes in the future.

Clinical Impact of this Research

A better understanding of the relationship between inflammation and nutrition would present several clinical advantages. Nutritional compounds may have significant effects on neurologic development through pro- and antioxidant activities and could be targeted through pre- and post-natal dietary interventions. This information could also be exploited to allow for earlier identification of infants at risk for neurodevelopmental disorders or other adverse inflammatory outcomes due to dietary insufficiencies during these crucial periods. These patients may require more aggressive or specifically targeted behavioral therapies in addition to nutritional supplementation to improve overall clinical outcomes. Thus, defining the factors that shape the inflammatory response in infants has the potential to significantly impact the health of neonates and infants.

This is the first study to describe the association between pro- and anti-inflammatory compounds and nutritional compounds in term and preterm infants. While some adult studies have demonstrated an inverse relationship between levels of vitamins C, E and D, and inflammatory markers, studies in infants are lacking[21]. As noted previously, evaluation of these associations in infants is of critical importance given the

increased susceptibility of preterm infants to neurologic injury associated with inflammation and nutritional deficiencies at birth that may exacerbate inflammation. Additionally, other micronutrients such as zeaxanthin, β-cryptoxanthin, *trans*-lycopene, *cis*-lycopene, total lycopene, α-carotene, *trans*-β-carotene, *cis*-β-carotene, total-β-carotene, and retinol have not been evaluated to determine their impact on systemic inflammation. Studying the associations between these nutritional compounds and inflammation may identify targets for pre- and post-natal dietary interventions in future clinical trials. Immunologic profiling may also allow us to risk-stratify infants in terms of long-term neurologic development, with those displaying a more neuroprotective IL-10 predominant response or higher levels of α-tocopherol having better long-term outcomes. This may also provide an immunomodulatory target in the future, by targeting components of the IL-10 pathway or dietary interventions.

CHAPTER TWO: METHODS

Design

This cross-sectional, descriptive study will evaluate the association between pro- and anti-inflammatory cytokines and nutritional antioxidants and pro-oxidants in term and pre-term infants. We will utilize existing samples from a cohort of 122 mother-infant pairs, which includes cord blood (infant) and maternal blood collected at birth.

Enrollment and Initial Sample Collection

Mothers of infants admitted to the neonatal intensive care unit (NICU) were approached for consent and enrollment in this study in summer 2016 and summer 2017. A maternal blood and cord blood sample were obtained from each infant enrolled. Maternal and cord blood samples are routinely drawn by the nursing staff during each delivery at Nebraska Medicine. After it was determined that the entire cord blood sample and a maternal blood sample was not needed for clinical purposes, it was collected by study personnel in subjects that have consented to participate in the study. This study was approved by the University of Nebraska Medical Center Institutional Review Board, Protocol 112-15-EP, Fatty Acids, Fat Soluble Vitamins, Infant Feeding and Inflammation during NICU Hospitalization.

Inflammatory and Nutritional Compound Analysis

Pro- and anti-inflammatory cytokine levels will be measured via a commercially available multi-analyte bead array (Millipore) per the manufacturers' instructions. This will include testing for interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10 (IL-10), and interleukin-2 (IL-2). Superoxide dismutase (SOD), an endogenous anti-oxidant enzyme that plays a role in the anti-

inflammatory effects of some nutritional compounds[22], was measured in maternal and infant samples using the SOD assay kit-WST (WST-1, 2-(4-Iodopheny)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt) from Dojindo[23]. This assay measures the percent inhibition of WST reduction by SOD, with one unit of SOD representing the amount of enzyme in 20μL of the sample that results in a 50% reduction of WST-1[23]. These cytokine levels were selected as they have been previously proposed to correlate with neurologic outcomes or have potential neuroprotective anti-inflammatory effects [8,24,25]. Serum nutrient levels will be assessed in the Biomarker Research Institute at Harvard University. Measurements of lutein + zeaxanthin, β-cryptoxanthin, *trans*-lycopene, *cis*-lycopene, total lycopene, α-carotene, *trans*-β-carotene, *cis*-β-carotene, total-β-carotene, retinol, α-tocopherol, and γ-tocopherol will be obtained. Concentrations of α- and gamma-tocopherol in plasma samples will be measured by high-performance liquid chromatography as previously described by Hanson, et al [19]. As outlined above, these nutritional compounds were selected based on their potential pro- and antioxidant properties; however, this not been previously studied in infants[18,19]. These analyses will allow us to investigate the proposed aims investigating the association between pro- and anti-inflammatory cytokines with pro- and anti-oxidant nutritional compounds in infants and in mother-infant pairs. We expect that levels of maternal and/or infant anti-inflammatory nutritional compounds (as outlined in **Table 1**) are inversely correlated with levels of pro-inflammatory compounds. We will additionally determine if levels of pro-oxidant nutritional compounds, such as γ-tocopherol, are associated with increases in pro-inflammatory cytokines and similarly if levels of anti-oxidant nutritional compounds are associated with an increase in anti-inflammatory IL-10.

Clinical Data and Evaluation

Demographic and clinical data will be collected from the mother and infant's record at delivery, including gestational age, birth weight, length, head circumference, race, ethnicity, maternal chorioamnionitis, maternal infections, maternal smoking/drug use, prenatal complications, Apgar scores and neonatal complications including seizures, infections, intraventricular hemorrhage, and respiratory distress syndrome (**Table 2**). Prenatal clinical measures, such as maternal infections and complications, and demographic factors such as race and ethnicity will be assessed as potential confounders affecting the association between nutritional compounds and cytokine responses. Post-natal outcomes, such as birth weight, length, and head circumference, Apgar scores and neonatal complications will be evaluated as outcomes potentially associated with increased levels of these nutritional compounds and cytokines. Gestational age, with prematurity defined as a gestational age less than 37 weeks and 0 days at the time of delivery, will also be included in analysis to determine if prematurity independently affects the association between nutritional compounds and inflammatory responses, suggesting that the altered immunity of a preterm infant may not respond in the same ways to nutritional manipulation as a more mature immune system.

Data Analysis Plan

This is a cross-sectional, descriptive study to collect pilot data for future NIH funding applications. All analyses were conducted using the intent-to-treat criterion. Descriptive statistics such as the mean, median, standard deviation, 95% CI, and range were used to summarize each outcome variable.[26] As the data are not normally distributed, the Wilcoxon-Mann-Whitney test was used to compare medians between smokers and non-smokers, term and pre-term infants, and infants with pre- and post-natal complications. The nutritional and inflammatory compounds are continuous variables and

Pro-inflammatory compounds	Anti-inflammatory compounds
IL-1β	IL-10
TNF-α	IL-2
IL-6	Lutein + zeaxanthin
IL-8	β-cryptoxanthin
γ-tocopherol	*trans*-lycopene, *cis*-lycopene, total lycopene
	α-carotene, *trans*-β-carotene, *cis*-β-carotene, total-β-carotene
	Retinol
	α-tocopherol
	Superoxide dismutase

Table 1: **Proposed pro- and anti-inflammatory dietary and immune compounds for evaluation.**

Potential Confounder/ Mediator / Moderator	**Secondary Outcome Measure**
Prenatal Complications: Maternal chorioamnionitis, infection, others	Birth weight
Maternal drug/tobacco use	Birth length
Gestational age	Head circumference
Prematurity *	Apgar scores
Race/ethnicity	Neonatal complications: Seizures, infection, others

Table 2: Clinical and demographic factors that may be associated with inflammatory and nutritional compounds. * Prematurity defined as gestational age less than 37 days and 0 days.

differences between compounds were evaluated using Wilcoxon rank sum as the data points were not normally distributed. Associations between compounds were evaluated via Spearman correlation coefficients, with hypothesis testing via Fisher's z-transformation to test the differences in the strength of this correlation in term versus pre-term infants[27]. We also used Fisher's z-transformation to test the differences in the strength of the correlation in infants born to mothers with current or prior tobacco use in comparison with those with no smoking tobacco history. The demographic and clinical data outlined above were evaluated as potential confounders affecting the association between nutritional compounds and cytokine responses, and we utilized multiple regression to address the effects of these factors on the observed relationships between inflammatory compounds and nutritional compounds. Because there were very few participants with documented prenatal complications, this was treated as a single variable defined as "prenatal complication" to include: pre-eclampsia, maternal diabetes, suspected clinical chorioamnionitis, placental evidence of chorioamnionitis and premature rupture of membranes. To evaluate the relationship between the nutritional compounds and cytokine responses and the neonatal complications, the Mann-Whitney-U-test was used as the only neonatal complication documented was respiratory distress syndrome (RDS) and the cytokine data is not normally distributed.[26, 28] $p < 0.05$ will be considered statistically significant. For all twin deliveries, only twin A was included in the analysis to avoid over-representation of twin infants in subsequent analyses. All statistical analyses were performed using Excel, SPSS, and VassarStats.

CHAPTER THREE: RESULTS

Description of Participants

One hundred and twenty-two maternal-infant pairs are included in this analysis, based on the availability of paired maternal and infant blood samples. This includes 120 singleton deliveries and two twin deliveries. As noted above, in the instance of twin deliveries, only the samples from twin A are included in future analyses. There are more male than female infants included in these analyses (n=74; 57.8% male; n=48; 37.5% female) (**Table 3**). 85 infants (66.4%) were delivered to Caucasian mothers; 19 African American mothers (14.8%); 7 Hispanic mothers (5.5%); 3 Asian or Pacific Islander (2.3%); 1 Native American (0.8%) and 7 unknown or unreported (5.5%) (**Table 3**).

This data set includes 14 (11.5%) preterm deliveries, defined as less than 37 weeks and 0 days estimated gestational age, and 15 deliveries (11.7%) with prenatal complications, as outlined in **Table 3**. Prenatal complications included maternal diabetes (n=8), suspected clinical chorioamnionitis (n=1), placental chorioamnionitis (n=1), pre-eclampsia (n=2) and premature rupture of membranes (n=5). Current or former maternal smoking was common, with 19 mothers (14.8%) reporting current tobacco use in this pregnancy and 28 (21.9%) reporting former tobacco use (**Table 3**).

Neonatal Complications

Ten of the pregnancies included in this dataset resulted in neonatal complications (7.85), including respiratory distress syndrome (n=10, 7.8%). There were no cases of retinopathy of prematurity, bacteremia as evidenced by positive blood cultures, intraventricular hemorrhage or necrotizing enterocolitis documented in this dataset.

Characteristic	***n* (% total)**
Gender	
Male	74 (61%)
Female	48 (39%)
Race/Ethnicity	
White	85 (66.4%)
African American	19 (14.8%)
Hispanic	7 (5.5%)
Asian / Pacific Islander	3 (2.3%)
Native American	1 (0.8%)
Other/Unknown	7 (5.5%)
Prematurity	
Term	108 (88.5%)
Pre-term	14 (11.5%)
Prenatal Complications	
Maternal diabetes	8 (6.6%)
Clinical chorioamnionitis	1 (0.8%)
Placental Chorioamnionitis	1 (0.8%)
Pre-eclampsia	2 (1.6%)
Premature rupture of membranes	5 (4%)
Maternal Tobacco	
No prior tobacco use	74 (57.8%)
Current tobacco use	19 (14.8%)
Former tobacco use	28 (21.9%)
Neonatal Complications	
Retinopathy of Prematurity	0
Intraventricular Hemorrhage	0
Necrotizing enterocolitis	0
Bacteremia	0
Respiratory Distress Syndrome	10 (8.2%)

Table 3: **Description of the participating mother-infant pairs in this study** (*N*=122).

Characteristic	Median (Q1, Q3)	Range
Gestational age (weeks)	39.57 (38.57, 40.43)	25-42.13
Birth weight (grams)	3458.5 (3050.75, 3744)	860-4617
Birth weight percentile	65.54 (36.38, 80.62)	3.26-99.82
Birth head circumference (cm)	34.9 (33.7, 35.6)	24-47.6
Birth head circumference percentile	50 (48.3, 52.1)	32-55.2
Birth length (cm)	49.25 (32, 52.1)	32-55.2
Birth length percentile	68.57 (29.62, 87.59)	0.01-99.77
Apgar 1 minute	8 (7, 8)	1-9
Apgar 5 minute	9 (9, 9)	3-10

Table 4: **Description of study population birth characteristics**. Data are presented as medians, with first (Q1) and third (Q3) quartiles noted because data were not normally distributed.

Median Apgar scores were 8 at 1-minute (range 1-9) and 9 at 5-minutes (range 3-10) (**Table 4**).

Maternal and Infant Inflammatory Compounds

Infant cord blood samples were noted to have higher levels of most inflammatory compounds measured, except for IL-2 which was slightly higher in the maternal samples (**Table 5, Figure 2**). Superoxide dismutase was measured in only 27 infant and 22 maternal samples as this was an exploratory measure added later in the study. Infant superoxide dismutase activity was also higher than maternal, with a median of 431.74 U/mL (range 176.62-1234.21) in infant samples and 172.77 U/mL (range 62.5-974.35) in maternal samples. There was no significant correlation between gestational age and any of the inflammatory compounds measured. As noted in **Tables 6-7**, there are significant correlations between many maternal and infant inflammatory compounds, reflecting the significant cross-talk between various components pro- and anti-inflammatory of the immune response. Because the primary focus of this project is the relationship between inflammatory compounds and nutritional compounds, subsequent analysis and results focus on this relationship. However, the factors that influence the correlation between cytokines in infancy will be analyzed in future studies of this dataset.

Cytokine (pg/mL)	Median (Q1, Q3)	Range
Infant samples		
IL-10	21.85 (12.26, 32.11)	2.94-472.95
IL-1β	8.52 (5.50, 12.18)	0-133.74
IL-2	5.72 (3.94, 10.54)	2.29-21.82
IL-6	13.72 (5.35, 50.82)	0-1997
IL-8	12.99 (7.36, 29.66)	3.14-1325
TNF-α	52.12 (42.52, 64.96)	16.39-298.79
Maternal samples		
IL-10	14.78 (7.68, 24.83)	-2e+308-105.42
IL-1β	6.3 (4.24, 10.21)	-2e+308-20.08
IL-2	7.43 (4.78, 10.96)	2.55-216.75
IL-6	11.05 (6.63, 46.16)	3.07-216.75
IL-8	7.84 (4.96, 16.285)	-2e+308-125.25
TNF-α	25.18 (17.42, 35.95)	-2e+308-473.96

Table 5: Median infant and maternal cytokine measurements (pg/mL). IL-10, IL-1β, IL-2, IL-6, IL-8, and TNF-α levels were measured in all 122 samples.

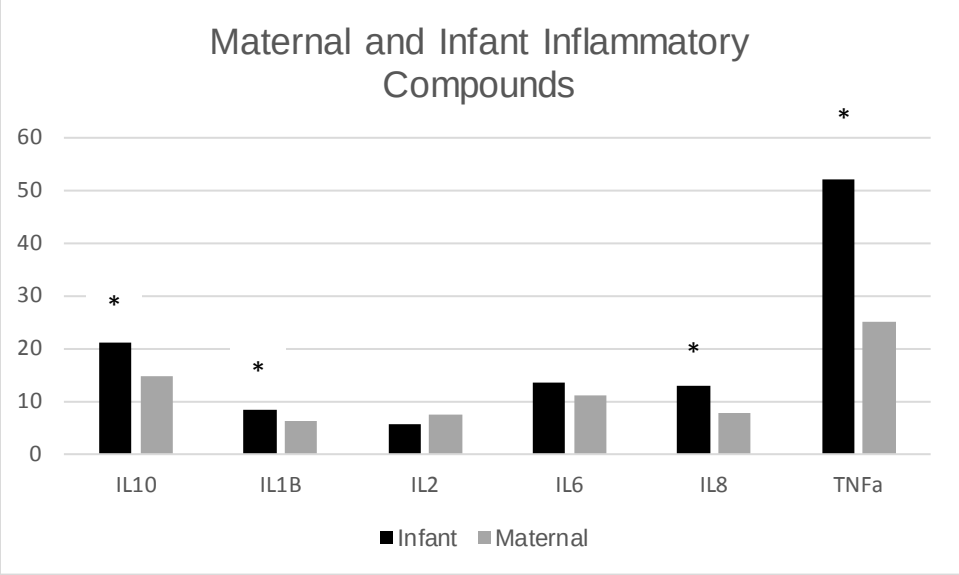

Figure 2: **Maternal and Infant Cytokines.** Median levels of infant (black) and maternal (grey) inflammatory compounds (pg/mL). *N*=122. * *p* <0.05

Inflammatory Compound Correlation with other Inflammatory Compound	**r_s (95% confidence interval)**
Infant IL-10	
Infant IL-10: Infant IL-1β	0.452 (0.298 to 0.583)
Infant IL-10: Infant IL-2	0.451 (0.297 to 0.582)
Infant IL-10: Infant IL-6	0.324 (0.155 to 0.474)
Infant IL-10: Infant TNF-α	0.312 (0.142 to 0.464)
Infant IL-10: Maternal Superoxide Dismutase	0.477 (0.327 to 0.604)
Infant IL-1β	
Infant IL-1β: Infant IL-10	0.452 (0.298 to 0.583)
Infant IL-1β: Infant IL-2	0.849 (0.791 to 0.892)
Infant IL-1β: Infant TNF-α	0.357 (0.191 to 0.503)
Infant IL-1β: Maternal IL-10	0.250 (0.076 to 0.41)
Infant IL-1β: Maternal TNF-α	0.266 (0.093 to 0.424)
Infant IL-2	
Infant IL-2: Infant IL-10	0.451 (0.297 to 0.582)
Infant IL-2: Infant IL-1β	0.849 (0.791 to 0.892)
Infant IL-2: Infant TNF-α	0.387 (0.225 to 0.528)
Infant IL-2: Maternal IL-10	0.231 (0.056 to 0.393)
Infant IL-2: Maternal TNF-α	0.234 (0.059 to 0.395)
Infant IL-6	
Infant IL-6: Infant IL-10	0.324 (0.155 to 0.474)
Infant IL-6: Infant IL-8	0.797 (0.721 to 0.854)
Infant IL-6: Infant TNF-α	0.302 (0.131 to 0.455)
Infant IL-6: Maternal IL-6	0.387 (0.225 to 0.528)
Infant IL-6: Maternal IL-8	0.341 (0.174 to 0.489)
Infant IL-8	
Infant IL-8: Infant IL-6	0.797 (0.721 to 0.854)
Infant IL-8: Infant TNF-α	0.290 (0.118 to 0.445)
Infant IL-8: Maternal IL-6	0.349 (0.183 to 0.496)
Infant IL-8: Maternal IL-8	0.219 (0.043 to 0.382)
Infant IL-8: Infant Superoxide Dismutase	0.463 (0.311 to 0.592)
Infant TNF-α	
Infant TNF-α: Infant IL-10	0.312 (0.142 to 0.464)
Infant TNF-α: Infant IL-1β	0.357 (0.191 to 0.503)
Infant TNF-α: Infant IL-2	0.387 (0.225 to 0.528)
Infant TNF-α: Infant IL-6	0.302 (0.131 to 0.455)
Infant TNF-α: Infant IL-8	0.290 (0.118 to 0.445)
Infant TNF-α: Maternal IL-6	0.231 (0.056 to 0.393)
Infant TNF-α: Maternal Superoxide Dismutase	0.440 (0.284 to 0.573)

Table 6: **Correlation between maternal and infant cytokines (infant)**. Spearman's correlation coefficients (r_s) were calculated for all inflammatory compounds in both infant and maternal samples (N=122). Significantly significant (p <0.05) relationships are included in this table. Other relationships were not statistically significant.

Inflammatory Compound Correlation with other Inflammatory Compound	**r_s (95% confidence interval)**
Maternal IL-10	
Maternal IL-10: Infant IL-1β	0.250 (0.076 to 0.41)
Maternal IL-10: Infant IL-2	0.231 (0.056 to 0.393)
Maternal IL-10: Maternal IL-1β	0.610 (0.485 to 0.711)
Maternal IL-10: Maternal IL-2	0.507 (0.362 to 0.628)
Maternal IL-10: Maternal IL-8	0.215 (0.039 to 0.378)
Maternal IL-10: Maternal TNF-α	0.548 (0.41 to 0.661)
Maternal IL-1β	
Maternal IL-1β: Maternal IL-10	0.610 (0.485 to 0.711)
Maternal IL-1β: Maternal IL-2	0.750 (0.66 to 0.819)
Maternal IL-1β: Maternal TNF-α	0.644 (0.527 to 0.737)
Maternal IL-2	
Maternal IL-2: Maternal IL-10	0.507 (0.362 to 0.628)
Maternal IL-2: Maternal IL-1β	0.750 (0.66 to 0.819)
Maternal IL-2: Maternal TNF-α	0.647 (0.53 to 0.74)
Maternal IL-6	
Maternal IL-6: Infant IL-6	0.387 (0.225 to 0.528)
Maternal IL-6: Infant IL-8	0.349 (0.183 to 0.496)
Maternal IL-6: Infant TNF-α	0.231 (0.056 to 0.393)
Maternal IL-6: Maternal IL-8	0.896 (0.854 to 0.926)
Maternal IL-6: Maternal Superoxide Dismutase	0.734 (0.64 to 0.807)
Maternal IL-8	
Maternal IL-8: Infant IL-6	0.341 (0.174 to 0.489)
Maternal IL-8: Infant IL-8	0.219 (0.043 to 0.382)
Maternal IL-8: Maternal IL-10	0.215 (0.039 to 0.378)
Maternal IL-8: Maternal IL-6	0.896 (0.854 to 0.926)
Maternal TNF-α	
Maternal TNF-α: Infant IL-1β	0.266 (0.093 to 0.424)
Maternal TNF-α: Infant IL-2	0.234 (0.059 to 0.395)
Maternal TNF-α: Maternal IL-10	0.548 (0.41 to 0.661)
Maternal TNF-α: Maternal IL-1β	0.644 (0.527 to 0.737)
Maternal TNF-α: Maternal IL-2	0.647 (0.53 to 0.74)

Table 7: **Correlation between maternal and infant cytokines (maternal).** Spearman's correlation coefficients (r_s) were calculated for all inflammatory compounds in both infant and maternal samples (N=122). Significantly significant (p <0.05) relationships are included in this table. Other relationships were not statistically significant.

Maternal and Infant Nutritional Compounds

In contrast to inflammatory compounds, in which infant measures tended to be higher than the maternal levels, the levels of nutritional compounds were significantly higher in mothers than in infants (**Table 8; Figures 3-4**). There were many statistically significant correlations between the cytokines and nutritional compounds evaluated, most frequently negative correlations between pro-inflammatory cytokines such as IL-1β and IL-8 and anti-inflammatory nutritional compounds such the lycopenes and carotenes (**Table 9**). However, these relationships were relatively weak (r_s = -0.213 to -0.308; r_s = 0.186 to 0.235). Additionally, there were several correlations that did not align with the predicted axis of anti-inflammatory compounds decreasing pro-inflammatory compounds and vice versa. These include infant TNF-α, which is a pro-inflammatory cytokine and was positively associated with maternal *cis*-lycopene (r_s = 0.186) and maternal total lycopene (r_s = 0.190), both anti-inflammatory nutritional compounds (**Table 9**). Maternal IL-6 and IL-8, both potent pro-inflammatory cytokines, were most often found to be negatively correlated with anti-inflammatory nutritional compounds. Maternal IL-6 and IL-8 are also positively correlated with infant levels of these pro-inflammatory compounds (**Table 7**), demonstrating the impact of maternal inflammation on the infant's inflammatory state and potential downstream effects of low levels of protective maternal nutritional compounds.

Nutritional Compound	Median (Q1, Q3)	Range
Infant samples		
Lutein + Zeaxanthin	34.84 (26.23, 50.66)	11.69-144.74
β-cryptoxanthin	17.22 (11.32, 25.49)	2.8-683.37
trans-lycopene	11.5 (8.30, 17.63)	0.63-223.62
cis-lycopene	12.74 (9.35, 18.58)	0.92-231.24
Total lycopene	24.17 (17.26, 35.60)	1.55-454.86
α-carotene	3.72 (1.48, 6.65)	0-939.42
trans-β-carotene	9.88 (5.18, 18.84)	0-262.34
cis-β-carotene	0 (0, 3.03)	0-24.23
Total β-carotene	10.2 (5.18, 21.29)	0-286.57
Retinol	166.76 (131.38, 208.1)	72.93-298.6
δ-tocopherol	17.06 (12, 29.3)	0-114.99
γ-tocopherol	207.22 (151.48, 275.3)	57.81-763.5
α-tocopherol	2941.48 (2192.66, 3654.24)	394.53-11904.2
Maternal samples		
Lutein + Zeaxanthin	216.51 (173.60, 273.11)	25.65-605.45
β-cryptoxanthin	140.68 (91.17, 196.96)	20.85-538.58
trans-lycopene	309.7 (234.20, 410.73)	10.33-609.44
cis-lycopene	263.94 (210.59, 354.02)	15.48-534.38
Total lycopene	574.83 (437.9, 764.01)	25.81-1128.24
α-carotene	44.31 (27.34, 77.46)	2.38-1022.34
trans-β-carotene	166.26 (92.41, 286.79)	7.08-2792.48
cis-β-carotene	12.99 (7.71, 25.97)	0-210.6
Total β-carotene	176.33 (99, 312.80)	7.08-3003.08
Retinol	297 (240.65, 365.55)	113.28-590.39
δ-tocopherol	110.07 (70.63, 182.21)	8.25-575.09
γ-tocopherol	1,751.91 (1274.53, 2315.96)	217.82-5,695.33
α-tocopherol	18,893.1 (15,057.95, 21,194.87)	3,898.27-35,538.29

Table 8: Median infant and maternal nutritional compounds (mcg/L). *N*=122

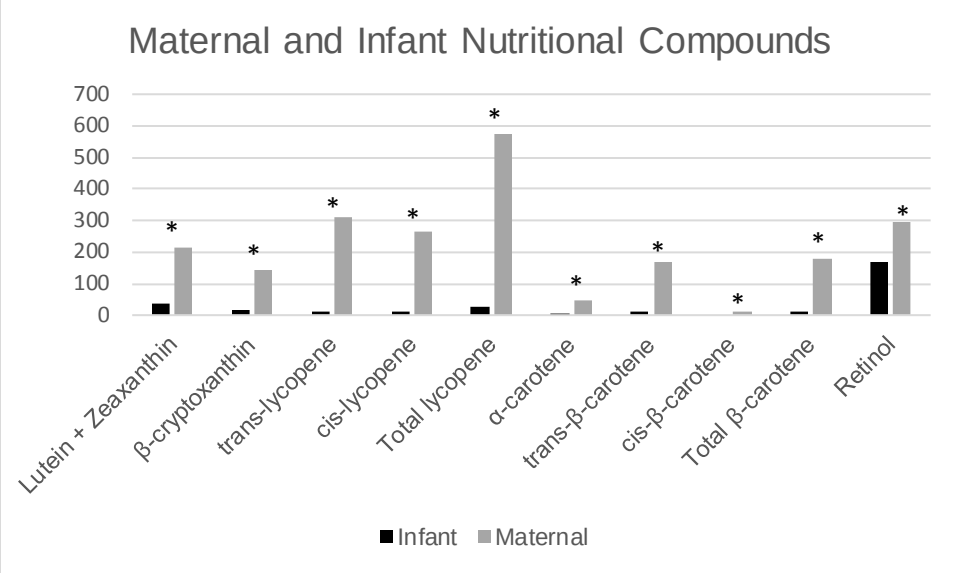

Figure 3: **Maternal and infant nutritional levels**. Median infant (black) and maternal (grey) levels of nutritional compounds (mcg/L). *N*=122 * $p < 0.001$

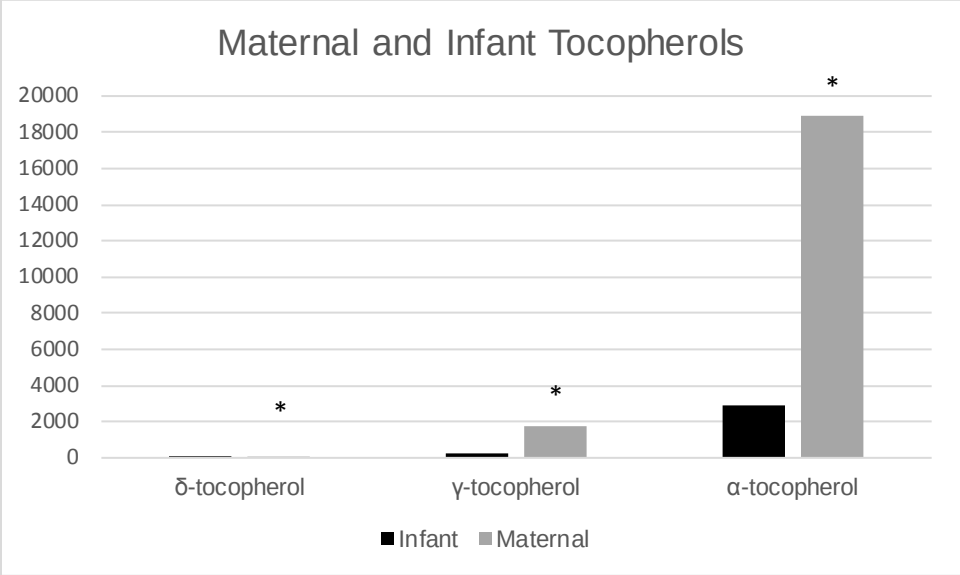

Figure 4: Maternal and infant tocopherols. Median maternal (grey) and infant (black) levels of δ-, γ-, and α-tocopherols (mcg/L). N=122 * $p < 0.001$

Cytokine: Nutritional Compound	r_s (95% confidence interval)
Infant IL-2	
Infant IL-2: Maternal α-tocopherol	0.235 (0.06 to 0.396)
Infant IL-8	
Infant IL-8: Infant δ-tocopherol	0.215 (0.039 to 0.378)
Infant IL-8: Infant retinol	-0.213 (-0.377 to -0.037)
Infant TNF-α	
Infant TNF-α: Maternal *cis*-lycopene	0.186 (0.009 to 0.352)
Infant TNF-α: Maternal total lycopene	0.190 (0.013 to 0.356)
Maternal IL-10	
Maternal IL-10: Infant α-carotene	0.226 (0.05 to 0.388)
Maternal IL-1β	
Maternal IL-1β: Infant β-cryptoxanthin	-0.218 (-0.381 to -0.042)
Maternal IL-1β: Maternal *trans*-β-carotene	-0.224 (-0.386 to -0.048)
Maternal IL-1β: Maternal total β-carotene	-0.220 (-0.383 to -0.044)
Maternal IL-2	
Maternal IL-2: Maternal δ-tocopherol	0.208 (0.031 to 0.372)
Maternal IL-6	
Maternal IL-6: Maternal δ-tocopherol	-0.231 (-0.393 to -0.056)
Maternal IL-6: Infant β-cryptoxanthin	-0.239 (-0.4 to -0.064)
Maternal IL-6: Infant *cis*-lycopene	-0.228 (-0.39 to -0.052)
Maternal IL-6: Infant total lycopene	-0.234 (-0.395 to -0.059)
Maternal IL-6: Maternal lutein + zeaxanthin	-0.224 (-0.386 to -0.048)
Maternal IL-8	
Maternal IL-8: Infant *trans*-lycopene	-0.254 (-0.413 to -0.08)
Maternal IL-8: Infant *cis*-lycopene	-0.308 (-0.461 to -0.138)
Maternal IL-8: Infant total lycopene	-0.296 (-0.45 to -0.125)
Maternal IL-8: Infant *trans*-carotene	-0.267 (-0.425 to -0.094)
Maternal IL-8: Infant total carotene	-0.263 (-0.421 to -0.089)
Maternal IL-8: Maternal *trans*-carotene	-0.206 (-0.37 to -0.029)
Maternal IL-8: Maternal *cis*-carotene	-0.267 (-0.425 to -0.094)
Maternal IL-8: Maternal total carotene	-0.213 (-0.377 to -0.037)

Table 9: **Correlation between cytokines and nutritional compounds**. Spearman's correlation coefficients (r_s) were calculated for all inflammatory and nutritional compounds in both infant and maternal samples (N=122). Statistically significant (p <0.05) relationships are included in this table. Other relationships were not statistically significant.

Factors Affecting the Correlation between Inflammatory and Nutritional Compounds

To address Specific Aim 3, we compared the inflammatory and nutritional compounds in term and preterm infants, and then used the Fisher z-transformation to determine if the correlation between cytokines and nutritional compounds were significantly different between term and preterm infants. There was no statistically significant difference between the inflammatory or nutritional compounds measured in term versus preterm infants in this dataset (**Tables 10-11**). However, we found that the positive correlation between anti-inflammatory infant IL-2 and anti-inflammatory maternal α-tocopherol was significantly stronger in preterm (r_s = 0.667) versus term infants (r_s = 0.185) (**Table 14**). The correlation between maternal IL-2, which is anti-inflammatory, and maternal δ-tocopherol, which is also anti-inflammatory, was also significantly different, with a strongly negative correlation (r_s = -0.661) in preterm infants and a weakly positive correlation in term infants (r_s = 0.308) (**Table 14**). None of the other correlations between inflammatory and nutritional compounds, as identified on analysis of the entire dataset, were significantly different between the term and preterm infants.

Given the large number of current or former tobacco users in this dataset (38.5%) and increased inflammatory responses associated with prenatal tobacco use[29-31], we also compared the levels of inflammatory and nutritional compounds in samples from mothers and infants with current or former tobacco use versus those without tobacco use. We found that infants born to mothers with current or former tobacco use had significantly higher levels of pro-inflammatory IL-8 (**Table 10**). Additionally, both infant and maternal samples had higher levels of anti-inflammatory δ-tocopherol and lower levels of anti-inflammatory

lutein, *trans*-β-carotene and total β-carotene than mother-infant pairs without tobacco use (**Table 11**). We then used the Fisher z-transformation to determine if the correlation between cytokines and nutritional compounds were significantly different between samples with no prior tobacco use versus current or former tobacco use. We found that the correlation between anti-inflammatory infant IL-2 and anti-inflammatory maternal α-tocopherol in infants born to mothers without tobacco use (r_S = 0.139) was significantly different from that observed in infants born to mothers with current or prior tobacco use (r_S = -0.390) (**Table 16**). The correlation between pro-inflammatory infant TNF-α and anti-inflammatory maternal *cis*- and total lycopene were also significantly different when comparing results from mother with no tobacco use and those with prior or current tobacco use (**Table 16**).

There were no statistically significant differences in the levels of inflammatory or nutritional compounds in infants with or without prenatal complications. Given the small number of participants with a prenatal complication (n=17, 13.9%), we did not evaluate the difference in correlation between inflammatory and nutritional compounds in this population versus those without complications. There were statistically significant differences in the levels of several nutritional compounds in white versus non-white participants (**Tables 12-13**). Therefore, race was included in the regression analyses going forward.

	Overall	Term	Pre-term	Non-smokers	Smokers
Infant samples					
IL-10	21.29	21.29	21.52	21.29	21.35
IL-1β	8.52	8.53	8.385	8.60	8.39
IL-2	5.72	5.77	5.62	6.82	5.62
IL-6	13.72	13.72	18.80	14.92	9.39
*IL-8	12.99	11.86	22.05	11.12*	15.71*
TNF-α	52.12	51.36	58.27	55.34	51.21
Maternal samples					
IL-10	14.78	14.62	22.485	14.87	14.78
IL-1β	6.3	6.07	7.16	7.135	5.35
IL-2	7.43	7.43	7.95	7.51	5.84
IL-6	11.05	10.695	46.16	10.695	14.79
IL-8	7.84	7.36	8.58	7.49	8.17
TNF-α	25.18	24.31	32.72	24.7	25.405

Table 10: **Median differences in cytokines in infants and mothers based on prematurity and tobacco use.** Median inflammatory compound levels (pg/mL) in the total participant dataset; term deliveries; pre-term (<37 weeks) deliveries; participants with no evidence of maternal tobacco use (non-smokers); and participants with current or prior tobacco use (smokers). Inflammatory compounds noted with * are statistically significant with p <0.05 by Mann-Whitney test. There were no statistically significant differences between the medians of term and pre-term deliveries. There were also no statistically significant differences between the medians of deliveries with or without prenatal complications.

Nutritional Compound	Overall	Term	Pre-term	Non-smokers	Smokers
Infant samples					
Lutein + Zeaxanthin	34.84	35.65	32.14	37.07	31.82
β-cryptoxanthin	17.22	18.82	13.865	19.59	15.26
trans-lycopene	11.5	11.92	10.84	11.64	11.325
cis-lycopene	12.74	13.17	11.38	13.06	12.375
Total lycopene	24.17	25.81	22.275	24.47	23.385
α-carotene	3.72	3.73	3.68	4.28	3.18
trans-β-carotene	9.88	10.01	7.75	11.37	7.695
cis-β-carotene	0	0	0	0	0
Total β-carotene	10.2	10.99	8	12.24	8.17
Retinol	166.76	170.39	131.29	167.53	162.64
*δ-tocopherol	17.06	16.89	18.86	15.01*	21.735*
*γ-tocopherol	207.22	204.67	255.905	172.16*	260.275*
α-tocopherol	2941.48	2916.82	3265.5	3062.24	2833.19
Maternal samples					
*Lutein + Zeaxanthin	216.51	219.37	191.26	240.91*	191.83*
β-cryptoxanthin	140.68	142.47	87.57	145.56	123.54
trans-lycopene	309.7	318.03	231.15	310.88	304.96
cis-lycopene	263.94	269.32	221.65	264.44	261.8
Total lycopene	574.83	608.41	449.38	568.95	584.67
α-carotene	44.31	44.31	38.75	47.93	37.66
**trans*-β-carotene	166.26	169.91	93.51	200.69*	137.42*
cis-β-carotene	12.99	13.44	8.46	14.03	11.47
*Total β-carotene	176.33	185.75	102.54	214.44*	147.08*
Retinol	297	300.63	249.4	300.46	292.75
*δ-tocopherol	110.07	110.71	91.15	94.50*	137.79*
γ-tocopherol	1751.91	1651.54	1851.08	1520.59	1978.86
α-tocopherol	18893.1	18893.1	19044.96	19007.65	18622.93

Table 11: **Median differences in nutritional compounds in infants and mothers based on prematurity and tobacco use.** Median nutritional levels (mcg/L) in the total participant dataset; term deliveries; pre-term (<37 weeks) deliveries; participants with no evidence of maternal tobacco use (non-smokers); and participants with current or prior tobacco use (smokers). Nutritional variables noted with * are statistically significant with p <0.05 by Mann-Whitney test. There were no statistically significant differences between the means of term and pre-term deliveries. There were also no statistically significant differences between the medians of deliveries with or without prenatal complications.

	Overall	White	Non-White
Infant samples			
IL-10	21.29	20.51	24.43
*IL-1β	8.52	8.26*	13.35*
IL-2	5.72	5.65	6.96
IL-6	13.72	12.12	16.85
IL-8	12.99	11.96	14.04
TNF-α	52.12	51.5	55.2
Maternal samples			
IL-10	14.78	15.825	12.58
IL-1β	6.3	6.605	5.78
IL-2	7.43	6.685	7.985
*IL-6	11.05	14.79*	9.12*
IL-8	7.84	8.95	6.73
TNF-α	25.18	25.775	23.72

Table 12: **Median differences in cytokines in infants and mothers based on race/ethnicity.** Median inflammatory compound levels (pg/mL) in the total participant dataset; mothers identifying as white/Caucasian (*n*=85); mothers identifying as a race/ethnicity other than white (*n*=37), including African American (19), Hispanic (7), Asian/Pacific Islander (3), Native American (1), and other/unknown (7). Nutritional variables noted with * are statistically significant with $p < 0.05$ by Mann-Whitney test.

Nutritional Compound	Overall	White	Non-white
Infant samples			
Lutein + Zeaxanthin	34.84	33.23	41.91
*β-cryptoxanthin	17.22	15.615*	22.39*
**trans*-lycopene	11.5	10.67*	14.03*
**cis*-lycopene	12.74	12.115*	14.2*
*Total lycopene	24.17	22.205*	30.64*
α-carotene	3.72	3.945	3.23
trans-β-carotene	9.88	9.8	10.68
cis-β-carotene	0	0	0
Total β-carotene	10.2	10.135	11.19
Retinol	166.76	167.145	166.14
δ-tocopherol	17.06	16.655	17.32
γ-tocopherol	207.22	197.15	254.67
α-tocopherol	2941.48	2857.575	3167.8
Maternal samples			
Lutein + Zeaxanthin	216.51	203.1	236.465
β-cryptoxanthin	140.68	134.97	181.58
trans-lycopene	309.7	294.31	355.075
cis-lycopene	263.94	249.42	339.44
Total lycopene	574.83	544.85	672.64
α-carotene	44.31	47.31	37.94
trans-β-carotene	166.26	174.8	130.125
cis-β-carotene	12.99	13.75	11.575
Total β-carotene	176.33	187.79	138.86
*Retinol	297	309.1*	261.345*
δ-tocopherol	110.07	103.84	138.74
γ-tocopherol	1751.91	1623.74	1779.21
α-tocopherol	18893.1	18960.17	17522.94

Table 13: **Median differences in nutritional compounds in infants and mothers based on race/ethnicity.** Median nutritional levels (mcg/L) in the total participant dataset; mothers identifying as white/Caucasian (*n*=85); mothers identifying as a race/ethnicity other than white (*n*=37), including African American (19), Hispanic (7), Asian/Pacific Islander (3), Native American (1), and other/unknown (7). Nutritional variables noted with * are statistically significant with $p < 0.05$ by Mann-Whitney test.

Cytokine: Nutritional Compound	Term Infants r_s (n= 108)	Preterm Infants r_s (n= 14)	Fisher z-transformation
Infant IL-2			
Infant IL-2: Maternal α-tocopherol	0.185	0.667*	-1.95*
Infant IL-8			
Infant IL-8: Infant δ-tocopherol	0.224*	0.332	-0.37
Infant IL-8: Infant retinol	-0.174	-0.284	0.37
Infant TNF-α			
Infant TNF-α: Maternal *cis*-lycopene	0.263*	-0.154	1.34
Infant TNF-α: Maternal total lycopene	0.284*	-0.176	1.48
Maternal IL-10			
Maternal IL-10: Infant α-carotene	0.192	0.538*	-1.28
Maternal IL-1β			
Maternal IL-1β: Infant β-cryptoxanthin	-0.248*	0.082	-1.06
Maternal IL-1β: Maternal *trans*-β-carotene	-0.265*	0.045	-1
Maternal IL-1β: Maternal total β-carotene	-0.266*	0.091	-1.15
Maternal IL-2			
Maternal IL-2: Maternal δ-tocopherol	0.308*	-0.661*	3.51*
Maternal IL-6			
Maternal IL-6: Infant β-cryptoxanthin	-0.254*	-0.200	-0.18
Maternal IL-6: Infant *cis*-lycopene	-0.177	-0.133	-0.14
Maternal IL-6: Infant total lycopene	-0.194	-0.133	-0.14
Maternal IL-6: Maternal lutein + zeaxanthin	-0.201	-0.167	-0.11
Maternal IL-6: Maternal δ-tocopherol	-0.183	-0.417	0.082

Table 14: **Difference in correlation between inflammation and nutritional compounds based on prematurity**. Spearman's correlation coefficients (r_s) were compared between samples in which the infant was born at term (n=108) versus preterm (<37 weeks estimated gestational age; n=14) for all statistically significant relationships identified on initial analysis. All statistically significant results (p<0.05) are indicated with *.

Cytokine: Nutritional Compound	Term Infants r_s (n= 108)	Preterm Infants r_s (n= 14)	Fisher z-transformation
Maternal IL-8			
Maternal IL-8: Infant *trans*-lycopene	-0.262*	-0.178	-0.28
Maternal IL-8: Infant *cis*-lycopene	-0.313*	-0.064	-0.82
Maternal IL-8: Infant total lycopene	-0.310*	-0.134	-0.59
Maternal IL-8: Infant *trans*-carotene	-0.306*	0.002	-1
Maternal IL-8: Infant total carotene	-0.303*	-0.011	-1.02
Maternal IL-8: Maternal *trans*-carotene	-0.273*	0.182	-1.46
Maternal IL-8: Maternal *cis*-carotene	-0.286*	-0.108	-0.59
Maternal IL-8: Maternal total carotene	-0.271	0.099	-1.19

Table 15: **Difference in correlation between inflammation and nutritional compounds based on prematurity (maternal IL-8)**. Spearman's correlation coefficients (r_s) were compared between samples in which the infant was born at term (n=108) versus preterm (<37 weeks estimated gestational age; n=14) for all statistically significant relationships identified on initial analysis. All statistically significant results (p<0.05) are indicated with *.

Cytokine: Nutritional Compound	**NonSmokers r_s** (*n*= 74)	**Smokers r_s** (*n*= 47)	**Fisher z-transformation**
Infant IL-2			
Infant IL-2: Maternal α-tocopherol	0.139	-0.390*	2.88*
Infant IL-8			
Infant IL-8: Infant δ-tocopherol	0.295*	0.048	1.33
Infant IL-8: Infant retinol	-0.283*	-0.145	-0.76
Infant TNF-α			
Infant TNF-α: Maternal *cis*-lycopene	0.204	-0.184	2.05*
Infant TNF-α: Maternal total lycopene	0.231	-0.169	2.12*
Maternal IL-10			
Maternal IL-10: Infant α-carotene	0.196	0.303*	-0.6
Maternal IL-1β			
Maternal IL-1β: Infant β-cryptoxanthin	-0.225	-0.327	0.58
Maternal IL-1β: Maternal *trans*-β-carotene	-0.253	-0.253	0
Maternal IL-1β: Maternal total β-carotene	-0.255	-0.243	-0.07
Maternal IL-2			
Maternal IL-2: Maternal δ-tocopherol	0.229	0.330	-0.57
Maternal IL-6			
Maternal IL-6: Infant β-cryptoxanthin	-0.410*	-0.023	-2.09
Maternal IL-6: Infant *cis*-lycopene	-0.314*	-0.026	-1.56
Maternal IL-6: Infant total lycopene	-0.336*	-0.084	-1.38
Maternal IL-6: Maternal lutein + zeaxanthin	-0.315*	-0.06	-1.39
Maternal IL-6: Maternal δ-tocopherol	-0.268	-0.171	-0.53

Table 16: **Difference in correlation between inflammation and nutritional compounds based on tobacco use.** Spearman's correlation coefficients (r_s) were compared between samples with no current or prior maternal tobacco use (*n*=74) and those with current or prior maternal tobacco use (*n*=47) for all statistically significant relationships identified on initial analysis. All statistically significant results ($p<0.05$) are indicated with *.

Cytokine: Nutritional Compound	NonSmokers r_s (n= 74)	Smokers r_s (n= 47)	Fisher z-transformation
Maternal IL-8			
Maternal IL-8: Infant *trans*-lycopene	-0.327*	-0.188	-0.78
Maternal IL-8: Infant *cis*-lycopene	-0.339*	-0.256	-0.048
Maternal IL-8: Infant total lycopene	-0.352*	-0.264	-0.51
Maternal IL-8: Infant *trans*-carotene	-0.262*	-0.305	0.24
Maternal IL-8: Infant total carotene	-0.249	-0.307	0.33
Maternal IL-8: Maternal *trans*-carotene	-0.230	-0.105	-0.67
Maternal IL-8: Maternal *cis*-carotene	-0.281*	-0.158	-0.67
Maternal IL-8: Maternal total carotene	-0.241	-0.110	-0.71

Table 17: **Difference in correlation between inflammation and nutritional compounds based on tobacco use (maternal IL-8).** Spearman's correlation coefficients (r_s) were compared between samples with no current or prior maternal tobacco use (n=74) and those with current or prior maternal tobacco use (n=47) for all statistically significant relationships identified on initial analysis. All statistically significant results (p<0.05) are indicated with *.

Based on the differences observed in the correlations between inflammatory and nutritional compounds based on prematurity and maternal tobacco use, we then used multiple regression to identify the relative impact of prematurity and tobacco use, as defined by current or former tobacco use, on this correlation. The cytokine and nutritional compound levels were first log10 transformed to allow for analysis of normalized data and Pearson coefficients were calculated for all inflammatory-nutritional compound relationship identified as significant in Spearman correlation analysis. For all statistically significant Pearson coefficients, multiple regression was performed with the outcome defined as the cytokine of interest and the nutritional compound defined as the predictor. Neither prematurity nor tobacco use were significant variables in any of the models. As expected, for many pro-inflammatory cytokines (IL-1β, IL-6, and IL-8), there is a reduction in the level of the pro-inflammatory cytokine relative to the level of the anti-inflammatory nutritional compound (**Table 18**).

Our analyses demonstrated significant correlations between the inflammatory outcome compounds, particularly IL-6 and IL-8, and several nutritional compounds. This could reflect the multifactorial influence of these nutritional compounds on maternal and infant inflammation. Therefore, the model for each of the cytokines with multiple significant nutritional compounds associations was expanded to include each of the nutritional compounds as potential variables in addition to prematurity, tobacco use, and non-white race. When both *cis*- and total lycopene are included in the model, as well as tobacco use, prematurity and non-white, neither nutritional compound is significant in the model (Log10 Maternal IL-6 = 1.249 – 0.313*non-white race + 0.360*prematurity). However, maternal IL-1β did retain an inverse relationship with maternal *trans*-carotene (Log10 Maternal IL-1β = 1.173 – 0.156*log10 maternal *trans*-carotene). Non-white race, tobacco

use and prematurity were not significant in the maternal IL-1β regression model. Similarly, maternal IL-8 retained an inverse relationship with anti-inflammatory nutritional compounds (Log10 Maternal IL-8 = 1.667 – 0.29*log10 maternal *cis*-carotene – 0.291*log10 infant *cis*-lycopene). Non-white race, tobacco use and prematurity were not significant in the maternal IL-8 regression model.

Association Between Inflammatory and Nutritional Compounds and Adverse Infant Outcomes

A small number of infants included in this dataset (n=10) were diagnosed with respiratory distress syndrome (RDS) in the neonatal period. These infants had significantly higher infant IL-8 (with RDS 62.55 pg/mL; without RDS 11.75 pg/mL; $p = 0.019$), a potent pro-inflammatory cytokine, and lower maternal *cis*-lycopene (with RDS 234.79 mcg/L; without RDS 269.04 mcg/L; $p = 0.022$), an anti-inflammatory nutritional compound, than those without respiratory distress syndrome. There were no other significant differences in inflammatory or nutritional compounds between those with RDS versus those without RDS. There were no other adverse infant outcomes reported with this study.

Cytokine	Intercept	Nutritional Variable
Infant IL-8		
Infant IL-8	3.495	-1.025*infant retinol
Infant IL-2		
Infant IL-2	0.618	0.184*infant *trans*-lycopene
Maternal IL-1β		
Maternal IL-1β	1.173	-0.156*maternal *trans*-carotene
Maternal IL-1β	1.175	-0.154*maternal total carotene
Maternal IL-6		
Maternal IL-6	1.660	-0.404*infant *cis*-lycopene
Maternal IL-6	1.808	-0.427*infant total lycopene
Maternal IL-8		
Maternal IL-8	1.185	-0.243*infant α-carotene
Maternal IL-8	1.385	-0.339*infant *cis*-lycopene
Maternal IL-8	1.460	-0.324*infant total lycopene
Maternal IL-8	1.298	-0.282*infant *trans*-carotene
Maternal IL-8	1.287	-0.272*infant total carotene
Maternal IL-8	1.382	-0.325*maternal *cis*-carotene

Table 18: **Regression models for inflammation and nutritional compounds**. Pearson coefficients were calculated for all inflammatory-nutritional compound relationships identified as significant on Spearman correlation analysis. For all statistically significant Pearson coefficients, multiple regression was performed to identify the impact of prematurity and of tobacco use (as defined by current or former tobacco use) on the relationship between the inflammatory and nutritional variable. Neither prematurity nor tobacco use were significant variables in any of the relationships observed.

CHAPTER FOUR: DISCUSSION

The "first 1000 days" have the potential to lay the foundation for the rest of a child's life.[7] Increased inflammation in the perinatal period has been associated with many adverse outcomes with lifelong impact, including poor growth, atopic diseases such as asthma, and neurologic complications such as cerebral palsy[8,9,18,32]. A better understanding of the relationship between inflammation and nutrition could identify key strategies for supporting optimal maternal and infant nutrition. However, the relationship between nutritional and inflammatory compounds in infants and mother in the peri-natal period is not well-defined. In this study, we confirmed our hypothesis that there are significant inverse relationships between anti-inflammatory nutritional compounds such as carotenoids and pro-inflammatory cytokines such as IL-1β, IL-6, and IL-8. This suggests that improving maternal and infant intake of carotenoids and other anti-inflammatory nutritional compounds could significant decrease inflammation.

Nutritional compounds including retinol, lutein, β-cryptoxanthin, tocopherols, lycopene and carotene were higher in maternal versus infant samples in this participant group. The correlation between these anti-inflammatory nutritional compounds and pro-inflammatory cytokines IL-6, IL-8, and IL-1β suggest that nutritional interventions could be used decrease perinatal inflammation in mothers and infants. This may be particularly important in preterm infants and in pregnancies complicated by tobacco use, as evidenced by the significant differences observed in these groups in this study (**Tables 14-17**). Interestingly, we did not observe an positive correlation between anti-inflammatory IL-10 and anti-inflammatory nutritional compounds, suggesting that the decreased inflammation observed is more attributable to direct effects on the pro-inflammatory cytokines rather than a bolstering of anti-inflammatory cytokines.

Our results demonstrate the complex interplay among pro- and anti-inflammatory cytokines in the immune response, as well as the potential for multi-factorial influences on immunity. It is promising that there was still a significant relationship between maternal inflammatory cytokines IL-1β and IL-8, even with multiple factors taken into account in the modeling, such as the role of other nutritional compounds, tobacco use, prematurity and race. We observed a proportional decrease in maternal inflammatory cytokines with increases in maternal *trans*-carotene and *cis*-carotene, as well as infant *cis*-lycopene. Given the association in our results between maternal and infant inflammation, increasing maternal levels of these carotenoids could therefore have significant impact on perinatal inflammation and its myriad adverse consequences.

Carotenes, vitamins C, E and D have shown promise as an anti-inflammatory compounds in a variety of adult disorders, including, prostate cancer and cardiovascular disease[21,33-39]. However, these studies in infants are lacking and this area of investigation has been highlighted as a key gap in pediatric knowledge by the National Institute of Child Health and Human Development[20]. Carotenoids were noted in many of our analyses to have significant protective potential. Carotenoids are vitamin-A related compounds, including α-, β-, *cis-,* and *trans*-carotene, lycopene, lutein and β-cryptoxanthin, found primarily in plant-based foods[33,35]. Many studies have shown that carotenoids may have anti-inflammatory and antioxidant properties that impact human health and disease, including in maternal-infant dyad studies such as this one.[33-36] Plasma levels of carotenoids in mothers are strongly related to the dietary intake of fruits and vegetables[33], making this an important target of both individual prenatal nutritional counseling as well as large-scale programmatic strategies for optimizing prenatal nutrition.

Limitations of this study include the small sample size, particularly in regards to the number of preterm mother-infant pairs enrolled. This may have limited our ability to detect

significant differences in preterm infants. This pilot data will be expanded to a larger sample size in the future to further evaluate the impact of prematurity and tobacco use on the relationship between inflammation and nutrition. Additionally, as this is an observational study, the effects observed here may be due to another variable that was not included in our data analysis. Inflammation is a multifactorial process and controlled studies will be essential for demonstrating definitive links between higher levels of carotenoids and decreased inflammation. Long-term studies will also be needed to evaluate the impact of these interventions on child development and health.

Developing nutritional approaches to mitigating inflammation offers the opportunity for both pre- and post-natal interventions. The results of this study are particularly promising in highlighting the relationship between maternal anti-inflammatory nutritional compounds and infant pro-inflammatory cytokines. The interactions between nutritional status and inflammation represent a potential therapeutic target for reducing inflammation in term and pre-term infants, who may be particularly susceptible to inflammation during the period of rapid neurodevelopment in infancy[7-9]. Additionally, this information could lead to earlier identification of infants at risk for neurodevelopmental disorders or other adverse inflammatory outcomes due to dietary insufficiencies during these crucial periods. Early identification could guide more aggressive or specifically targeted behavioral therapies in addition to nutritional supplementation to improve overall clinical outcomes. Thus, defining the factors that shape the inflammatory response in infants has the potential to significantly impact the health of neonates and infants in the US and throughout the world, particularly in developing countries.

REFERENCES

1. March of Dimes. “Premature Birth Report Card of the United States 2016.” Accessed at http://www.marchofdimes.org/materials/premature-birth-report-card-united-states.pdf on 9/11/17.
2. Ochiai M, Kinjo T, Takahata Y, et al. Survival and neurodevelopmental outcome of preterm infants born at 22-24 weeks of gestational age. *Neonatology* 2014; 105(2):79-84.
3. Serenius F, Ewald U, Farooqi A, et al. Neurodevelopmental Outcomes Among Extremely Preterm Infants 6.5 Years After Active Perinatal Care in Sweden. *JAMA Pediatr* 2016; 170:954.
4. Larroque B, Ancel PY, Marret S, et al. Neurodevelopmental disabilities and special care of 5-year-old children born before 33 weeks of gestation (the EPIPAGE study): a longitudinal cohort study. *Lancet* 2008; 371:813.
5. Kiechl-Kohlendorfer U, Ralser E, Pupp Peglow U, et al. Adverse neurodevelopmental outcome in preterm infants: risk factor profiles for different gestational ages. *Acta Paediatr* 2009; 98:792.
6. Kerstjens JM, de Winter AF, Bocca-Tjeertes IF, et al. Developmental delay in moderately preterm-born children at school entry. *J Pediatr* 2011; 159:92.
7. UNICEF. “The first 1,000 days of life: The brain’s window of opportunity.” Accessed at https://www.unicef-irc.org/article/958/ on 9/11/17.
8. Armstrong-Wells J, Donnelly M, Post MD, et al. Inflammatory predictors of neurologic disability after preterm premature rupture of membranes. *Am J Obstet Gynecol*. 2015 Feb;212(2):212.e1-9.

9. Bi D, Chen M, Zhang X, et al. The association between sex-related interleukin-6 gene polymorphisms and the risk for cerebral palsy. *J Neuroinflammation*. 2014;11:100.
10. Bilbo SD, Rudy JW, Watkins LR, Maier SF. A behavioural characterization of neonatal infection-facilitated memory impairment in adult rats. *Behav Brain Res*. 2006;169(1):39-47.
11. Bilbo SD, Newsum NJ, Sprunger DB, et al. Differential effects of neonatal handling on early life infection-induced alterations in cognition in adulthood. *Brain Behav Immun*. 2007;21(3):332-342.
12. Williamson LL, Sholar PW, Mistry RS, et al. Microglia and memory: Modulation by early-life infection. *J Neurosci*. 2011;31(43):15511-15521.
13. Bilbo SD, Levkoff LH, Mahoney JH, et al. Neonatal infection induces memory impairments following an immune challenge in adulthood. *Behav Neurosci*. 2005;119(1):293-301.
14. Gutierrez-Murgas Y, Skar G, Ramirez D, et al. IL-10 plays an important role in the control of inflammation but not in the bacterial burden in *S. epidermidis* CNS catheter infection. *J Neuroimmunol.* 2016 Oct 13; 13(1):271.
15. Wu YQ, Shen J, Zhou QL, et al. Interleukin-6 and interleukin-8 in diagnosing neonatal septicemia. *J Biol Regul Homeost Agents*. 2016; Oct-Dec;30(4):1107-1113.
16. Kocabas E, Sarikcioglu A, Aksaray N, et al. Role of pro-calcitonin, C-reactive protein, interleukin-6, interleukin-8 and tumor necrosis factor-alpha in the diagnosis of neonatal sepsis. *Turk J Pediatr*. 2007 Jan-Mar; 49(1):7-20.
17. Shah MD, Shah SR. Nutrient deficiencies in the premature infant. *Pediatr Clin North Am*. 2009 Oct;56(5):1069-83.

18. Ozsurekci Y, Aykac K. Oxidative stress related diseases in newborns. *Oxid Med Cell Longev*. 2016; 2016:2768365.
19. Hanson C, Lyden E, Furtado J, et al. A comparison of nutritional antioxidant content in breast milk, donor milk, and infant formulas. *Nutrients*. 2016 Oct 28;8(11):pii:E681.
20. Kutlesic V, Brewinski Isaacs, M, et al. Executive Summary: Research Gaps at the Intersection of Pediatric Neurodevelopment, Nutrition, and Inflammation in Low-Resource Settings. *Pediatrics*. 2017; 139:s1.
21. Garcia-Bailo B, Roke K, Mutch DM, El-Sohemy A, Badawi A. Association between circulating ascorbic acid, α-tocopherol, 25-hydroxyvitamin D, and plasma cytokine concentrations in young adults: a cross-sectional study. *Nutrition & Metabolism*. 2012; 9:102.
22. Yahfoufi N, Alsodi N, Jambi M, Matar C. The immunology and anti-inflammatory role of polyphenols. *Nutrients*. 2018; 10(11): 1618.
23. Dojindo Molecular Technologies. “SOD Assay Kit-WST” Accessed at https://www.dojindo.com/store/p/203-SOD-Assay-Kit-WST.html on 11/11/18.
24. Zhou ZQ, Li F, Zheng ZT, Li YD, et al. Erythropoietin regulates immune/inflammatory reaction and improves neurological function outcomes in traumatic brain injury. *Brain Behav* 2017 Oct 3;7(11):e00827.
25. Machova Urdzikova L, Ruzicka J, Karova K, Kloudova A, et al. A green tea polyphenol epigallocatechin-3-gallate enhances neuroregeneration after spinal cord injury by altering levels of inflammatory cytokines. *Neuropharmacology* 2017 Nov; 126:213-223.
26. Littell RC, Stroup WW, Freund RJ. SAS for Linear Models, 4th Edition. SAS Institute Inc., Gary, NC: 2002.

27. Zar JH. Biostatistical Analysis. Second Edition. Prentice-Hall. 1984.Englewood Cliffs, New Jersey.

28. Randles RH, Wolfe DA. Introduction to the Theory of Nonparametric Statistics. Krieger Pub., Malabar, FL; 1991.

29. Niu Z, Xie C, Wen X, Tian F, et al. Potential pathways by which maternal second-hand smoke exposure during pregnancy causes full-term low birth weight. *Scientific Reports*. 2016; 6:24987.

30. Xiao R, Perveen Z, Rouse R, Le Donne V, et al. *In utero* exposure to second-hand smoke aggravates the response to ovalbumin in adult mice. *Am J Respir Cell Mol Biol*. 2013; 49(6) 1102-1109.

31. Niu Z, Xie C, Wen X, Tian F, et al. Mediating role of maternal serum interleukin-1beta and tumor necrosis factor-alpha in the association between environmental tobacco smoke exposure in pregnancy and low birth weight at term. *J Maternal-Fetal and Neonatal Medicine*. 2017; 31(10): 1251-1258.

32. Yanni D, Korzeniewski SJ, Allred EN, Fichorova RN, et al. Both antenatal and postnatal inflammation contribute information about the risk of brain damage in extremely preterm newborns. *Pediatr Res*. 2017 Oct; 82(4): 691-696.

33. Zielinska M, Wesolowska A, Pawlus B, Hamulka J. Health effects of carotenoids during pregnancy and lactation. *Nutrients*. 2017; 9(8): 838.

34. Kaulmann A, Bohn T. Carotenoids, inflammation, and oxidative stress-implications of cellular signaling pathways and relation to chronic disease prevention. *Nutr Res*. 2014; 34(11):907-929.

35. Hanson C, Lyden E, Anderson-Berry A, Kocmich N, et al. Status of retinoids and carotenoids and association with clinical outcomes in maternal-infant pairs in Nigeria. *Nutrients*. 2018; 10(9): 1286.

36. Jiang L, Liu Y, Li B. Lycopene exerts anti-inflammatory effect to inhibit prostate cancer progression. *Asian J Androl*. 2018; Sep 7. Epub ahead of print.
37. PetyaevIM, Dovgalevsky PY, Klochokov VA, Chalyk NE, et al. Effect of lycopene supplementation on cardiovascular parameters and markers of inflammation and oxidation in patients with coronary vascular disease. *Food Sci Nutr*. 2018; 6(6):1770-1777.
38. Maslova E, Hansen S, Strom M, Halldorsson T, Olsen S. Maternal intake of vitamins A, E and K in pregnancy and child allergic disease: a longitudinal study from the Danish National Birth Cohort. *Br J Nutr*. 2014; 111(6): 1096-1108.
39. Bry K, Lappalainen U. Pathogenesis of bronchopulmonary dysplasia: The role of interleukin 1 beta in the regulation of inflammation-mediated pulmonary retinoic acid pathways in transgenic mice. *Seminars in Perinatology*. 2006; 30:121-128.