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Vitamin A Compounds in Mothers and Infants at Birth

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VITAMIN A COMPOUNDS IN MOTHERS AND INFANTS AT BIRTH

by

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VITAMIN A COMPOUNDS IN

MOTHERS AND INFANTS AT BIRTH

Jenna M. Paseka, MS, RD, LMNT

University of Nebraska, 2016

Advisor: Corrine K Hanson, PhD, RD, LMNT

OBJECTIVE: Little is known about the vitamin A status in mothers and infants at birth in the United States. The main objective of this study is to determine associations between maternal and infant serum retinol, provitamin A carotenoids, and non-provitamin A carotenoids. The secondary aim is to explore the relationship between maternal intake and maternal and infant serum levels of vitamin A compounds.

METHODS: This was a prospective cohort of 34 mothers and their infants admitted to the neonatal intensive care unit (NICU). Maternal and cord blood samples were collected at delivery. Serum retinol, lutein + zeaxanthin, β-cryptoxanthin, lycopene, α-carotene, and β-carotene concentrations were measured using high-performance liquid chromatography (HPLC). Food frequency questionnaires (FFQs) were obtained to assess maternal dietary vitamin A intake. Descriptive statistics and Spearman correlation coefficients were calculated. P-value <0.05 was considered statistically significant.

RESULTS: The mean birth weight was 2738.0 ± 835.7 gm and mean gestational age was 36.7 ± 3.4 weeks. Vitamin A deficiency (VAD) was present in 71.9% of infants and 12.9% of mothers. Significant positive correlations were found between maternal-infant serum lutein + zeaxanthin ($r=0.50$, $p=0.004$), β-cryptoxanthin ($r=0.83$, $p=<0.001$), trans-lycopene ($r=0.68$, $p=<0.001$), cis-lycopene ($r=0.52$, $p=0.003$), total lycopene ($r=0.63$, $p=<0.001$), α-carotene ($r=0.67$, $p=<0.001$), trans-β-carotene ($r=0.74$, $p=<0.001$), cis-β-carotene ($r=0.44$, $p=0.013$), total-β-carotene ($r=0.71$, $p=<0.001$), and retinol ($r=0.42$, $p=0.018$).

CONCLUSION: VAD is a significant health problem in infants in the NICU. Infant vitamin A status is correlated with maternal levels and may be influenced by maternal dietary intake throughout pregnancy.

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LIST OF ABBREVIATIONS

AI	Adequate Intake
BMI	body mass index
BPD	bronchopulmonary dysplasia
DRI	Dietary Reference Intakes
EAR	Estimated Average Requirement
FFQ	food frequency questionnaire
GDP	gross domestic product
HPLC	high-performance liquid chromatography
IOM	Institute of Medicine
IU	international unit
IVH	intraventricular hemorrhage
LDL	low density lipoprotein
NEC	necrotizing enterocolitis
NHANES III	Third National Health and Nutrition Examination Survey
NICU	neonatal intensive care unit
RAE	retinol activity equivalents
RBP	retinol binding protein
RDA	Recommended Dietary Allowance
RDR	relative dose-response
RDS	respiratory distress syndrome
ROP	retinopathy of prematurity
UL	Tolerable Upper Intake Level
VAD	vitamin A deficiency
VLDL	very low density lipoprotein
WHO	World Health Organization

CHAPTER 1: INTRODUCTION

Vitamin A is a key micronutrient for growth and development, making it especially important for developing fetuses and infants. Vitamin A plays an important role in lung and vision development, immune function, and overall cellular growth.[1-4] Various provitamin A carotenoids are thought to have specific characteristics including antioxidant properties, immunoenhancement, and decreasing the risk of disorders that impact vision, aside from retinol alone.[5,6] Low dietary vitamin A intake is most strongly associated with health consequences during infancy, childhood, pregnancy, and lactation due to the high nutritional demands at these times of increased growth and development.[7]

Retinol levels of infants have been well-documented and have been considered the standard serum measurement that determines vitamin A adequacy or deficiency. The World Health Organization (WHO) estimates there are up to 122 countries with vitamin A deficiency (VAD), affecting 190 million preschool-age children. In addition to children, it is estimated that 19.1 million pregnant women are deficient, thus putting their infants at risk of deficiency at birth.[8] Worldwide, the focus has been to identify and target poorer countries. VAD is commonly identified in the United States among the malnourished or chronically sick populations, but has often been overlooked in pregnant women and infants. The presence of VAD within the population of the United States has not been surveyed or identified by WHO. Because those living in the United States have higher gross incomes and better access to food, it is assumed that VAD is not a public health concern.[7] This gap in knowledge warrants an evaluation of the vitamin A status in pregnant women and infants in the United States.

In addition, little is known about the way carotenoids influence the outcomes of pregnant women and their infants, recommendations for adequate intake, and normal serum levels. The main objective of this study is to evaluate and determine associations between maternal and infant

serum retinol, lutein + zeaxanthin, β-cryptoxanthin, lycopene, α-carotene, and β-carotene levels at birth. The secondary aim is to explore the relationship between maternal intake and maternal and infant serum levels of vitamin A compounds.

CHAPTER 2: BACKGROUND & REVIEW OF LITERATURE

OVERVIEW OF VITAMIN A

Structure, Metabolism and Storage:

Vitamin A is a fat soluble-vitamin that can be obtained from the diet in two forms, preformed vitamin A or provitamin A carotenoids. Preformed vitamin A includes retinol and its esterified form, retinyl ester. These can be found in animal sources such as dairy products, fish, meat, and eggs and comprise ≥70% of the daily vitamin A intake in the United States.[9] Provitamin A carotenoids are precursors for retinol and include β-carotene, α-carotene, and β-cryptoxanthin. These must be obtained from the diet and are found in fruits, vegetables, and oils.[10] Provitamin A carotenoids provide ≤30% of the daily vitamin A intake in the United States.[9] Another group of carotenoids, non-provitamin A carotenoids, includes lutein, zeaxanthin, and lycopene. This subset of carotenoids, though, cannot be converted into retinol.[7,10,11] In general, the term "vitamin A" is defined as the all-*trans*-retinol form.[12]

Retinol, retinal, and retinoic acid are the three active forms and retinyl ester is the storage form of vitamin A. Absorption of preformed vitamin A occurs in the small intestine after retinyl esters are compiled with triglycerides in micelles.[10] Products of fat digestion and bile secretions are essential in this process.[13] If dietary fat intake is low, retinol and carotenoid absorption is markedly reduced.[13,14] Dietary retinoids can also be converted into retinoic acid in the intestine. Efficiency of absorption of preformed vitamin A is 70-90%. As intake of these forms increases, the absorbability remains high. Carotenoids are absorbed similarly to preformed vitamin A in the intestinal lumen, but efficiency of absorption decreases as the amount of dietary carotenoids increases. It has been reported that 9-22% of β-carotene ingested is absorbed. Once the vitamin A forms are absorbed, they are transported to the liver via chylomicron remnants and mainly stored as retinyl esters.[10] The body stores approximately 80-90% of vitamin A in the liver as

retinyl ester.[12] Retinyl esters can be hydrolyzed into retinol as needed and transported to other tissues when bound to retinol-binding protein (RBP). Carotenoids are integrated into very low density lipoproteins (VLDL) to be sent throughout the bloodstream to other tissues.[10] Figure 1 shows the metabolic pathways and conversion capabilities of different vitamin A forms.

Figure 1: Vitamin A Metabolism

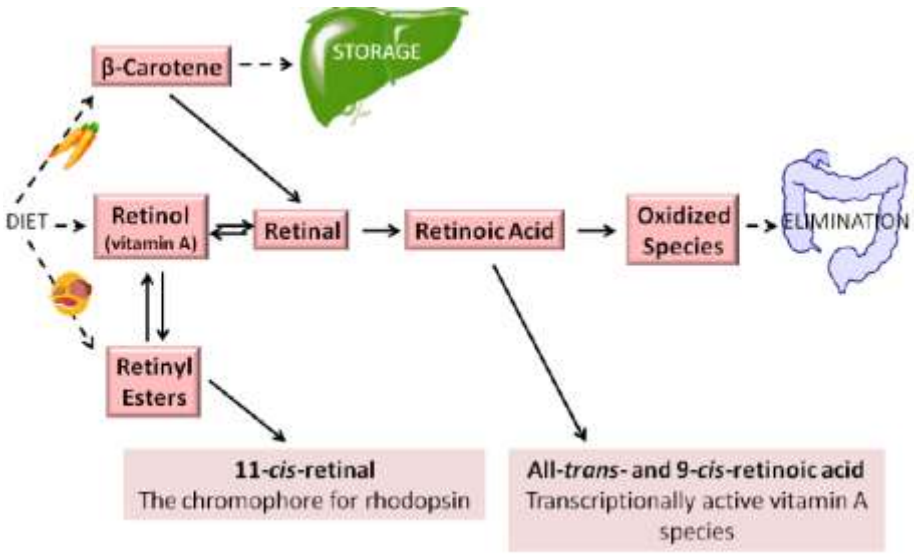

Figure 1: Vitamin A Metabolism. Generalized pathways from intake of provitamin A carotenoids, retinol, or retinyl esters for use in the body, storage, or elimination.[12]

Functions:

Vitamin A serves many important functions in the body and is crucial during periods of growth and development. It is involved in regulating and promoting cell growth and differentiation,[3] maintaining the integrity of respiratory epithelial cells,[4] visual development, reproductive functions, and improving the immune system. Vitamin A is especially important in fetal lung development and surfactant synthesis. In regards to vision, it is an important part of the

photosensitive visual pigment complex in the retina.[1] It is required for differentiation of the cornea, conjunctival membranes, and photoreceptor rod and cone cells.[10]

According to the Institute of Medicine (IOM), the only known function of provitamin carotenoids is vitamin A activity. Carotenoids are thought to have "antioxidant activity, immunoenhancement, inhibition of mutagenesis and transformation, inhibition of premalignant lesions, quenching of nonphotochemical fluorescence, and activity as a pigment in primate macula."[15] β-carotene, specifically, has been documented to have antioxidant properties by trapping free radicals and reducing oxidative stress or damage.[1,2,5]

The non-provitamin A carotenoids lutein, zeaxanthin, and lycopene have been shown to function in the body, despite not being converted into retinol. It is thought that lutein and zeaxanthin influence the maturation of cells in the macular region of the retina[16] and protect against stress and oxidation in the retinal pigment epithelium.[6] This region is responsible for developing central visual acuity. There also appears to be a link between lutein and zeaxanthin and improved cognitive function. Vishwanathan et al. determined that the mean concentration of lutein was significantly greater than the other carotenoids in brain tissue samples of infants who died within the first 18 months of life ($p=<0.05$). Preterm infants also had significantly lower concentrations of lutein and zeaxanthin compared to term infants in most of the brain regions ($p=<0.05$). These findings in addition to previous research help support the role lutein and zeaxanthin play in visual and cognitive development.[16] Little is known about the function of lycopene in pregnant women and infants. It is thought that lycopene can act as an antioxidant,[11] impact immune development, and protect against inflammatory diseases.[6] In general, lycopene has been associated with benefits in cardiovascular disease, neurological disorders, and cancer.[17]

Oxidative stress that commonly occurs in infants in the neonatal intensive care unit (NICU) is associated with many conditions such as bronchopulmonary dysplasia (BPD), respiratory distress, retinopathy of prematurity (ROP), and necrotizing enterocolitis (NEC), as

well as an increased risk of infection.[18] Ensuring adequate vitamin A status at birth, particularly specific vitamin A forms found to decrease oxidative stress, may provide protective benefits to infants at an increased risk of developing these conditions or positively impact an infant's recovery from these complications.

Dietary Reference Intakes:

The Dietary Reference Intakes (DRIs) established by the IOM are available for vitamin A for different age categories ranging from infants to older adults. The DRIs are reported in retinol activity equivalents (RAE). Retinol and provitamin A carotenoids are converted into common units of RAE to accommodate the different bioactivities. Lutein, zeaxanthin, and lycopene, on the other hand, cannot be converted into RAE, thus they do not possess vitamin A activity. Conversion rates are used to determine RAE in food and supplements as nutrition facts labels and supplement labels currently report vitamin A in international units (IUs).[7] Knowing the source is necessary to determine appropriate conversions to RAE. The following are conversion rates between IUs and RAE:

- 1 IU retinol = 0.3 μg RAE
- 1 IU β-carotene from dietary supplements = 0.15 μg RAE
- 1 IU β-carotene from food = 0.05 μg RAE
- 1 IU α-carotene or β-cryptoxanthin = 0.025 μg RAE[7]

Vitamin A intake may also be measured in μg which can also be converted into RAE. The following are conversion rates between an RAE and μg for specific vitamin A forms:

- 1 μg RAE = 1 μg retinol
- 1 μg RAE = 2 μg β-carotene in oil
- 1 μg RAE = 12 μg β-carotene in mixed foods

- 1 µg RAE = 24 µg other provitamin A carotenoids in mixed foods[19]

The DRIs for vitamin A are displayed in Table 1. In adult females 19 years and older, the Estimated Average Requirement (EAR) and Recommended Dietary Allowance (RDA) for vitamin A is 500 µg RAE/day and 700 µg RAE/day, respectively. In pregnant women, vitamin A needs increase to 550 µg RAE/day for the EAR and 770 µg RAE/day for the RDA. Increased needs during pregnancy take into account accumulation of vitamin A in the liver of the fetus during gestation. The Tolerable Upper Intake Level (UL) for non-pregnant and pregnant women is 3000 µg/day of preformed vitamin A.[10]

The Adequate Intake (AI) for vitamin A in infants 0-6 months is 400 µg RAE/day. The AI for infants is established from the mean human milk intake in healthy breastfed infants, assuming an average total human milk intake of 0.78 L/day. The average concentration of vitamin A in human milk is 1.70 mmol/L. The UL for infants is defined at a level of 600 µg RAE/day of preformed vitamin A.[10]

Table 1: Dietary Reference Intakes for Infants and Women

Age Group	EAR	RDA	AI	UL
Infants 0-6 months			400	600
Women ≥19 years	500	700		3000
Pregnant Women ≥19 years	550	770		3000
Lactation ≥19 years	900	1300		3000

The Dietary Reference Intakes for vitamin A as reported by the Institute of Medicine. Values are reported in µg RAE/day of vitamin A.[10]

There are currently no established DRIs for carotenoids. The IOM concluded in their most recent report in 2000 that existing evidence was insufficient to establish recommended intake values. Current research cannot exclude the possibility of other substances found in carotenoid-rich food being the parts that are contributing to health benefits. The IOM, though,

does support recommendations for increasing consumption of carotenoid-rich fruits and vegetables.[15]

In a report by Strobel et al., β-carotene needs are increased during pregnancy and the breastfeeding period. It was recommended that β-carotene intake should be one third higher during pregnancy and 0.7 mg/day higher during lactation than that for non-pregnant or non-breastfeeding women.[14]

VITAMIN A DEFICIENCY

VAD is associated with a high risk of xerophthalmia and can cause childhood blindness.[20] Immunity can also be compromised if deficient, especially in infants, due to the reduction in the function of neutrophils, macrophages, and natural killer cells as well as interference with the regeneration of damaged mucosal barriers.[9] VAD contributes to morbidity and mortality from infections.[8]

VAD can be diagnosed by a clinical presentation of xerophthalmia and may be the only assessment method if serum retinol concentrations cannot be obtained. Xerophthalmia encompasses night blindness, conjunctival xerosis, Bitot's spots, corneal xerosis, corneal ulceration and necrosis, corneal scarring, and xerophthalmic fundus.[8]

Although an individual may not be showing clinical signs of deficiency such as xerophthalmia and night blindness, subclinical VAD can also be present. This can contribute to increased susceptibility to infections, reduced physical growth, and decreased survival from serious illness. Populations at risk of subclinical VAD may include those living at or below the poverty level, those with inadequate health care and immunizations, those with other nutritional deficiencies, recent immigrants or refugees from countries with a high incidence of deficiency, and those with ineffective fat digestion.[21]

Assessing liver reserves is considered the gold standard to measure adequacy or deficiency of vitamin A, but this method is not feasible in humans.[22] Plasma retinol levels, though, can be indicative of vitamin A status and liver reserves. If hepatic vitamin A reserves are adequate, there will be little change in the concentration of retinol in the blood. The hepatic stores must be depleted before a decline in plasma levels is detected. If serum retinol levels are <1.05 µmol/L, storage levels are likely reduced.[12]

In 1980, a serum retinol level <0.35 µmol/L was used by WHO to define VAD among children younger than six years of age and was associated with an increased prevalence of clinical signs of deficiency. The cut-off level was then raised in 1996 after consensus by members of WHO. It was believed that using <0.35 µmol/L was too low to identify individuals at risk for subclinical consequences of deficiency, but had not yet displayed clinical signs of severe deficiency. Serum retinol levels <0.70 µmol/L are now considered deficient, while levels <0.35 µmol/L are indicative of a severe deficiency where liver stores have been depleted.[23]

A serum retinol level >1.05 µmol/L has been proposed by WHO as an adequate concentration for vitamin A status among pregnant and lactating women.[8] This cut-off value has been utilized in various research studies to determine adequate vs. inadequate vitamin A levels in mothers and infants.[20,24-26] Radhika et al. discovered that spontaneous preterm delivery and anemia were significantly associated with serum retinol <20 µg/dL (<0.70 µmol/L) and recommended maintaining levels >20 µg/dL (>0.70 µmol/L) at a minimum.[27]

An additional test that has been used to assess vitamin A status in infants is the relative dose-response (RDR) test. Weinman et al. used it to assess vitamin A status of 124 newborn preterm infants <37 weeks gestational age. To perform the RDR test, blood samples in newborn infants were obtained before and 5 hours after an intramuscular injection of 5000 IU of vitamin A. It is expected that this amount would increase the serum retinol levels by 10-20% if hepatic reserves are normal. A positive RDR test was defined as retinol levels increasing >20% at >/= 5

hours after administration, indicative of deficient liver vitamin A stores. When deficiency was defined as <20 µg/dL (0.70 µmol/L), the test underestimated the number of deficient infants. Using <30 µg/dL (1.05 µmol/L) as a deficient level, the test overestimated VAD. Weinman et al. concluded that <25 µg/dL (0.87 µmol/L) was more appropriate for defining VAD.[28]

Although clear worldwide deficiency standards are lacking for infants and pregnant women, recommendations by WHO are generally recognized internationally. Overall, populations are defined as severely deficient (<0.35 µmol/L), deficient (0.35-0.70 µmol/L), low (0.70-1.05 µmol/L), or adequate (>1.05 µmol/L) based upon their serum retinol levels.[8,25,29]

The level of public health significance of VAD in a population has been established by WHO. Deficiency is considered mild if ≥2-<10%, moderate if ≥10-<20%, or severe if ≥20% of children 6-71 months of age have serum retinol concentrations 0.70 µmol/L or lower.[23] Figures 2-3 demonstrate the public health significance of VAD in preschool-age children and pregnant women in the world when evaluating serum retinol concentrations. A clinical presentation of night blindness can also be used to define the level of public health significance of VAD. A mild, moderate, and severe deficiency affects >0-<1%, ≥1-<5%, and ≥5% of children 24-71 months of age, respectively, if night blindness is present.[8]

Figure 2.1: Vitamin A Deficiency in Preschool-age Children

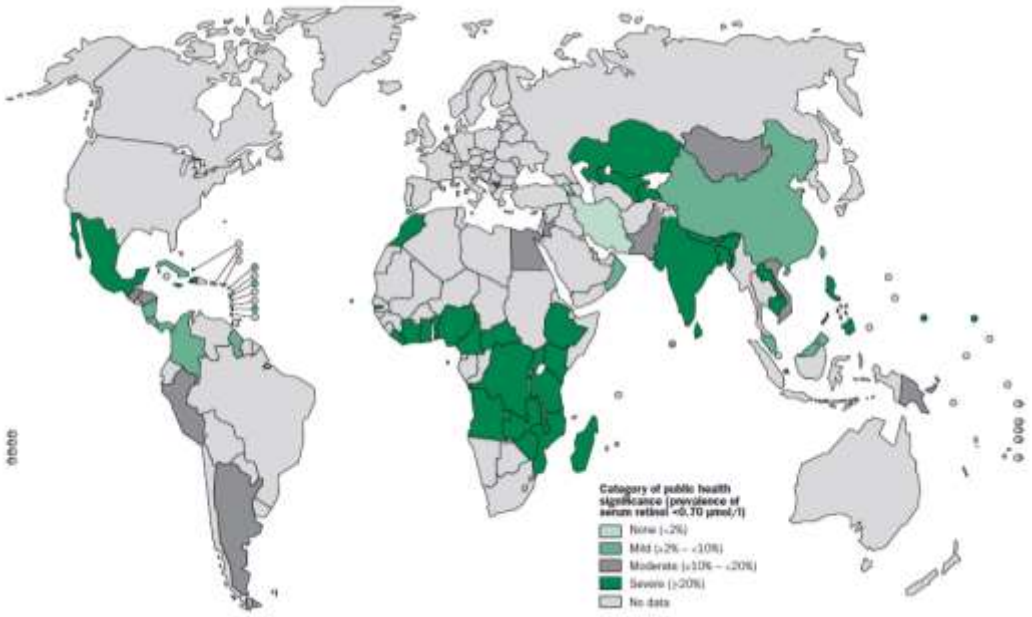

Figure 2.1: Vitamin A Deficiency in Preschool-age Children. Public health significance of global vitamin A deficiency in preschool-age children based upon the World Health Organization's survey data of retinol levels <0.70 µmol/L from 1995-2005.[8]

Figure 2.2: Estimated Vitamin A Deficiency in Preschool-age Children

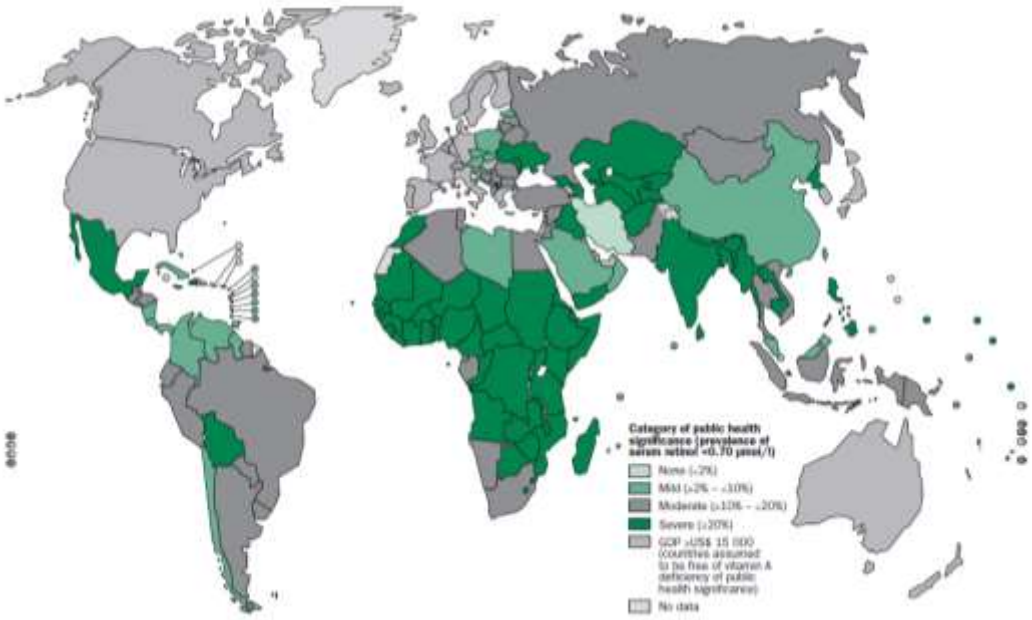

Figure 2.2: Estimated Vitamin A Deficiency in Preschool-age Children. Public health significance of global vitamin A deficiency in preschool-age children based upon the World Health Organization's survey data and regression-based estimates of retinol levels <0.70 µmol/L from 1995-2005.[8]

Figure 3.1: Vitamin A Deficiency in Pregnant Women

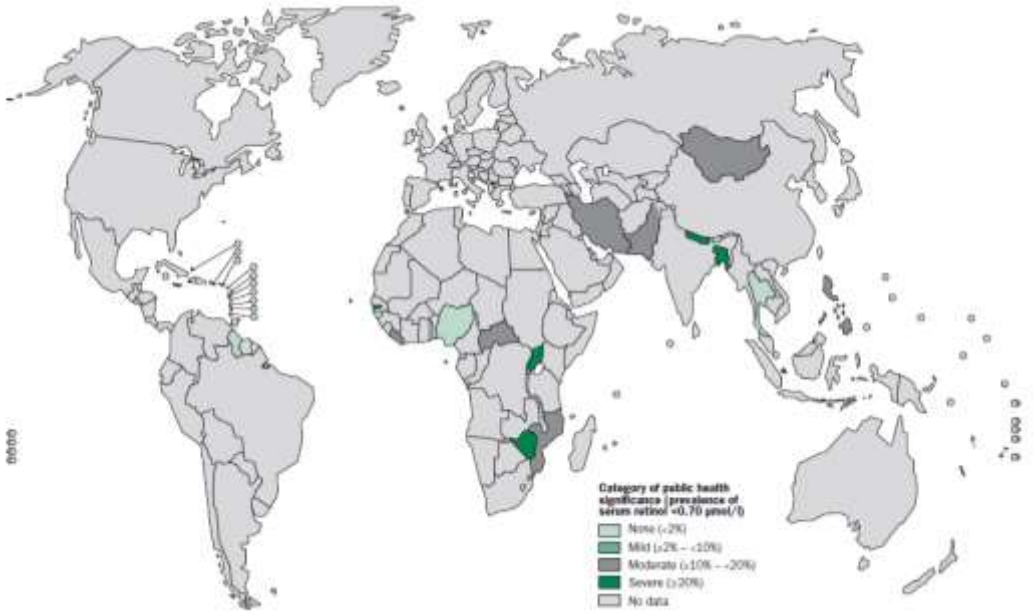

Figure 3.1: Vitamin A Deficiency in Pregnant Women. Public health significance of global vitamin A deficiency in pregnant women based upon the World Health Organization's survey data of retinol levels <0.70 µmol/L from 1995-2005.[8]

Figure 3.2: Estimated Vitamin A Deficiency in Pregnant Women

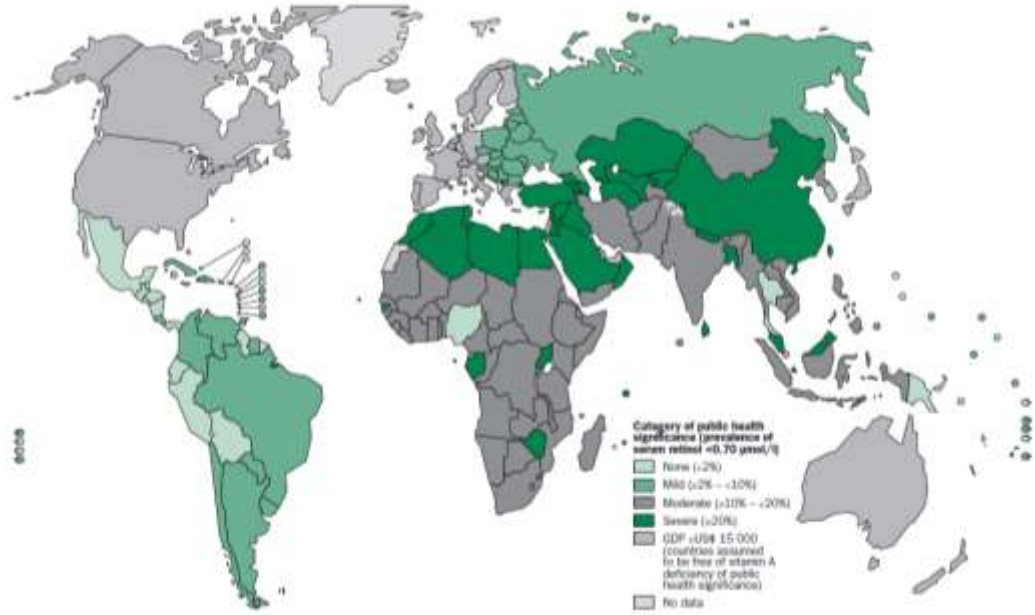

Figure 3.2: Estimated Vitamin A Deficiency in Pregnant Women. Public health significance of global vitamin A deficiency in pregnant women based upon the World Health Organization's survey data and regression-based estimates of retinol levels <0.70 µmol/L from 1995-2005.[8]

Efforts have been made in poorer countries to increase vitamin A intake through diet, supplementation, and fortification. In 1995, WHO estimated that deficiency was a public health significance in 60 countries and likely a problem in an additional 13 countries. Newer estimates reflecting the time period of 1995-2005 indicate that 45 countries have VAD based on the prevalence of night blindness and 122 countries based on serum retinol concentrations <0.70 µmol/L in preschool children.[8]

In a survey conducted between 1995 and 2005 in countries with a 2005 gross domestic product (GDP) <$15,000, 15.3% of pregnant women (95% CI: 7.4-23.2) had serum retinol levels below 0.70 µmol/L. This accounts for 19.1 million pregnant women. Preschool-age children were also evaluated with 33.3% (95% CI: 31.1-35.4) deficient which translated into 190 million children affected. When evaluating the six WHO regions, the prevalence of deficiency in pregnant women was highest in the Western Pacific region (21.5%; 4.90 million), South-East Asia (17.3%; 6.69 million), the Eastern Mediterranean region (16.1%; 2.42 million), Africa (13.5%; 4.18 million), Europe (11.6%; 0.72 million), and the Americas (2.0%; 0.23 million). In preschool-age children, the prevalence was highest in South-East Asia (49.9%; 91.5 million), Africa (44.4%; 56.4 million), the Eastern Mediterranean region (20.4%; 13.2 million), Europe (19.7%; 5.81 million), the Americas (15.6%; 8.68 million), and the Western Pacific region (12.9%; 14.3 million).[8] VAD contributes to 1.2-3.0 million deaths in children, and leads to the development of xerophthalmia in 4.4 million children and 6.2 million mothers each year.[30]

The main underlying cause of deficiency is a diet insufficient in vitamin A. The risk of health consequences is greatly increased if there is low vitamin A intake during periods of growth and development such as pregnancy, infancy, and childhood.[8] VAD in infants can develop when their mothers are deficient during pregnancy or during lactation if infants are breastfed, when infants receive diets offering very few sources of vitamin A, and/or when infants suffer from ongoing illnesses contributing to malnutrition, malabsorption, and increased catabolism.[31]

MATERNAL AND CORD BLOOD VITAMIN A LEVELS

Maternal-fetal Transfer:

The growing embryo and fetus require circulating maternal vitamin A for developmental needs since there is no *de novo* synthesis of vitamin A. This transfer occurs through the placenta, the maternal-fetal barrier. The placenta is thought to be the storage site for vitamin A until the embryonic liver is functional.[32] Fetal liver stores then tend to increase with increasing gestational age, typically doubling between the 25th and 37th week gestation.[18] Building fetal liver stores is still strongly dependent on the vitamin A status of the mother.[14] The placenta also serves to release retinol to the fetus when maternal dietary intake is low or to store extra retinol to prevent a toxic excess from maternal retinoids. The process of placental transfer is not clear. Possible mechanisms include direct transfer of the retinol-RBP complex, transfer of free retinol, or cellular uptake of retinol by a specific receptor Stra6.[32]

β-carotene from maternal blood may also reach the fetus through placental uptake of β-carotene-containing chylomicrons, VLDL, and low density lipoproteins (LDL). Spiegler et al. hypothesized that "maternally circulating β-carotene can be delivered to the developing tissues and there cleaved to generate retinoic acid *in situ*."[32] This may be beneficial in times of insufficient dietary intake of other vitamin A compounds.

Relationship of Vitamin A Status in Mothers and Infants:

Various studies have evaluated retinol concentrations in mothers and infants at birth. It has been reported that maternal and cord blood retinol and carotenoid concentrations are directly correlated[14] and are significantly higher in mothers than in cord blood levels ($p<0.0001$).[33] β-carotene levels, specifically, in mothers have been found to be significantly higher and correlate with cord blood levels.[32] Limited research is available describing the relationship of the other provitamin A and non-provitamin A carotenoids between mothers and infants.

Saunders et al. described retinol blood levels in 217 mothers and 222 infants born at a mean gestational age of 39 ± 1.7 weeks in Rio de Janeiro, Brazil. VAD was defined as serum levels <1.05 μmol/L in both mothers and infants. The mean postpartum level in mothers was 1.70 ± 0.86 μmol/L with 24.4% of the mothers being vitamin A deficient. The mean concentration in umbilical cord blood was 1.29 ± 0.75 μmol/L, and the prevalence of VAD was 45.5%. Placental transfer of vitamin A, or the ratio of infant to maternal serum concentration, was 75.9%.[24] Another study in Recife, Brazil of 65 term normal birth weight infants (>2500 grams) determined the mean serum retinol levels at birth to be 1.46 ± 0.63 μmol/L in mothers and 1.13 ± 0.60 μmol/L in infants. Placental transfer was similar to the previous study in Rio de Janeiro, Brazil at 77.4%. Although the mean retinol concentrations were considered normal in both groups, 23.1% of infants and 23% of mothers were considered deficient in the sample when VAD was defined as <0.70 μmol/L in infants and <1.05 μmol/L in mothers. The serum retinol concentrations of infants was also positively correlated with maternal serum retinol concentrations ($r=0.27$, $p=0.04$).[25]

Comparisons have been made between serum retinol levels in mothers after delivery and non-pregnant women from the same community in South Africa as well as newborns with a mean gestational age of 38.9 ± 1.9 weeks and preschool children from the same community. The mean serum retinol level for mothers was 1.03 ± 0.40 μmol/L, while non-pregnant women had a mean level of 1.93 ± 0.50 μmol/L. Newborns and preschool children had mean retinol levels of 0.73 ± 0.24 μmol/L and 1.10 ± 0.27 μmol/L, respectively. There were 49.3% of infants and 21.4% of mothers with retinol levels <0.70 μmol/L. A significant positive correlation was found between serum retinol concentrations in the mothers and newborns ($r=0.227$, $p=0.001$).[34]

In healthy mothers and infants with a mean gestational age of 39.5 ± 1.6 weeks in China, mean maternal vitamin A concentration was 1.13 ± 0.371 μmol/L and cord blood levels were 0.69 ± 0.162 μmol/L. Of the mothers, 16.8% were considered to be deficient with levels below

0.70 µmol/L as well as 36.4% had low levels ranging between 0.70 and 1.05 µmol/L. Of the infants, 41.3% were deficient and 56.6% had a low vitamin A status. The mean placental transfer of vitamin A was 78.3 ± 35.2%.[20]

Infant birth weight has thought to also influence vitamin A levels in infants. Agarwal et al. discovered mean serum retinol levels were 13.3 ± 8.2 µg/dL (0.47 ± 0.29 µmol/L) in 146 low birth weight infants (<2500 grams) and 14.0 ± 6.2 µg/dL (0.49 ± 0.22 µmol/L) in 79 normal birth weight infants (≥2500 grams) in India (p=0.51). Deficiency in infants was defined as retinol levels <10 µg/dL (<0.35 µmol/L). Of the low birth weight and normal birth weight infants, 41.1% and 24.1%, respectively, were vitamin A deficient. Mean maternal levels were 39.0 ± 15.5 µg/dL (1.36 ± 0.54 µmol/L) for mothers of low birth weight infants and 38.1 ± 16.3 µg/dL (1.33 ± 0.57 µmol/L) for mothers of normal birth weight infants (p=0.69). VAD defined as <20 µg/dL (0.70 µmol/L) in mothers was present in 11.5% of low birth weight and 9.0% of normal birth weight infants' mothers. Placental transfer was 34.1% for low birth weight infants and 36.7% in normal birth weight infants.[35]

Vitamin A Status – Data Exclusive to Infants Only:

In a study evaluating 350 infants weighing <1250 grams at birth at the University of Washington who had a serum retinol concentration measured in the first 28 days of life, 55% had vitamin A levels <0.35 µmol/L. Mean retinol concentrations were not reported.[4] Kositamongkol et al. also evaluated 31 very low birth weight infants (<1500 grams) at birth in Thailand. The median retinol level at birth was 15.5 µg/dL (IQR 10.5-24.9 µg/dL) (0.54 µmol/L (IQR 0.37-0.87 µmol/L)). The deficiency rate in this group was 67.7% when VAD was defined as retinol levels <20 µg/dL (<0.70 µmol/L). Severe VAD with concentrations <10 µg/dL (<0.35 µmol/L) was present in 19% of the infants.[3]

In a study of 56 infants <33 weeks gestational age in Finland, 22 infants were found to have low vitamin A levels (<1.05 µmol/L) in umbilical cord samples with two of those subjects

being deficient (<0.70 μmol/L). Tammela et al. also compared serum levels of infants whose mothers either received or did not receive multivitamin supplementation during pregnancy. The serum levels of infants of mothers who did not use supplements were not significantly different from those that did with serum levels of 1.19 μmol/L and 1.29 μmol/L, respectively (p=0.273).[26]

Vitamin A Status – Data Exclusive to Mothers Only:

Throughout pregnancy, serum vitamin A levels in mothers tend to decrease as plasma volume expands in the later trimesters. Normal maternal serum levels were found to be 25.6 ± 7.6 μg/dL (0.90 ± 0.27 μmol/L) in the first trimester, 24.0 ± 7.8 μg/dL (0.84 ± 0.27 μmol/L) in the second trimester, 24.6 ± 9.3 μg/dL (0.86 ± 0.33 μmol/L) in the third trimester, and 35.3 ± 8.9 μg/dL (1.23 ± 0.31 μmol/L) postpartum.[36] The Third National Health and Nutrition Examination Survey (NHANES III) collected from 1988-1994 reported mean serum retinol levels in pregnant women to be 1.47 μmol/L.[10] In a study by Radhika et al., 736 women in their third trimester of pregnancy in India were enrolled to evaluate VAD. The mean serum retinol level of mothers was 27.1 ± 11.4 μg/dL (0.95 ± 0.40 μmol/L). Thirty-five percent of mothers had an adequate vitamin A status when defined as serum retinol levels ≥30 μg/dL (≥1.05 μmol/L). A small portion of the mothers (3.5%) were considered severely deficient with levels <10 μg/dL (<0.35 μmol/L). It was recommended that mothers obtain serum retinol levels >20 μg/L (>0.70 μmol/L) during pregnancy to avoid increased complications including preterm delivery, moderate to severe anemia, and pregnancy-induced hypertension.[27]

Relationship of Carotenoids in Mothers and Infants:

Although many research studies have evaluated serum retinol concentration levels, information on the serum levels of carotenoids is limited. A study in Germany evaluated 151 maternal and 200 cord blood samples to assess the different vitamin A forms in maternal and cord blood at birth. Infants had a mean gestational age of 39.1 ± 1.5 weeks, 10% of the infants were born preterm (<37 weeks), and 8.5% had a low birth weight (<2500 grams). The mean maternal

and cord retinol levels, respectively, were 1.15 μmol/L (95% CI: 1.09, 1.21) and 0.90 μmol/L (95% CI: 0.85, 0.95) (r=0.099, p=0.228), lutein levels were 0.528 μmol/L (95% CI: 0.494, 0.563) and 0.021 μmol/L (95% CI: 0.014, 0.030) (r=0.368, p=<0.001), zeaxanthin levels were 0.111 ± 0.056 μmol/L and undetectable, β-cryptoxanthin levels were 0.399 ± 0.259 μmol/L and undetectable, lycopene levels were 1.08 ± 0.429 μmol/L and undetectable, α-carotene levels were 0.323 μmol/L (95% CI: 0.277, 0.376) and 0.004 μmol/L (95% CI: 0.003, 0.005) (r=0.668, p=<0.001), and β-carotene levels were 0.755 μmol/L (95% CI: 0.675, 0.845) and 0.013 μmol/L (95% CI: 0.010, 0.017) (r=0.832, p=<0.001). Comparisons were also made between preterm and term infants. The only statistically significant finding between preterm and term infants was retinol concentrations, 0.70 vs. 0.90 μmol/L (p<0.001) for preterm and term infants, respectively. Lutein (0.01 vs 0.03 μmol/L, p=0.268), α-carotene (0.00 vs. 0.00 μmol/L, p=0.963), and β-carotene (0.02 vs. 0.01 μmol/L, p=0.531) were not significantly different in the preterm and term infants, respectively. Zeaxanthin, β-cryptoxanthin, and lycopene were all below the limit of detection in the cord blood samples. Maternal lutein (r=0.368, p=<0.001), α-carotene (r=0.668, p=<0001), and β-carotene (r=0.832, p=<0.001) levels were positively correlated with cord blood samples.[18]

A study in Ireland assessed retinol and carotenoid concentrations in 66 samples from mothers and 40 cord blood samples from infants with a mean birth weight of 3439 ± 486 grams. The mean levels in mothers and cord blood, respectively, were 1.88 ± 0.4 μmol/L and 1.01 ± 0.3 μmol/L of retinol, 0.46 ± 0.2 μmol/L and 0.13 ± 0.1 μmol/L of lutein + zeaxanthin (n=38 detectable cord blood samples), 0.14 ± 0.1 μmol/L and 0.04 ± 0.03 μmol/L of β-cryptoxanthin, 0.40 ± 0.2 μmol/L and 0.04 ± 0.03 μmol/L of lycopene (n=12 detectable cord blood samples), 0.23 ± 0.1 μmol/L and 0.04 ± 0.03 μmol/L of β-carotene (n=18 detectable cord blood samples), and 0.05 ± 0.03 μmol/L and 0.01 ± 0.01 μmol/L of α-carotene (n=8 detectable cord blood samples). Cord retinol concentrations were 53.7% of maternal concentrations.[33]

From the NHANES III data, the mean serum level of each specific carotenoid was reported for adult females. Women ages 19-30 and 31-50, respectively, had mean β-carotene levels of 15.0 and 21.7 μg/dL, mean α-carotene levels of 3.44 and 5.37 μg/dL, mean β-cryptoxanthin levels of 7.33 and 8.84 μg/dL, mean lutein + zeaxanthin levels of 18.6 and 21.4 μg/dL, and mean lycopene levels of 24.8 and 22.9 μg/dL.[15]

Carotenoid Status – Data Exclusive to Infants Only:

In a study assessing vitamin A status in 100 cord blood samples from normal birth weight infants in Hawaii (mean birth weight was 3411.6 ± 464.3 grams), mean retinol was 209 ± 55 ng/mL (0.73 ± 0.19 μmol/L), total trans lutein + trans zeaxanthin was 30 ± 16 ng/mL (30 ± 16 μg/L), α-cryptoxanthin was 5 ± 2 ng/mL (5 ± 2 μg/L), trans-β-cryptoxanthin was 12 ± 15 ng/mL (12 ± 15 μg/L), total lycopene was 12 ± 6 ng/mL (12 ± 6 μg/L), trans-α-carotene was 2 ± 2 ng/mL (2 ± 2 μg/L), trans-β-carotene was 7 ± 17 ng/mL (7 ± 17 μg/L), cis-β-carotene was 0.9 ± 2 ng/mL (0.9 ± 2 μg/L), and total carotenoids was 99 ± 56 ng/mL (99 ± 56 μg/L).[2] Carotenoids were also measured in 83 preterm and term infants at birth in Germany in a study by Sommerburg et al. Median plasma concentrations at birth of cryptoxanthin were 11 μg/L (8-12 μg/L), lycopene were 16 μg/L (14-18 μg/L), α-carotene were 5 μg/L (0-8 μg/L), and β-carotene were 24 μg/L (19-31 μg/L).[5]

MATERNAL VITAMIN A INTAKE

The nutritional status of mothers throughout pregnancy and at birth is one of the most important factors that influence the nutritional status of infants at birth. Vitamin A stores in infants are affected by maternal vitamin A intake while pregnant.[37] Changes in maternal vitamin A intake affect the circulating vitamin A levels, thus influencing the availability of vitamin A to

transfer to the fetus via the placenta.[32] Mothers can also influence their infant's vitamin A status postpartum if breast feeding their infant.[37]

Little is known about maternal intake of vitamin A during pregnancy. Weinman et al. administered a qualitative questionnaire reflecting intake during gestation to 124 mothers at birth in Brazil. It assessed intake from green, orange or yellow vegetables, fruits, milk, dairy products, and meat (liver and fish). Information on vitamin A supplementation during pregnancy was also obtained and showed that 7.3% of mothers took oral vitamin A supplementation. The questionnaire revealed that 74.2% of mothers consumed a satisfactory amount of food rich in vitamin A, although the satisfactory criteria was not defined.[28]

Another study in Finland by Tammela et al. administered 10-item structured questionnaires to 11 mothers of preterm infants with low (<1.05 μmol/L) concentrations of vitamin A in cord blood and 25 mothers of preterm infants with normal (>1.05 μmol/L) concentrations of vitamin A in cord blood. The questionnaires assessed the frequency of consumption of liver products, carrots and vegetables, and butter and vegetable fats. The dietary habits did not differ between mothers whose infants had low vitamin A concentrations and those that had normal vitamin A concentrations. The questionnaire revealed that 91% of mothers of infants with low vitamin A status and 56% of mothers of infants with normal vitamin A status consumed liver products 0-1 times/month. It also showed that 45% of mothers of infants with low vitamin A status and 28% of mothers of infants with normal vitamin A status ate carrots 0-1 times/week. Vegetables were consumed less than 5 times/week in 18% of mothers of infants with low vitamin A status and in 56% of mothers of infants with normal vitamin A status. Lastly, the questionnaire showed that 54% of mothers of infants with low vitamin A status and 44% of mothers of infants with normal vitamin A status consumed vegetable fat.[26]

In a sample of 683 pregnant mothers in Israel, 24 hour dietary questionnaires were given to assess nutritional status during pregnancy. Mean and median vitamin A intake was 531.0 ±

94.1 μg RAE and 237.1 μg RAE, respectively. The IOM's EAR of 550 μg RAE was used for adequate vitamin A intake. Of the mothers, 88% had vitamin A intake below the EAR.[38]

The NHANES III data estimated the mean vitamin A intake for pregnant women to be 757 μg RAE/day. Dietary intake information was also obtained on carotenoids. Pregnant women consumed on average 376 μg of α-carotene, 1531 μg β-carotene, 159 μg β-cryptoxanthin, 1455 μg lutein + zeaxanthin, and 8713 μg lycopene based on the usual intake from food for one day.[10]

INFANT VITAMIN A INTAKE

Estimations of vitamin A intake have been reported in various populations of infants after birth. A prospective cohort of 35 very low birth weight infants (<1500 grams) in Thailand estimated vitamin A intake from parenteral nutrition, enteral feedings of breast milk or formula, and supplementation. Median daily vitamin A intake was 1066 IU/day (IQR 893.7-1337 IU/day) or 820 IU/kg/day (IQR 698-1122 IU/kg/day) from birth to the time of full feeding. From time of full feeding to term postmenstrual age, the median vitamin A intake was 1585 IU/day (IQR 1169-1893 IU/day) or 832 IU/kg/day (IQR 516.3-1087 IU/kg/day). Information was not available to differentiate the percentage of vitamin A intake from each source of nutrition.[3]

Souza et al. described differences in vitamin A intake between term and preterm infants (<37 weeks gestation) who were exclusively breastfed with no supplements by mothers in Brazil. Breast milk samples were analyzed to determine the vitamin A intake. Term and preterm infants had an average intake of 352.64 ± 152.72 μg of retinol/day and 217.65 ± 105.65 μg of retinol/day, respectively. With these groups, 46.3% of term and 72.5% of preterm infants did not consume adequate amounts of vitamin A, which was defined as 400 μg/day, as established by the IOM's DRIs. This is likely influenced by the concentration of retinol in the breast milk of mothers who had term vs. preterm infants and the volumes consumed. The mean retinol concentration of

breast milk was significantly higher in mothers of term infants (1.87 ± 0.81 μmol/L) compared to preterm infants (1.38 ± 0.67 μmol/L) ($p<0.0001$).[39] The American Academy of Pediatrics has recommended that preterm infants should consume 210-450 μg retinol/kg each day.[14]

In a research study conducted in China, the vitamin A status of infants aged 0-2 months was evaluated. Infants were evaluated based upon their form of nutrition received. The groups included mix-fed, artificially fed, and breast fed infants. For mix-fed infants, the mean and median intakes of vitamin A were 662 ± 12.8 μg/day and 543 μg/day, respectively. Only 0.4% of the infants in this group were inadequate for vitamin A when compared to the EAR in China of 375 μg/day. When evaluating possible toxicity levels in mix-fed infants, 38.2% were above the UL of 600 μg/day. Infants who were solely receiving artificial formula had a mean vitamin A intake of 627 ± 26.8 μg/day and median intake of 644 μg/day. The artificially fed group had 21.5% who did not meet the EAR, but also had over half of the group exceeding the UL (53.5%). Breast fed infants had mean vitamin A intakes lower than that of the artificially fed and mix-fed infants, although the specific data for this group was not reported.[40]

In order to appropriately provide recommendations and care to pregnant women and infants to prevent VAD, an evaluation of maternal and infant serum vitamin A compound levels, prevalence of VAD, and maternal dietary intake of vitamin A compounds in the United States is needed.

CHAPTER 3: METHODS

STUDY DESIGN AND POPULATION

This prospective cohort was conducted to evaluate vitamin A status in infants and mothers at birth. All infants admitted to the NICU at Nebraska Medicine and their mothers were eligible for enrollment. Written informed consent was obtained from a parent for each infant enrolled. Exclusion criteria included infants with congenital abnormalities, inborn errors of metabolism, gastrointestinal, liver, or kidney disease, anemia, and those that required blood transfusions. Infants were also excluded if the parents were under the age of 19 years or if infants were made wards of the State of Nebraska. The study protocol was approved by the University of Nebraska Medical Center Institutional Review Board.

DATA AND BLOOD COLLECTION

Thirty-four mother-infant pairs were enrolled into the study. Maternal and umbilical cord blood samples were collected at the time of delivery. Samples were processed efficiently after collection, protected from heat and light, and stored in -80˚ F freezers until they were analyzed. Analysis of samples was performed at the Biomarker Research Institute at the Harvard School of Public Health. Measurements of lutein + zeaxanthin, β-cryptoxanthin, trans-lycopene, cis-lycopene, total lycopene, α-carotene, trans-β-carotene, cis-β-carotene, total-β-carotene, and retinol were obtained. Concentrations in plasma samples were measured as described by El-Sohemy et al. Plasma samples (250 µL) were mixed with 250 mL ethanol containing 10 ug *rac*-tocopherol/mL (Tocol) as an internal standard, extracted with 4 mL hexane, evaporated to dryness under nitrogen, and reconstituted in 100 mL ethanol-dioxane (1:1, by vol) and 150 mL acetonitrile. Samples are quantitated by high-performance liquid chromatography (HPLC) on a Restek Ultra C_{18} 150 mm X 4.6 mm column with a 3-µm particle size encased in a column oven

(Hitachi L-2350, Hitachi, San Jose, CA) to prevent temperature fluctuations, and equipped with a trident guard cartridge system (Restek, Corp. Bellefonte, PA). A mixture of acetonitrile, tetrahydrofuran, methanol, and a 1% ammonium acetate solution (68:22:7:3) was used as the mobile phase at a flow rate of 1.1 mL/min, with a Hitachi L-2130 pump in isocratic mode, a Hitachi L-2455 diode array detector (300 nm and 445 nm), and a Hitachi L-2200 auto-sampler with water-chilled tray. The Hitachi System Manager software (D-2000 Elite, Version 3.0) was used for peak integration and data acquisition. Internal quality control was monitored with four control samples analyzed within each run. These samples consisted of two identical high-level plasmas and two identical low-level plasmas. Comparison of data from these samples allowed for within-run and between-run variation estimates. In addition, external quality control was monitored by participation in the standardization program for carotenoid analysis from the National Institute of Standards and Technology U.S.A.[41]

Maternal baseline and clinical data was collected and included race, body mass index (BMI), delivery mode, birth month, gestational diabetes mellitus, preeclampsia, placental chorioamnionitis, serum vitamin A levels, and dietary intake. Infant baseline and clinical data was collected and included sex, gestational age, birth weight and percentile, birth head circumference and percentile, birth length and percentile, ROP, supplemental oxygen use, intubation on admission, days on ventilator, BPD, respiratory distress syndrome (RDS), NEC, intraventricular hemorrhage (IVH), positive blood cultures, and cord blood vitamin A levels. A subject was noted to have any of the above conditions if a medical diagnosis was made during the NICU stay in the electronic medical record.

Dietary intake throughout pregnancy was assessed by administering food frequency questionnaires (FFQ) to the mothers. The FFQs encompassed typical intake from supplements, food from all of the major food groups, and beverages over the past year. FFQs were analyzed

using the Harvard nutrient-composition database which contains food composition values from the United States Department of Agriculture.

VAD was defined according to WHO standards and categories used by Fernandes et al. Infants and mothers were considered severely deficient (<0.35 µmol/L or <100 µg/L), deficient (0.35-0.70 µmol/L or 100-200 µg/L), low (0.70-1.05 µmol/L or 200-300 µg/L), or adequate (>1.05 µmol/L or >300 µg/L) based upon their serum retinol levels.[8,25,29] Other investigators have used similar concentrations as an indicator of VAD in infants and pregnant women.[1,3,20,25,26]

STATISTICAL ANALYSIS

Descriptive statistics were used for continuous and categorical variables. Continuous variables (maternal BMI, serum vitamin A levels, and dietary intake information and infant gestational age, birth weight and percentile, birth head circumference and percentile, birth length and percentile, days on ventilator, and serum vitamin A levels) were described using means ± standard deviations, medians, and ranges. For categorical variables (maternal race, delivery mode, birth month, gestational diabetes mellitus, preeclampsia, and placental chorioamnionitis and infant sex, ROP, supplemental oxygen use, intubation on admission, BPD, RDS, NEC, IVH, and positive blood cultures), frequencies and percentages were reported.

Retinol levels were measured in µg/L and converted to µmol/L to reflect adequacy and deficiency standards established by WHO. The conversion factor used was 286 µg retinol is equivalent to 1 µmol retinol.[19] Conversion factors were also used for carotenoids to make comparisons to previous research findings according to the NHANES 2005-2006 data processing protocol. The following conversions factors were used:

- Lutein + zeaxanthin results in µg/L were converted into µmol/L by multiplying by 0.001758

- β-cryptoxanthin results in μg/L were converted into μmol/L by multiplying by 0.001810
- Lycopene results in μg/L were converted into μmol/L by multiplying by 0.001863
- α-carotene results in μg/L were converted into μmol/L by multiplying by 0.001863 and
- β-carotene results in μg/L were converted into μmol/L by multiplying by 0.001863.[42]

Spearman correlation coefficients were used to describe associations between maternal and cord blood vitamin A levels, maternal dietary intake and maternal and cord blood vitamin A levels, gestational age and maternal and cord blood vitamin A levels, and birth weight and maternal and cord blood vitamin A levels.

All tests were two-sided and a P-value <0.05 was considered statistically significant. All statistical analyses were carried out using the Statistical Package for the Social Sciences software (IBM SPSS Statistics; Version 22.0).

CHAPTER 4: RESULTS

SUBJECT CHARACTERISTICS

Maternal and infant demographics and clinical information were obtained for all 34 mother-infant pairs. Characteristics of the study mothers and infants are presented in Tables 2-3.

Maternal Characteristics

Of the mothers enrolled, 64.7% were Caucasian, 17.6% Hispanic, 14.7% African American, and 2.9% other/unknown race. Gestational diabetes mellitus, preeclampsia, and placental chorioamnionitis were present in 17.6%, 20.6%, and 11.8% of mothers, respectively. The mean BMI was 30.7 ± 6.3 kg/m2. Vaginal deliveries occurred in 47.1% of the births. All of the births took place in the summer (29.4% in June, 41.2% in July, and 29.4% in August).

Infant Characteristics

The group of infants was comprised of 58.8% males and 41.2% females. The mean gestational age was 36.7 ± 3.4 weeks. The mean birth weight was 2738.0 ± 835.7 gm (45.5 ± 33.3 %ile), mean length was 47.0 ± 4.6 cm (47.2 ± 34.4 %ile), and mean head circumference was 32.3 ± 2.6 cm (45.4 ± 33.5 %ile). Development of complications after birth in the infants included ROP (2.9%), RDS (38.2%), BPD (2.9%), intubation on admission (26.5%), supplemental oxygen use (41.2%), and a positive blood culture result (2.9%). Nine subjects were on a ventilator for an average of 2.0 ± 2.3 days throughout their NICU stay.

Table 2: Maternal Baseline Characteristics

Categorical Demographics (n=34)	**n (%)**		
Race			
Caucasian	22 (64.7)		
Hispanic	6 (17.6)		
African American	5 (14.7)		
Other/Unknown	1 (2.9)		
Delivery Mode			
Vaginal	16 (47.1)		
Cesarean	18 (52.9)		
Birth Month			
June	10 (29.4)		
July	14 (41.2)		
August	10 (29.4)		
Gestational Diabetes Mellitus	6 (17.6)		
Preeclampsia	7 (20.6)		
Placental Chorioamnionitis	4 (11.8)		
Continuous Demographics	**Mean ± SD**	**Median**	**Range**
BMI (kg/m2) (n=34)	30.7 ± 6.3	29.6	18.0-42.1
Serum Vitamin A Levels (n=31)			
lutein + zeaxanthin (µg/L)	163.3 ± 91.7	140.3	45.1-443.8
β-cryptoxanthin (µg/L)	73.5 ± 53.0	59.9	8.7-285.7
trans-lycopene (µg/L)	243.9 ± 107.4	222.8	35.6-438.5
cis-lycopene (µg/L)	200.9 ± 81.4	194.9	36.7-378.8
total lycopene (µg/L)	444.9 ± 186.2	424.1	72.3-795.8
α-carotene (µg/L)	48.4 ± 72.9	18.1	4.6-388.4
trans-β-carotene (µg/L)	186.4 ± 221.6	104.6	21.4-968.9
cis-β-carotene (µg/L)	14.5 ± 16.1	7.5	1.7-67.8
total-β-carotene (µg/L)	200.9 ± 237.5	113.2	23.1-1036.7
retinol (µg/L)	326.3 ± 140.8	288.2	146.5-648.9
retinol (µmol/L)	1.14 ± 0.49	1.01	0.51-2.27
Dietary Intake (n=20)			
Calorie Intake (kcals)	2600.3 ± 991.3	2323.7	1314.0-4415.6
Protein Intake (gm)	104.1 ± 32.3	108.7	44.9-157.4
Carbohydrate Intake (gm)	343.1 ± 157.5	299.0	165.6-710.7
Fat Intake (gm)	95.1 ± 35.4	89.9	39.0-161.7
Retinol Activity Equivalents (RAE) (µg)	1799.9 ± 613.6	1937.4	422.3-2721.7
lutein + zeaxanthin (µg)	2761.7 ± 1894.5	2060.2	852.4-6937.0
β-cryptoxanthin (µg)	218.5 ± 183.2	130.7	45.0-629.5
total lycopene (µg)	5362.8 ± 5273.4	3637.2	1072.0-24654.1
α-carotene (µg)	620.5 ± 1056.6	267.8	17.4-4719.5
total-β-carotene (µg)	5039.2 ± 3806.7	4336.6	430.8-15661.6
retinol (IU)	3403.6 ± 1837.1	3243.3	756.1-7490.8

Table 3: Infant Baseline Characteristics

Categorical Demographics (n=34)	**n (%)**		
Sex			
Male	20 (58.8)		
Female	14 (41.2)		
Retinopathy of Prematurity (ROP)	1 (2.9)		
Supplemental Oxygen Use	14 (41.2)		
Intubation on Admission	9 (26.5)		
Bronchopulmonary Dysplasia (BPD)	1 (2.9)		
Respiratory Distress Syndrome (RDS)	13 (38.2)		
Necrotizing Enterocolitis (NEC)	0 (0)		
Intraventricular hemorrhage (IVH)	0 (0)		
Positive blood culture	1 (2.9)		
Continuous Demographics (n=34)	**Mean ± SD**	**Median**	**Range**
Gestational Age (weeks)	36.7 ± 3.4	37.1	30.3-42.0
Birth weight (gm)	2738.0 ± 835.7	2850.0	840.0-4109.0
Birth weight Percentile	45.5 ± 33.3	43.4	0.0-98.8
Birth Head Circumference (cm)	32.3 ± 2.6	32.7	25.0-37.0
Birth Head Circumference Percentile	45.4 ± 33.5	43.3	0.0-99.6
Birth Length (cm)	47.0 ± 4.6	47.0	35.0-55.0
Birth Length Percentile	47.2 ± 34.4	48.5	0.0-99.7
Days on Ventilator (n=9)	2.0 ± 2.3	1.0	1.0-8.0
Cord Blood Vitamin A Levels (n=32)			
lutein + zeaxanthin (µg/L)	25.4 ± 11.4	24.6	9.5-57.7
β-cryptoxanthin (µg/L)	9.3 ± 5.7	7.9	2.4-25.8
trans-lycopene (µg/L)	11.5 ± 6.3	10.5	3.6-26.0
cis-lycopene (µg/L)	10.3 ± 5.3	9.2	2.9-24.0
total lycopene (µg/L)	21.8 ± 11.4	19.5	6.5-48.5
α-carotene (µg/L)	5.4 ± 4.5	4.5	0.0-18.8
trans-β-carotene (µg/L)	15.1 ± 15.4	10.1	0.0-70.3
cis-β-carotene (µg/L)	3.1 ± 2.5	2.3	0.0-10.0
total-β-carotene (µg/L)	18.3 ± 17.3	12.4	0.0-75.9
retinol (µg/L)	172.0 ± 43.0	180.5	98.1-255.9
retinol (µmol/L)	0.60 ± 0.15	0.63	0.34-0.89

VITAMIN A STATUS

Maternal and cord blood samples were collected and analyzed for 31 mothers and 32 infants. Maternal and cord blood levels are displayed in Tables 2-3. Vitamin A status of mothers and infants according to WHO classification is shown in Table 4 and Figure 4.

Maternal Status

The mean maternal serum retinol level was 326.3 ± 140.8 µg/L (1.14 ± 0.50 µmol/L). Categorical placement of retinol levels revealed 41.9%, 45.2%, and 12.9% of mothers had adequate, low, and deficient vitamin A status, respectively. The mean lutein + zeaxanthin level was 163.3 ± 91.7 µg/L, β-cryptoxanthin was 73.5 ± 53.0 µg/L, trans-lycopene was 243.9 ± 107.4 µg/L, cis-lycopene was 200.9 ± 81.4 µg/L, total lycopene was 444.9 ± 186.2 µg/L, α-carotene was 48.4 ± 72.9 µg/L, trans-β-carotene was 186.4 ± 221.6 µg/L, cis-β-carotene was 14.5 ± 16.1 µg/L, and total-β-carotene was 200.9 ± 237.5 µg/L.

Infant Status

At birth, 71.9% of infants were vitamin A deficient with 3.1% of those being severely deficient. The remaining 28.1% of infants had low vitamin A status. None of the subjects had levels >1.05 µmol/L. The mean cord retinol level was 172.0 ± 43.0 µg/L (0.60 ± 0.15 µmol/L). The mean lutein + zeaxanthin level was 25.4 ± 11.4 µg/L, β-cryptoxanthin was 9.3 ± 5.7 µg/L, trans-lycopene was 11.5 ± 6.3 µg/L, cis-lycopene was 10.3 ± 5.3 µg/L, total lycopene was 21.8 ± 11.4 µg/L, α-carotene was 5.4 ± 4.5 µg/L, trans-β-carotene was 15.1 ± 15.4 µg/L, cis-β-carotene was 3.1 ± 2.5 µg/L, and total-β-carotene was 18.3 ± 17.3 µg/L.

Table 4: Prevalence of Vitamin A Deficiency

Infants (n = 32)	**n (%)**
Severely Deficient (<0.35 µmol/L or <100 µg/L)	1 (3.1)
Deficient (0.35-0.70 µmol/L or 100-200 µg/L)	22 (68.8)
Low (0.70-1.05 µmol/L or 200-300 µg/L)	9 (28.1)
Adequate (>1.05 µmol/L or >300 µg/L)	0 (0)
Mothers (n = 31)	
Severely Deficient (<0.35 µmol/L or <100 µg/L)	0 (0)
Deficient (0.35-0.70 µmol/L or 100-200 µg/L)	4 (12.9)
Low (0.70-1.05 µmol/L or 200-300 µg/L)	14 (45.2)
Adequate (>1.05 µmol/L or >300 µg/L)	13 (41.9)

Figure 4: Maternal and Infant Serum Vitamin A Status

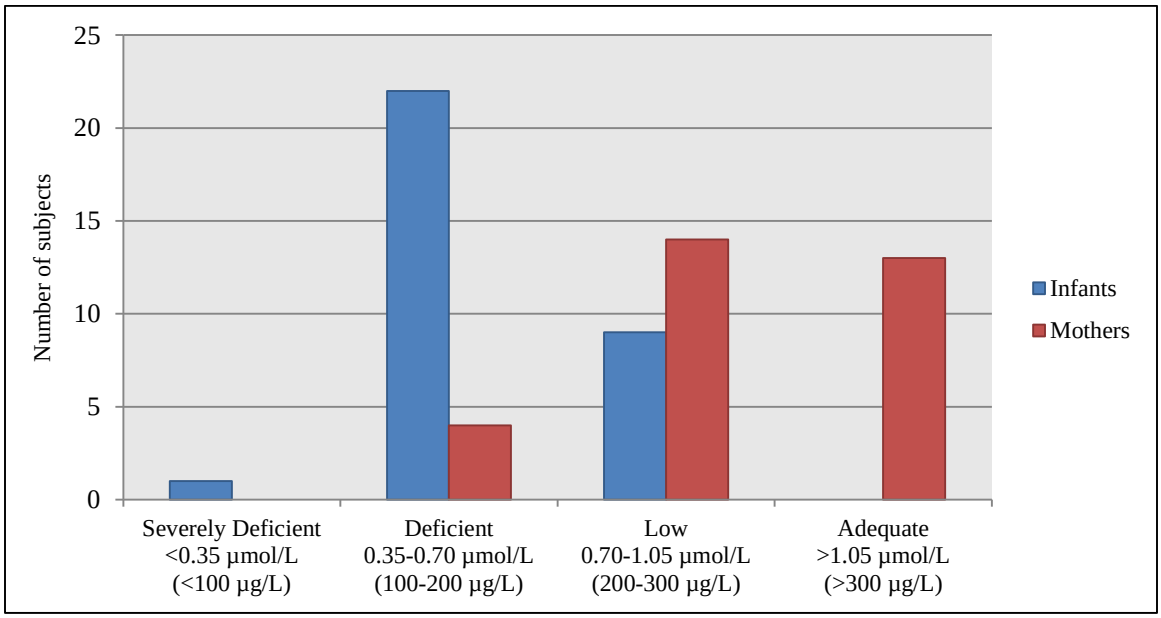

Figure 4: Maternal and Infant Serum Vitamin A Status. Maternal and infant retinol levels categorized into severely deficient, deficient, low, and adequate vitamin A status.

Relationship between Serum Vitamin A Levels of Mothers and Infants:

Associations between maternal and cord blood were determined and are displayed in Table 5. Levels were positively correlated for lutein + zeaxanthin (r=0.50, p=0.004), β-cryptoxanthin (r=0.83, p=<0.001), trans-lycopene (r=0.68, p=<0.001), cis-lycopene (r=0.52, p=0.003), total lycopene (r=0.63, p=<0.001), α-carotene (r=0.67, p=<0.001), trans-β-carotene (r=0.74, p=<0.001), cis-β-carotene (r=0.44, p=0.013), total-β-carotene (r=0.71, p=<0.001), and retinol (r=0.42, p=0.018). Figures 5-10 display the significant correlations for the main carotenoids (lutein + zeaxanthin, β-cryptoxanthin, total lycopene, α-carotene, and total-β-carotene) and retinol.

Table 5: Correlations of Maternal and Infant Vitamin A Concentrations

	Correlation Coefficient	P-value
lutein + zeaxanthin (µg/L)	0.50	0.004
β-cryptoxanthin (µg/L)	0.83	<0.001
trans-lycopene (µg/L)	0.68	<0.001
cis-lycopene (µg/L)	0.52	0.003
total lycopene (µg/L)	0.63	<0.001
α-carotene (µg/L)	0.67	<0.001
trans-β-carotene (µg/L)	0.74	<0.001
cis-β-carotene (µg/L)	0.44	0.013
total-β-carotene (µg/L)	0.71	<0.001
retinol (µg/L)	0.42	0.018

Figure 5: Relationship of Maternal-Infant Serum Lutein + Zeaxanthin Concentrations

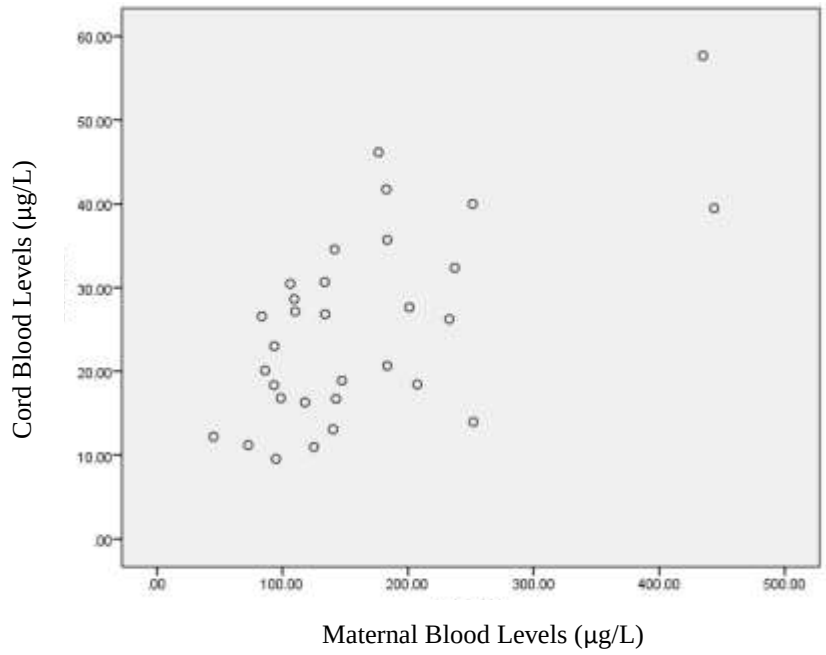

Figure 5: Relationship of Maternal-Infant Serum Lutein + Zeaxanthin Concentrations. Positive correlation between maternal and infant cord blood lutein + zeaxanthin levels (r=0.50; p=0.004).

Figure 6: Relationship of Maternal-Infant Serum β-Cryptoxanthin Concentrations

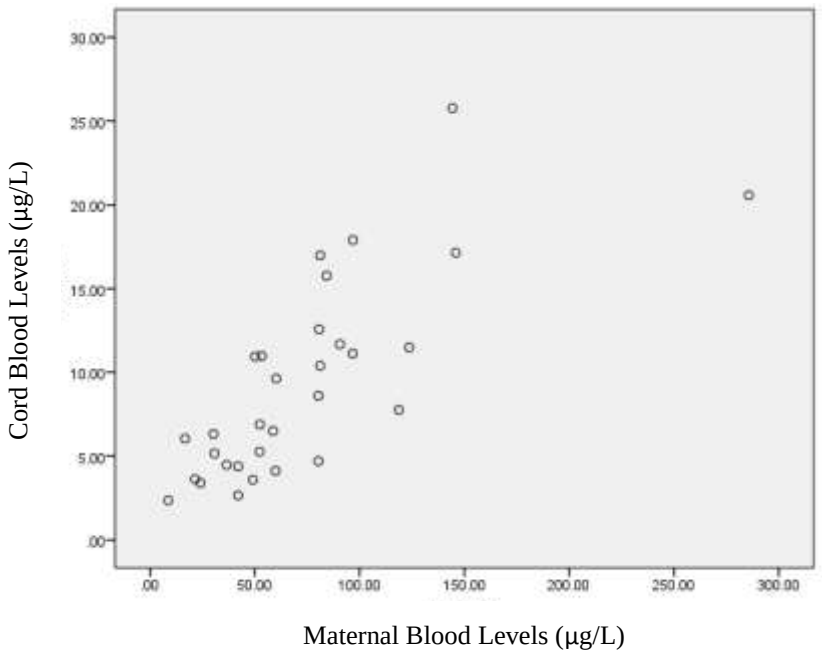

Figure 6: Relationship of Maternal-Infant Serum β-Cryptoxanthin Concentrations. Positive correlation between maternal and infant cord blood β-cryptoxanthin levels ($r=0.83$; $p=<0.001$).

Figure 7: Relationship of Maternal-Infant Serum Total Lycopene Concentrations

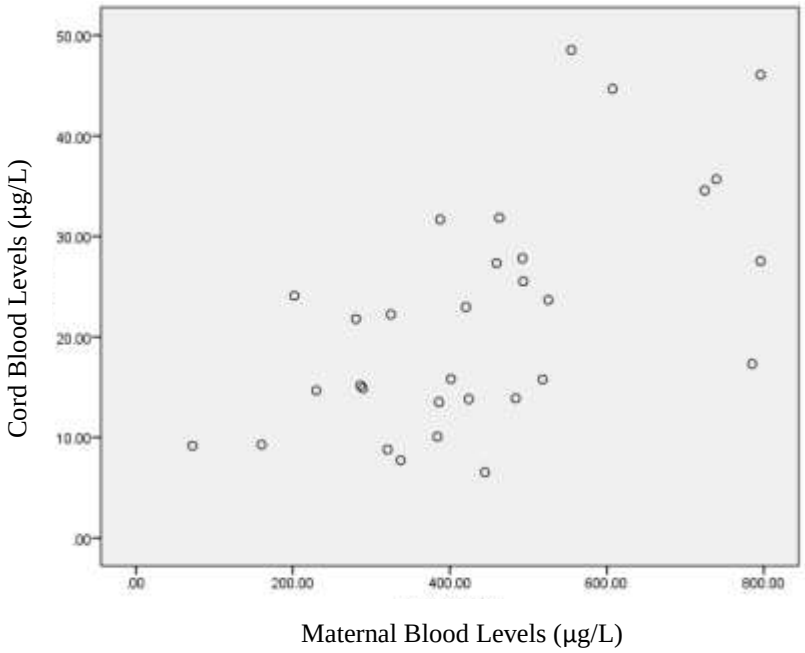

Figure 7: Relationship of Maternal-Infant Serum Total Lycopene Concentrations. Positive correlation between maternal and infant cord blood total lycopene levels (r=0.63; p=<0.001).

Figure 8: Relationship of Maternal-Infant Serum α-Carotene Concentrations

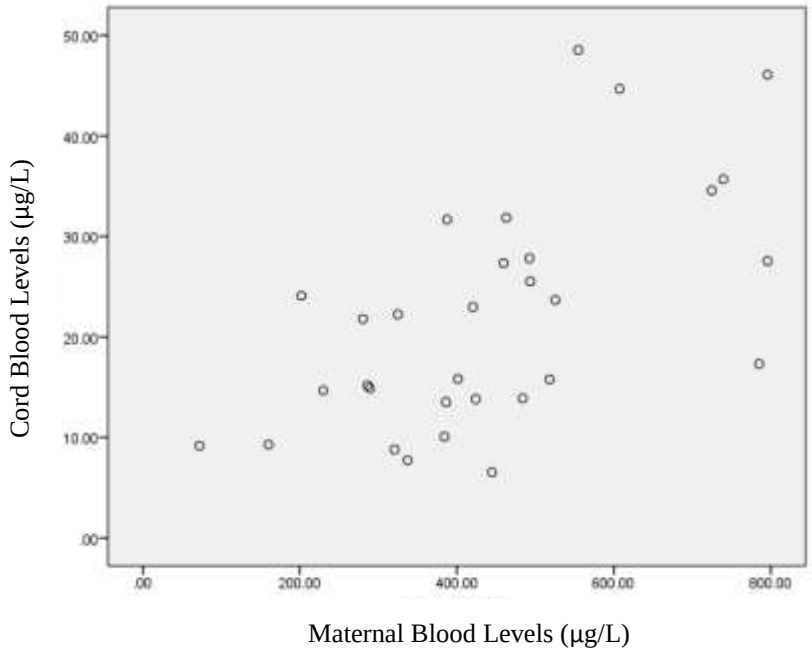

Figure 8: Relationship of Maternal-Infant Serum α-Carotene Concentrations. Positive correlation between maternal and infant cord blood α-Carotene levels (r=0.67; p=<0.001).

Figure 9: Relationship of Maternal-Infant Serum Total-β-Carotene Concentrations

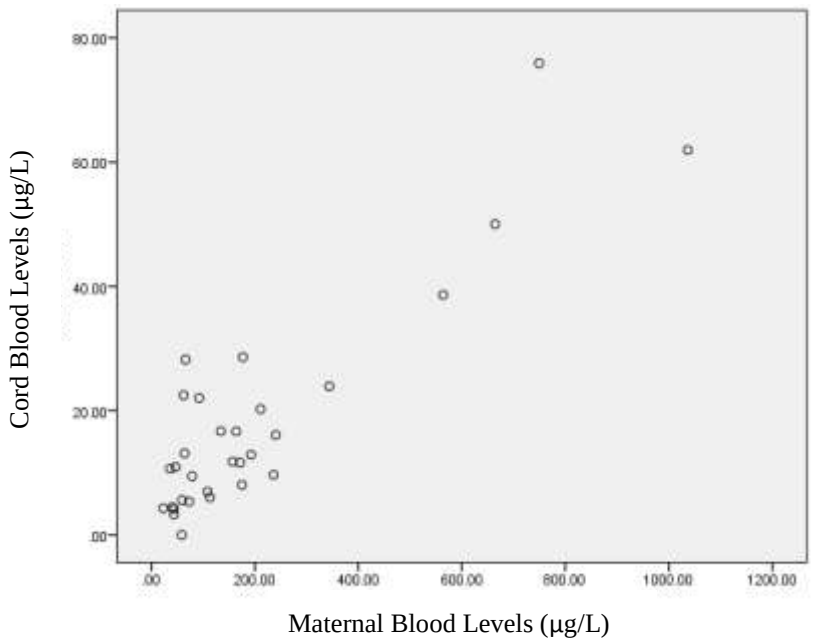

Figure 9: Relationship of Maternal-Infant Serum Total-β-Carotene Concentrations. Positive correlation between maternal and infant cord blood total β-carotene levels (r=0.71; p=<0.001).

Figure 10: Relationship of Maternal-Infant Serum Retinol Concentrations

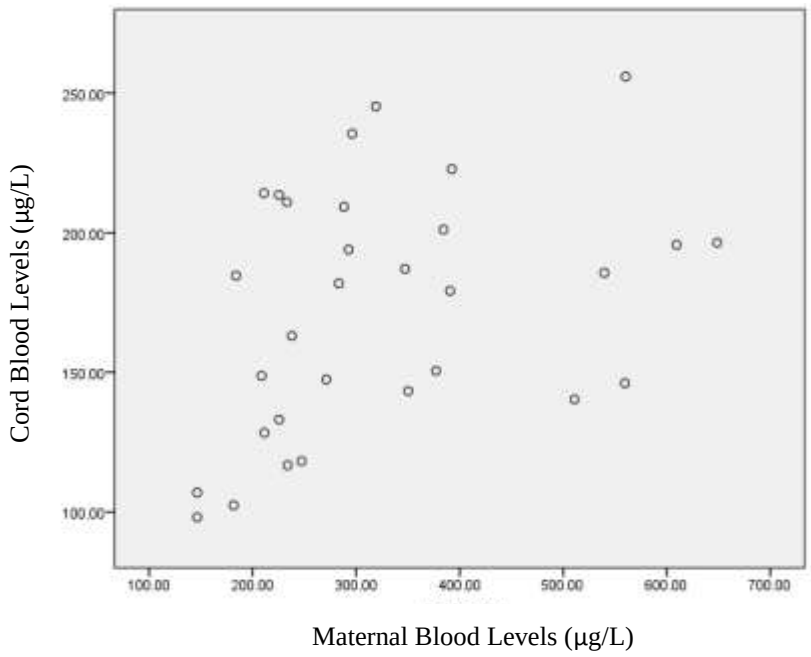

Figure 10: Relationship of Maternal-Infant Serum Retinol Concentrations. Positive correlation between maternal and infant cord blood retinol levels (r=0.42; p=0.018).

Positive significant associations between birth weight and maternal serum vitamin A concentrations were found for β-cryptoxanthin (r=0.36, p=0.048), α-carotene (r=0.44, p=0.012), trans-β-carotene (r=0.48, p=0.007), cis-β-carotene (r=0.50, p=0.004), and total-β-carotene (r=0.49, p=0.005). Table 6 displays all correlations between birth weight and maternal serum vitamin A concentrations. Figures 11-13 illustrate the associations between the main carotenoids (β-cryptoxanthin, α-carotene, and total-β-carotene) with significant correlations. Significant associations between birth weight and cord blood concentrations were also found for β-cryptoxanthin (r=0.39, p=0.026), α-carotene (r=0.36, p=0.042), trans-β-carotene (r=0.42, p=0.015), cis-β-carotene (r=0.44, p=0.012), total-β-carotene (r=0.44, p=0.012), and retinol (r=0.73, p=<0.001). All correlations are shown in Table 7 for birth weight and cord blood concentrations. Retinol was most strongly associated with birth weight and is presented in Figure 14.

Table 6: Correlations of Birth Weight and Maternal Vitamin A Concentrations

	Correlation Coefficient	P-value
lutein + zeaxanthin (µg/L)	0.34	0.065
β-cryptoxanthin (µg/L)	0.36	0.048
trans-lycopene (µg/L)	-0.12	0.532
cis-lycopene (µg/L)	-0.08	0.665
total lycopene (µg/L)	-0.11	0.570
α-carotene (µg/L)	0.44	0.012
trans-β-carotene (µg/L)	0.48	0.007
cis-β-carotene (µg/L)	0.50	0.004
total-β-carotene (µg/L)	0.49	0.005
retinol (µg/L)	0.17	0.361

Figure 11: Relationship of Birth Weight and Maternal β-Cryptoxanthin Concentrations

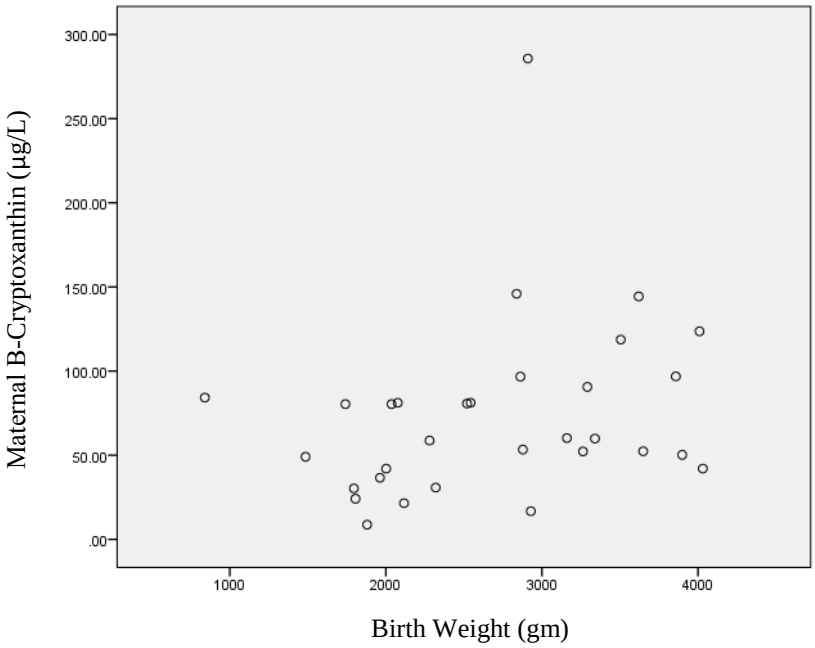

Figure 11: Relationship of Birth Weight and Maternal β-Cryptoxanthin Concentrations. Positive correlation between birth weight and maternal serum β-cryptoxanthin levels (r=0.36; p=0.048).

Figure 12: Relationship of Birth Weight and Maternal α-Carotene Concentrations

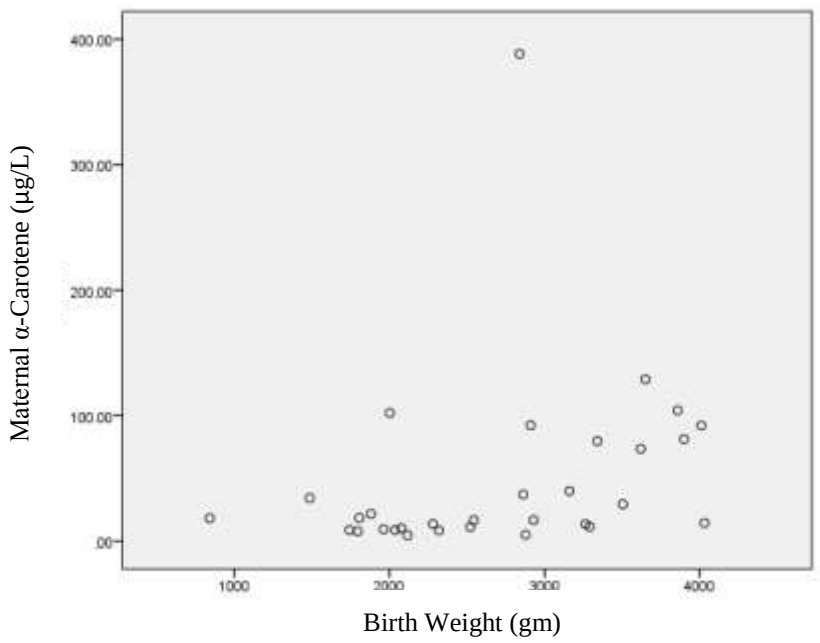

Figure 12: Relationship of Birth Weight and Maternal α-Carotene Concentrations. Positive correlation between birth weight and maternal serum α-carotene levels (r=0.44; p=0.012).

Figure 13: Relationship of Birth Weight and Maternal Total-β-Carotene Concentrations

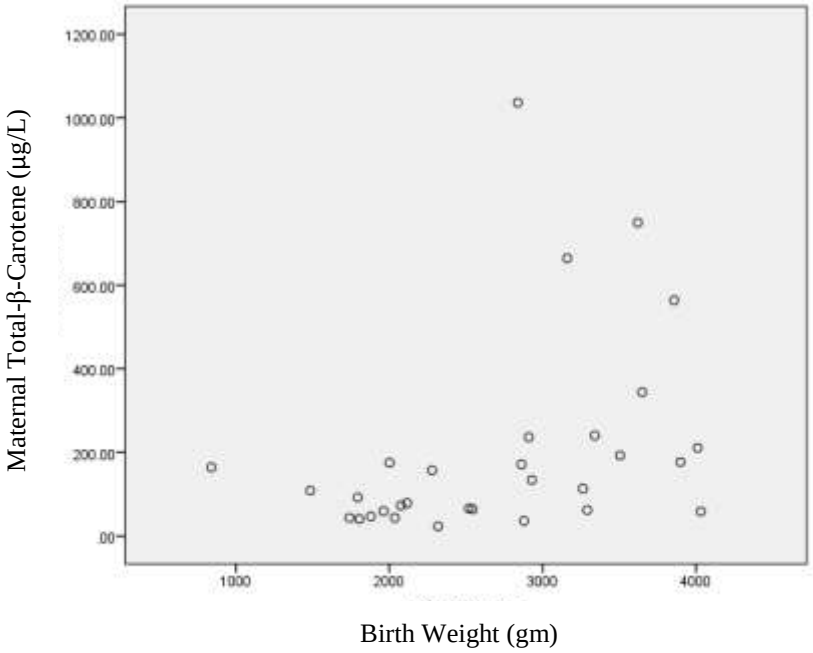

Figure 13: Relationship of Birth Weight and Maternal Total-β-Carotene Concentrations. Positive correlation between birth weight and maternal serum total-β-carotene levels (r=0.49; p=0.005).

Table 7: Correlations of Birth Weight and Infant Vitamin A Concentrations

	Correlation Coefficient	P-value
lutein + zeaxanthin (µg/L)	0.21	0.250
β-cryptoxanthin (µg/L)	0.39	0.026
trans-lycopene (µg/L)	-0.11	0.553
cis-lycopene (µg/L)	-0.10	0.600
total lycopene (µg/L)	-0.07	0.715
α-carotene (µg/L)	0.36	0.042
trans-β-carotene (µg/L)	0.42	0.015
cis-β-carotene (µg/L)	0.44	0.012
total-β-carotene (µg/L)	0.44	0.012
retinol (µg/L)	0.73	<0.001

Figure 14: Relationship of Birth Weight and Infant Retinol Concentrations

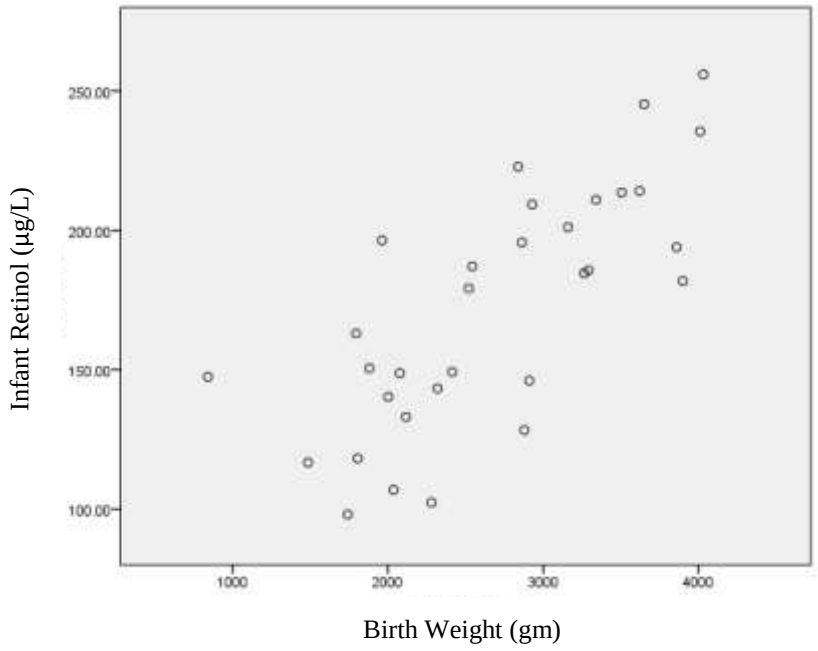

Figure 14: Relationship of Birth Weight and Infant Retinol Concentrations. Positive correlation between birth weight and cord blood retinol levels (r=0.73; p=0.005).

Gestational age and maternal β-cryptoxanthin was significantly positively correlated (r=0.39, p=0.031) (Figure 15) as well as gestational age and infant β-cryptoxanthin (r=0.39, p=0.026), cis-β-carotene (r=0.37, p=0.039), and retinol (r=0.55, p=0.001) (Figure 16). Table 8 and Table 9 show all correlations between gestational age and serum vitamin A concentrations for mothers and infants, respectively.

Table 8: Correlations of Gestational Age and Maternal Vitamin A Concentrations

	Correlation Coefficient	P-value
lutein + zeaxanthin (µg/L)	0.23	0.221
β-cryptoxanthin (µg/L)	0.39	0.031
trans-lycopene (µg/L)	-0.11	0.571
cis-lycopene (µg/L)	-0.09	0.636
total lycopene (µg/L)	-0.11	0.568
α-carotene (µg/L)	0.22	0.236
trans-β-carotene (µg/L)	0.27	0.144
cis-β-carotene (µg/L)	0.35	0.053
total-β-carotene (µg/L)	0.28	0.123
retinol (µg/L)	-0.12	0.519

Figure 15: Relationship of Gestational Age and Maternal β-Cryptoxanthin Concentrations

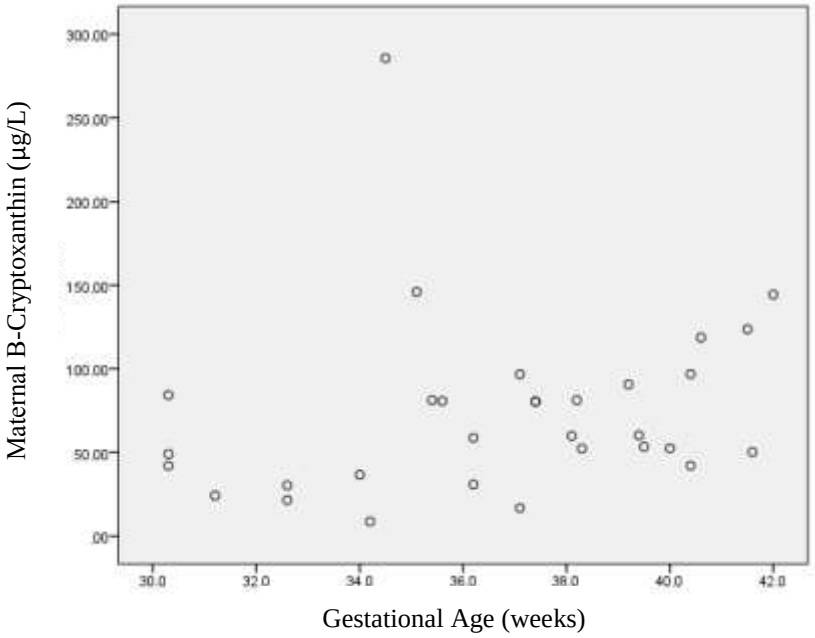

Figure 15: Relationship of Gestational Age and Maternal β-Cryptoxanthin Concentrations. Positive correlation between gestational age and maternal serum β-cryptoxanthin levels (r=0.39; p=0.031).

Table 9: Correlations of Gestational Age and Infant Vitamin A Concentrations

	Correlation Coefficient	P-value
lutein + zeaxanthin (µg/L)	0.25	0.173
β-cryptoxanthin (µg/L)	0.39	0.026
trans-lycopene (µg/L)	0.01	0.949
cis-lycopene (µg/L)	0.02	0.929
total lycopene (µg/L)	0.04	0.826
α-carotene (µg/L)	0.18	0.323
trans-β-carotene (µg/L)	0.31	0.083
cis-β-carotene (µg/L)	0.37	0.039
total-β-carotene (µg/L)	0.33	0.063
retinol (µg/L)	0.55	0.001

Figure 16: Relationship of Gestational Age and Infant Retinol Concentrations

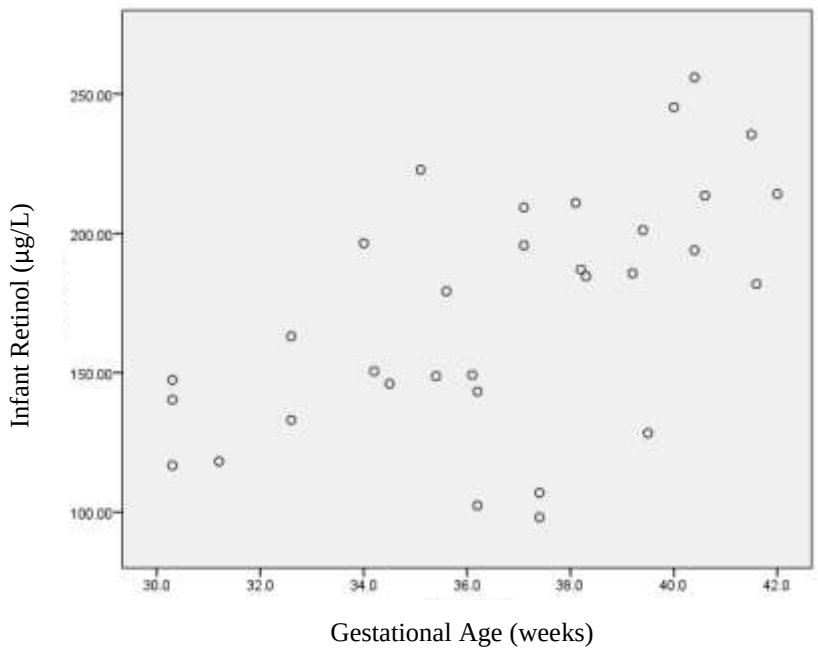

Figure 16: Relationship of Gestational Age and Infant Retinol Concentrations. Positive correlation between gestational age and cord blood retinol levels (r=0.55; p=0.001).

MATERNAL VITAMIN A INTAKE

FFQs were completed for 21 mothers, but data from 20 FFQs were used for analysis (Table 2). FFQ data from one mother was excluded due to results of calorie intake (31727 kcals) exceeding expected amount. The median daily energy intake was 2323.7 kcals (1314.0-4415.6 kcals). Median macronutrient intake of protein, carbohydrates, and fat were 108.7 gm (44.9-157.4 gm), 299.0 gm (165.6-710.7 gm), and 89.9 gm (39.0-161.7 gm), respectively. Median daily RAE was 1937.4 µg (422.3-2721.7 µg). Median retinol intake was 3243.3 IU (756.1-7490.8 IU. Specific median carotenoid intake was 2060.2 µg (52.4-6937.0 µg) of lutein + zeaxanthin, 130.7 µg (45.0-629.5 µg) of β-cryptoxanthin, 3637.2 µg (1072.0-24654.1 µg) of lycopene, 267.8 µg (17.4-4719.5 µg) of α-carotene, and 4336.6 µg (430.8-15661.6 µg) of β-carotene.

Correlations between dietary intake and maternal and cord blood concentrations of vitamin A compounds are displayed in Tables 10-11. Maternal intake of specific vitamin A forms was found to be significantly associated with maternal and cord blood levels. Intake of lutein + zeaxanthin was positively associated with maternal serum α-carotene ($r=0.47$, $p=0.047$) and β-carotene ($r=0.48$, $p=0.045$). Dietary α-carotene was positively associated with maternal serum α-carotene ($r=0.52$, $p=0.029$). β-carotene intake was also positively associated with maternal serum β-carotene ($r=0.47$, $p=0.050$) and α-carotene ($r=0.50$, $p=0.034$). One significant negative association was found between RAE intake and maternal serum β-cryptoxanthin ($r=-0.51$, $p=0.031$). Maternal intake of lutein + zeaxanthin and β-carotene was significantly correlated with cord blood α-carotene levels ($r=0.55$, $p=0.015$ and $r=0.63$, $p=0.004$, respectively).

Table 10: Correlations of Maternal Intake of Vitamin A and Maternal Blood Levels

Dietary intake vs. Serum Level	Correlation Coefficient	P-value (*significant)
lutein + zeaxanthin (µg) vs. lutein + zeaxanthin (µg/L)	0.31	0.205
lutein + zeaxanthin (µg) vs. β-cryptoxanthin (µg/L)	0.27	0.278
lutein + zeaxanthin (µg) vs. total lycopene (µg/L)	-0.15	0.550
lutein + zeaxanthin (µg) vs. α-carotene (µg/L)	0.47	0.047*
lutein + zeaxanthin (µg) vs. total-β-carotene (µg/L)	0.48	0.045*
lutein + zeaxanthin (µg) vs. retinol (µg/L)	-0.05	0.858
β-cryptoxanthin (µg) vs. β-cryptoxanthin (µg/L)	0.16	0.518
β-cryptoxanthin (µg) vs. lutein + zeaxanthin (µg/L)	-0.11	0.677
β-cryptoxanthin (µg) vs. total lycopene (µg/L)	0.27	0.281
β-cryptoxanthin (µg) vs. α-carotene (µg/L)	-0.12	0.633
β-cryptoxanthin (µg) vs. total-β-carotene (µg/L)	-0.02	0.942
β-cryptoxanthin (µg) vs. retinol (µg/L)	-0.07	0.772
lycopene (µg) vs. total lycopene (µg/L)	0.38	0.119
lycopene (µg) vs. lutein + zeaxanthin (µg/L)	0.19	0.458
lycopene (µg) vs. β-cryptoxanthin (µg/L)	0.21	0.404
lycopene (µg) vs. α-carotene (µg/L)	0.14	0.573
lycopene (µg) vs. total-β-carotene (µg/L)	0.31	0.216
lycopene (µg) vs. retinol (µg/L)	0.09	0.738
α-carotene (µg) vs. α-carotene (µg/L)	0.52	0.029*
α-carotene (µg) vs. lutein + zeaxanthin (µg/L)	0.33	0.188
α-carotene (µg) vs. β-cryptoxanthin (µg/L)	0.13	0.598
α-carotene (µg) vs. total lycopene (µg/L)	-0.17	0.499
α-carotene (µg) vs. total-β-carotene (µg/L)	0.46	0.058
α-carotene (µg) vs. retinol (µg/L)	0.32	0.203
β-carotene (µg) vs. total-β-carotene (µg/L)	0.47	0.050*
β-carotene (µg) vs. lutein + zeaxanthin (µg/L)	0.25	0.326
β-carotene (µg) vs. β-cryptoxanthin (µg/L)	0.19	0.450
β-carotene (µg) vs. total lycopene (µg/L)	-0.16	0.407
β-carotene (µg) vs. α-carotene (µg/L)	0.50	0.034*
β-carotene (µg) vs. retinol (µg/L)	0.28	0.263
retinol (IU) vs. retinol (µg/L)	-0.07	0.779
retinol (IU) vs. lutein + zeaxanthin (µg/L)	-0.20	0.435
retinol (IU) vs. β-cryptoxanthin (µg/L)	-0.18	0.486
retinol (IU) vs. total lycopene (µg/L)	-0.22	0.378
retinol (IU) vs. α-carotene (µg/L)	-0.14	0.592
retinol (IU) vs. total-β-carotene (µg/L)	-0.03	0.909
RAE (µg) vs. lutein + zeaxanthin (µg/L)	-0.30	0.222
RAE (µg) vs. β-cryptoxanthin (µg/L)	-0.51	0.031*
RAE (µg) vs. total lycopene (µg/L)	0.03	0.906
RAE (µg) vs. α-carotene (µg/L)	0.00	0.997
RAE (µg) vs. total-β-carotene (µg/L)	0.06	0.804
RAE (µg) vs. retinol (µg/L)	0.14	0.581

Table 11: Correlations of Maternal Intake of Vitamin A and Cord Blood Levels

Dietary intake vs. Serum Level	Correlation Coefficient	P-value (*significant)
lutein + zeaxanthin (µg) vs. lutein + zeaxanthin (µg/L)	0.09	0.726
lutein + zeaxanthin (µg) vs. β-cryptoxanthin (µg/L)	0.22	0.359
lutein + zeaxanthin (µg) vs. total lycopene (µg/L)	-0.09	0.716
lutein + zeaxanthin (µg) vs. α-carotene (µg/L)	0.55	0.015*
lutein + zeaxanthin (µg) vs. total-β-carotene (µg/L)	0.39	0.103
lutein + zeaxanthin (µg) vs. retinol (µg/L)	0.29	0.226
β-cryptoxanthin (µg) vs. β-cryptoxanthin (µg/L)	0.01	0.977
β-cryptoxanthin (µg) vs. lutein + zeaxanthin (µg/L)	-0.09	0.729
β-cryptoxanthin (µg) vs. total lycopene (µg/L)	0.04	0.875
β-cryptoxanthin (µg) vs. α-carotene (µg/L)	-0.03	0.901
β-cryptoxanthin (µg) vs. total-β-carotene (µg/L)	-0.16	0.518
β-cryptoxanthin (µg) vs. retinol (µg/L)	-0.16	0.502
lycopene (µg) vs. total lycopene (µg/L)	0.06	0.814
lycopene (µg) vs. lutein + zeaxanthin (µg/L)	0.26	0.288
lycopene (µg) vs. β-cryptoxanthin (µg/L)	0.23	0.351
lycopene (µg) vs. α-carotene (µg/L)	0.05	0.844
lycopene (µg) vs. total-β-carotene (µg/L)	0.05	0.836
lycopene (µg) vs. retinol (µg/L)	0.17	0.488
α-carotene (µg) vs. α-carotene (µg/L)	0.34	0.149
α-carotene (µg) vs. lutein + zeaxanthin (µg/L)	0.12	0.616
α-carotene (µg) vs. β-cryptoxanthin (µg/L)	0.15	0.542
α-carotene (µg) vs. total lycopene (µg/L)	-0.29	0.232
α-carotene (µg) vs. total-β-carotene (µg/L)	0.18	0.450
α-carotene (µg) vs. retinol (µg/L)	0.23	0.355
β-carotene (µg) vs. total-β-carotene (µg/L)	0.38	0.110
β-carotene (µg) vs. lutein + zeaxanthin (µg/L)	0.06	0.803
β-carotene (µg) vs. β-cryptoxanthin (µg/L)	0.24	0.333
β-carotene (µg) vs. total lycopene (µg/L)	-0.13	0.611
β-carotene (µg) vs. α-carotene (µg/L)	0.63	0.004*
β-carotene (µg) vs. retinol (µg/L)	0.27	0.257
retinol (IU) vs. retinol (µg/L)	-0.01	0.969
retinol (IU) vs. lutein + zeaxanthin (µg/L)	0.00	0.997
retinol (IU) vs. β-cryptoxanthin (µg/L)	-0.07	0.770
retinol (IU) vs. total lycopene (µg/L)	-0.23	0.355
retinol (IU) vs. α-carotene (µg/L)	-0.32	0.179
retinol (IU) vs. total-β-carotene (µg/L)	0.02	0.943
RAE (µg) vs. lutein + zeaxanthin (µg/L)	-0.09	0.710
RAE (µg) vs. β-cryptoxanthin (µg/L)	-0.29	0.223
RAE (µg) vs. total lycopene (µg/L)	0.07	0.764
RAE (µg) vs. α-carotene (µg/L)	-0.04	0.864
RAE (µg) vs. total-β-carotene (µg/L)	0.11	0.658
RAE (µg) vs. retinol (µg/L)	0.11	0.658

CHAPTER 5: DISCUSSION

VITAMIN A STATUS IN MOTHERS AND INFANTS

The mean retinol level in infants, indicative of vitamin A adequacy or deficiency, was 172.0 μg/L (0.60 μmol/L). This is considered deficient according to WHO standards.[8] None of the infants had adequate levels >1.05 μmol/L. These findings are concerning considering WHO has not acknowledged VAD as a problem and has not evaluated the prevalence in infants or preschool-age children in the United States. Some studies evaluating infants with mean normal birth weights or mean gestational ages >37 weeks, similar to our subjects, have reported retinol cord levels of 0.49-0.73 μmol/L in the United States (Hawaii), China, and India,[2,20,43] which are consistent with our findings. Others have found higher levels at 0.90-1.29 μmol/L in Germany, Brazil, and Ireland.[18,24,25,33] Preterm infants have even been found to have higher levels ranging from 1.19-1.29 μmol/L in Finland.[26] Very low or low birth weight infants, though, have been found to be deficient with cord blood concentrations of 0.47-0.54 μmol/L in India and Thailand.[3,43] Retinol concentrations in cord blood are still variable throughout the world and between infants of different birth weights and gestational ages. It has been proposed that the normal retinol concentration for newborn infants is between 0.7-2.5 μmol/L.[44] Assessing levels in the United States, especially different regions of the country, will help define the level of public health significance and thus allow health professionals to appropriately screen and provide interventions to prevent or treat VAD.

VAD is more prevalent than expected in the infants in our study. The prevalence of VAD when defined as serum retinol concentrations <0.70 μmol/L has been previously reported to affect 23.1-49.3% of infants with normal birth weights and gestational ages in Brazil, China, and South Africa,[20,25,34] 3.6% of infants <33 weeks gestational age in Finland,[26] and 67.7% of very low birth weight infants in Thailand.[3] In addition, 45.5% of healthy infants in Brazil had VAD

when retinol levels were <1.05 μmol/L.[24] Roughly 3 out of 4 infants in our study had VAD, despite nearly 42% of mothers having adequate retinol levels. Low retinol levels in infants at birth could be expected because the fetal liver is only able to store a small amount of vitamin A during pregnancy,[14] but does not explain the degree of deficiency in our study.

The maternal-fetal placental transfer was 53%, consistent with 52-55% placental transfers previously reported[33,43,44] and a proposed estimated ratio of maternal to fetal plasma concentrations in healthy pregnancies of 2:1.[1] Cord retinol levels at 50-60% of maternal levels have been suggested to represent a normal range for infants at birth.[44] Researchers in Brazil and China, though, have reported higher retinol placental transfer rates of 75-78%.[20,24,25] Even when comparing infants of low and normal birth weights, rates have not varied between the two groups. Agarwal et al. discovered a placental transfer of 34% in low birth weight infants and 37% in normal birth weight infants, both findings much lower than the results of our study.[35]

WHO has expanded the serum range for what is considered deficient to include those with retinol levels <0.70 μmol/L, which identifies more mothers and infants with VAD. This cutoff value was raised from <0.35 μmol/L in 1996 after members of WHO thought it was too low to identify those with subclinical signs of deficiency.[23] It has been suggested that defining VAD as retinol levels <0.70 μmol/L may not apply for mothers and infants right after delivery due to the acute phase response as a result of the birth process and the hemodilution of pregnancy.[34] However, this change appears to be appropriate as even a subclinical deficiency that can be present with retinol levels <0.70 μmol/L has been shown to negatively impact health and increase the risk of infections, reduce growth, and decrease survival from serious illnesses.[21] Early identification could lead to earlier interventions and potentially improve health outcomes. Even a low vitamin A level should be acted upon to decrease the likelihood of a deficiency from developing, especially in infants. Infants already have low liver stores at birth to begin with and

these can be quickly depleted in a couple of days if experiencing a sudden stressful health event, malabsorption state, or fasting state.[14]

In mothers, low retinol levels throughout pregnancy influence the developing fetus and contribute to VAD in infants at birth. The mean maternal retinol level in our study was 326.3 μg/L (1.14 μmol/L). Despite this adequate vitamin A concentration, nearly 13% of mothers were deficient (<0.70 μmol/L), with another 45% with low levels (0.70-1.05 μmol/L). This is especially concerning given the placental transfer is only approximately 50%. A few studies have found similar maternal retinol levels between 1.13-1.23 μmol/L[18,20,36] while others have found higher concentrations of 1.33-1.88 μmol/L[24,25,33,43] including the NHANES III data where pregnant women had mean retinol levels of 1.47 μmol/L, reflective of the United States population.[10] Retinol levels in mothers in our study and previous research appear to be variable, which may be a result of environmental factors such as vitamin A intake. It may be crucial to evaluate maternal nutritional status throughout pregnancy in order to optimize vitamin A intake and thus vitamin A status in the mother and the developing fetus.

Carotenoid Status:

The carotenoid status in mothers and infants at birth has not been evaluated nearly to the extent that retinol levels have been, especially in the United States. Reference intervals are not currently established for carotenoid concentrations in plasma. There is no data available to our knowledge reporting maternal and infant carotenoid concentrations in the Midwest region. All carotenoids evaluated were measurable in all subjects except one who had undectable levels of α-carotene and β-carotene. Weber et al. was unable to detect zeaxanthin, β-cryptoxanthin, and lycopene levels in their cord blood samples.[18] Over half the subjects in a study by Kiely et al. also had undectable levels of lycopene, β-carotene, and α-carotene.[33] Being able to identify levels of all of the carotenoids in nearly all of the subjects may be a result of improvements in technology used or be reflective of truly higher carotenoid levels in our sample.

Compared to data obtained in Hawaii, this study demonstrated lower lutein + zeaxanthin levels (25.4 μg/L vs. 30 μg/L) in infants, although Franke et al. measured the trans form only and not total lutein + zeaxanthin. B-cryptoxanthin levels were also lower in infants in this study (9.3 μg/L vs. 12 μg/L). The other carotenoid cord blood levels, total lycopene (21.8 μg/L vs. 12 μg/L), α-carotene (5.4 μg/L vs. 2 μg/L), and β-carotene (18.3 μg/L vs. 7.9 μg/L), were all higher in the infants in the present study.[2] More variability in carotenoid levels was found when compared to a study by Sommerburg et al. Mean lycopene (21.8 μg/L vs. 16 μg/L), α-carotene (5.4 μg/L vs. 5 μg/L), and β-carotene (18.3 μg/L vs. 24 μg/L) in this study were found to differ when compared to median levels in infants in Germany.[5]

In our sample of mothers, all carotenoid levels measured were lower than those found in a study by Weber et al. in Germany. Carotenoids in our study compared to the results of Weber et al. for mothers at birth, respectively, are 0.287 μmol/L vs. 0.639 μmol/L for lutein + zeaxanthin, 0.133 μmol/L vs. 0.399 μmol/L for β-cryptoxanthin, 0.829 μmol/L vs. 1.08 μmol/L for total lycopene, 0.090 μmol/L vs. 0.323 μmol/L for α-carotene, and 0.374 μmol/L vs. 0.755 μmol/L for total β-carotene.[18] However, our results compared to mothers in Ireland were mixed. Our study demonstrated higher levels of total lycopene (0.829 μmol/L vs. 0.40 μmol/L), total β-carotene (0.374 μmol/L vs. 0.23 μmol/L) and α-carotene (0.090 μmol/L vs. 0.05 μmol/L) in mothers and lower levels of lutein + zeaxanthin (0.287 μmol/L vs. 0.46 μmol/L). β-cryptoxanthin concentrations, interestingly, were very similar (0.133 μmol/L vs. 0.14 μmol/L).[33]

The NHANES III serum carotenoids levels of general adult women, although not exclusive to pregnant women, aligned fairly closely to the results of this study for β-carotene (200.9 μg/L vs. 150-217 μg/L in NHANES III), α-carotene (48.4 μg/L vs. 34.4-53.7 μg/L in NHANES III), β-cryptoxanthin (73.5 μg/L vs. 73.3-88.4 μg/L in NHANES III), and lutein + zeaxanthin (163.3 μg/L vs. 186-214 μg/L in NHANES III). The one exception was the mothers

in this study had higher lycopene levels at 444.9 μg/L compared to 229-248 μg/L in adult females in the samples collected in NHANES III.[15]

Due to limited previous research on serum carotenoid levels in pregnant women and infants at birth and no established reference values, the adequacy or deficiency in our sample was difficult to determine.

Relationship between Serum Vitamin A Levels of Mothers and Infants:

Not only were maternal retinol levels significantly positively correlated with cord blood levels, all measured carotenoids were as well. These significant findings provide further evidence of the placental transfer of vitamin A compounds *in utero*. Fernandes et al. also found a significant correlation with serum retinol levels ($r=0.27$, $p=0.04$),[25] although Weber et al. ($r=0.099$, $p=0.228$)[18] and Kiely et al. did not ($r=0.19$).[33] Previous research has also shown positive correlations of maternal and infant lutein ($r=0.368$, $p=<0.001$), α-carotene ($r=0.668$, $p=<0.001$), and β-carotene ($r=0.832$, $p=<0.001$).[18] These results support the importance of improving maternal levels throughout pregnancy in order to benefit the developing fetus.

MATERNAL VITAMIN A INTAKE

Because mothers influence the retinol and carotenoid concentrations of their infant via placental transfer, it is important to determine which dietary vitamin A compounds influence serum concentrations. This is the first study to date to our knowledge that thoroughly evaluates the diets of mothers throughout pregnancy using a detailed FFQ and evaluates how dietary intake of specific vitamin A compounds influences serum levels of mothers and infants. The median vitamin A intake measured in RAE in our sample of mothers was 3.5 times the EAR and 2.5 times the RDA for pregnant women. It was also 2.5 times higher than the NHANES III intake reports for pregnant women (1937.4 μg vs. 757 μg).[10] Median RAE intake in our study was also

well above the intake in mothers in Israel (1937.4 μg vs. 237.1 μg).[38] Given that maternal vitamin A intake greatly exceeded the DRIs, it could be expected to translate into adequate serum levels. However, deficiency was still prevalent in our study population. Also RAE intake did not correlate with serum retinol levels in both mothers and infants in our sample. The current DRIs take into account a combination of all vitamin A compounds by converting to a common unit of RAE, but this may not be a sufficient measure to assume adequate intake and thus adequate serum levels.

In comparison to the NHANES III data, median intake of β-cryptoxanthin (130.7 μg vs. 159 μg), lycopene (3637.2 μg vs. 8713 μg), and α-carotene (267.8 μg vs. 376 μg) were all lower in this study. Intake of lutein + zeaxanthin (2060.2 μg vs. 1455 μg) and β-carotene (4336.6 μg vs. 1531 μg) were the only carotenoids consumed in higher amounts in this study population than the pregnant women evaluated in NHANES III.[10] There may be specific dietary vitamin A compounds that are important in optimizing vitamin A serum status. This has not been previously explored. Interestingly, maternal dietary intake of lutein + zeaxanthin did not statistically correlate with maternal serum lutein + zeaxanthin, but it did positively correlate with serum α-carotene and β-carotene. Other correlations observed included maternal intake of α-carotene being significantly positively correlated with maternal serum α-carotene as well as being positively correlated with serum β-carotene, although not statistically significant ($p=0.058$). β-carotene was also significantly positively correlated with both serum β-carotene and α-carotene. None of the other dietary intake measures directly correlated with its corresponding serum measure. These findings suggest that β- and α-carotenes influence serum levels similarly and could be obtained in the diet from the same food source. This also brings into question how the metabolism of each vitamin A compound may affect the others when consumed together. With our earlier results that all serum levels of the vitamin A compounds correlated positively in cord blood, it can be assumed that enhancing the serum vitamin A status in mothers will enhance the

infant's vitamin A status at birth. Due to only obtaining dietary intake information on 59% of our subjects, dietary vitamin A intake in mothers may not be representative of the study population. Further evaluations with larger samples need to be completed before stronger conclusions can be drawn.

Not only is maternal dietary intake important throughout pregnancy, it continues to be crucial after birth if infants are breast fed. Low dietary vitamin A intake and low serum concentrations in mothers may lead to the development of VAD after birth or contribute to a failure to form adequate vitamin A liver stores in later infancy and childhood.

IMPROVING VITAMIN A STATUS

The method to increase vitamin A status after birth is controversial. Various forms of supplementation given to mothers and infants have been assessed. Pregnant women are advised to avoid supplementing their diet with >10,000 IU of preformed vitamin A. These high dose supplements have been associated with malformation of the fetus.[21] Also, supplementation to mothers during pregnancy has not shown beneficial effects on neonatal birth weight, length, and head circumference.[20] Given the risk of negative consequences of vitamin A toxicity from supplementation, WHO does not recommend vitamin A supplementation during pregnancy to prevent maternal and infant morbidity and mortality. However, in areas where VAD is a public health issue, WHO recommends supplementing pregnant women with vitamin A to help prevent gestational night blindness. In addition, WHO does not recommend supplementation for nursing mothers, but encourages proper nutrition through a balanced diet.[39] Improving breast milk concentrations may help increase vitamin A intake for infants who are breast fed. Retinoids and carotenoids are not homeostatically regulated in breast milk, thus higher dietary intake can contribute to a higher composition in breast milk.[45] Haftel et al. showed 130% and 200%

increases in lycopene and β-carotene breast milk content, respectively, when mothers supplemented their diet with carrot puree or mashed tomatoes.[9]

Infant intake through formula may also be an area to target to improve infant vitamin A status. Sommerburg et al. evaluated breast milk and formula content and found that four major carotenoids (α-carotene, β-carotene, lycopene, and cryptoxanthin) were detected in breast milk samples, but formulas were lacking these vitamin A compounds. Of the eight formulas tested, 50% did not contain any of the carotenoids, 50% contained β-carotene, and 38% contained β-cryptoxanthin.[5] Rubin et al. determined that infants who received supplemented formula with lutein + zeaxanthin, lycopene, and β-carotene had considerably higher corresponding plasma levels.[46] Adding vitamin A compounds to formula may be a feasible and safe way to improve vitamin A status in infants. If a more aggressive treatment is needed for severe VAD, it may be appropriate to administer higher doses of vitamin A. Supplementing infants with 5000 IU vitamin A intramuscularly, three times per week for 28 days, has also been shown to improve vitamin A levels and decrease the risk of chronic lung disease in very low birth weight infants.[3] Vitamin A supplementation is still debatable due to inconclusive research and may need to be considered on an individual basis. Supplementation through carotenoids may be more appropriate compared to supplementation of preformed vitamin A, but would need to be evaluated further before general recommendations could be made.

LIMITATIONS

The limitations of this study should be considered. A larger sample size would have been beneficial to establish more solid findings that could be generalized to a larger population. In addition to the small sample of 34 mother-infant pairs enrolled, serum samples from three mothers and cord blood samples from two infants were not obtained. Also, this study looked at

subjects in the NICU, who are often under higher levels of stress due to an increase in health complications. These results may not be consistent with healthy mother-infant pairs at birth.

The FFQs were also completed and analyzed in only 20 of the mothers. Almost half of the mothers did not complete a FFQ, making it difficult to draw solid conclusions related to the intake of vitamin A compounds. As with other intake assessment forms, self-reporting can lead to both under or overestimations of intake. The FFQ also asked questions regarding the frequency of intake of specific foods on average over the past year. With many vitamin A-containing foods being seasonal, it may have been difficult for subjects to accurately fill out the FFQ. The FFQ used, though, did provide a thorough intake assessment and has been used in many other adult populations.

CHAPTER 6: CONCLUSION

This is one of the first studies to not only evaluate vitamin A status from retinol levels, but more importantly, assess the carotenoid status in mothers and infants at birth. Previously, limited data has been available regarding the current status of the various vitamin A compounds in the United States. This prospective study determined VAD is a significant problem in this population of infants and is influenced by the maternal vitamin A status. In addition, maternal intake may influence the serum levels of mothers and infants, although further research needs to be completed to determine the influence of specific carotenoid intake on serum concentrations of vitamin A compounds. Additional research evaluating vitamin A intake after birth including dietary intake, supplementation of preformed vitamin A vs. carotenoids, or a combination would provide an insight into how VAD can be prevented or corrected in early infancy and childhood. With a larger sample in the future, comparisons could be made between different gestational ages and birth weights, which may help target high risk populations.

Evaluating correlations of retinol and carotenoid concentrations between mothers and infants provides important information to help determine appropriate strategies and specific vitamin A components to target to improve vitamin A status in both parties. By increasing awareness of the significant VAD present in NICU babies in the Midwest, appropriate assessments and interventions can be implemented to provide optimal care and improve health outcomes.

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