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Dynamics of Maternal and Infant Vitamin E Tocopherols During NICU Hospitalization

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DYNAMICS OF MATERNAL AND INFANT VITAMIN E TOCOPHEROLS DURING NICU HOSPITALIZATION

by

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University of Nebraska, 2017

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OBJECTIVE: The main objective of this prospective cohort is to investigate serum levels of vitamin E tocopherol isoforms (specifically alpha and gamma) in mothers and infants admitted to the Neonatal Intensive Care Unit (NICU) in relation to infant feeding modality.

METHODS: This was a prospective cohort of 34 mothers and their infants admitted to the neonatal intensive care unit (NICU). Samples of maternal and cord blood were collected at the time of delivery, and a food frequency questionnaire (FFQ) was administered to the mother to measure maternal vitamin E tocopherol intake. After nutrition treatment of each feeding modality had been stable for 72 hours, blood samples were collected from participating infants during the administration. Serum tocopherols were measured in the Biomarker Research laboratory at the Harvard School of Public Health using high-performance liquid chromatography (HPLC). Descriptive statistic 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 E deficiency was present in 64.5% of infants and 41.9% of mothers. No significant correlations were found between gestation age or birth weight and infant α- and γ-tocopherol levels or between maternal and infant serum α- and γ-tocopherol levels. Additionally, no significant correlations were found between maternal serum or maternal intake and α- and γ-tocopherol levels in maternal breast milk samples. No significant difference was found between groups when comparing α- and γ-tocopherol levels of infants after 3 days stable on a feeding modality.

CONCLUSION: There is a high prevalence of vitamin E deficiency in mothers and infants. Further research is needed to identify how to safely raise levels to prevent complications of deficiency in infants.

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LIST OF ABBREVIATIONS

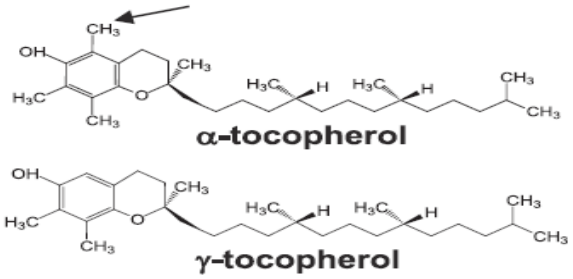
AI	Adequate Intake
AVED	Ataxia with Vitamin E Deficiency
BMI	Body Mass Index
BPD	Bronchopulmonary Dysplasia
CEHC	Carboxyethylchromans
CVD	Cardiovascular Disease
EAR	Estimated Average Requirement
FEV_1	Forced Expiratory Volume in the First Second
FFQ	Food Frequency Questionnaire
FVC	Forced Vital Capacity
IFNγ/PMA	Interferon-gamma/phorbol myristate acetate
IL8	Interleukin 8
IOM	Institute of Medicine
IRB	Internal Review Board
IU	International Units
mg alpha-TE	Alpha-tocopherol Equivalents
NICU	Neonatal Intensive Care Unit
NOX2	NADPH Oxidase
PKCα	Protein Kinase Cα
PS	Phosphatidylserine
RBC	Red Blood Cell
RDA	Recommended Daily Allowance
RDS	Respiratory Distress Syndrome
RNOS	Reactive Nitrogen Oxide Species

ROS	Reactive Oxygen Species
SGA	Small for Gestational Age
UL	Upper Limit of Tolerable Intakes
UNMC	University of Nebraska Medical Center
VCAM -1	Vascular Cell Adhesion Molecule 1
VLDLs	Very Low Density Lipoproteins
α-TTP	Alpha-Tocopherol Transfer Protein

CHAPTER 1: INTRODUCTION

Vitamin E is a major chain-breaking antioxidant thought to function by preventing the propagation of lipid peroxidation.[1] Vitamin E occurs naturally as eight different isoforms, including alpha and gamma tocopherol which differ by one methyl group (Figure 1).[2] Isoforms are not interconvertible in humans and, as a result, increased intakes of alpha- or gamma-tocopherol in the diet will cause a rise in serum tissue concentrations of that specific tocopherol.[3] In tissues, serum α-tocopherol levels are approximately 10

Figure 1: Structures of Alpha- and Gamma-Tocopherol Isoforms



times higher than γ-tocopherol due to a preferential transfer of α-tocopherol to lipid particles by liver α-tocopherol transfer protein (αTTP) and a higher rate of production of γ-tocopherol metabolites for excretion.[4] Importantly, serum and tissue levels of vitamin E isoforms correlate, meaning dietary intake of tocopherols has the potential to influence biological mechanisms.[5,6]

Vitamin E regulation of disease has been extensively studied in humans, animal models, and cell systems. Clinical studies primarily focus on the α-tocopherol isoform without adjustment for the dietary contribution of γ-tocopherol to the outcome. It has been recently reported that supplementation with physiological levels of purified natural forms of the Vitamin E isoforms α-tocopherol is anti-inflammatory and γ-tocopherol is pro-inflammatory.[4,6-8] The contradictory outcomes in vitamin E in research may be due, in part, to the different and possibly opposing inflammatory properties of isoforms.

Deficiency is not often seen in adults, but is more frequently found in children due to limited stores and rapid growth.[9] Vitamin E is of critical importance in early infancy, as deficiency has devastating consequences such as hemolytic anemia, retrolental fibroplasia, intraventricular hemorrhage (IVH), bronchopulmonary dysplasia (BPD), and delays in the development of the central nervous system.[10] Placental transfer of vitamin E to the fetus is limited and the expectant mother has little influence on the delivery of this substance to the fetus.[11] Therefore, maintenance and repletion of vitamin E stores after birth through other inputs is required. In clinical practice, there is a dependence on dietary intake to supply this crucial nutrient.[12,136]

Infant formula is designed to be an alternative food source similar to that of human milk for newborns. However, infant formula is highly distinct from that of human milk as human milk contains high concentrations of α-tocopherol while γ-tocopherol is the dominant isoform in infant formula. The potentially distinct vitamin E profile between human milk and infant formula could be a contributing factor to the greater susceptibility of formula-fed infants to contract inflammatory conditions compared to breast-fed infants.[14]

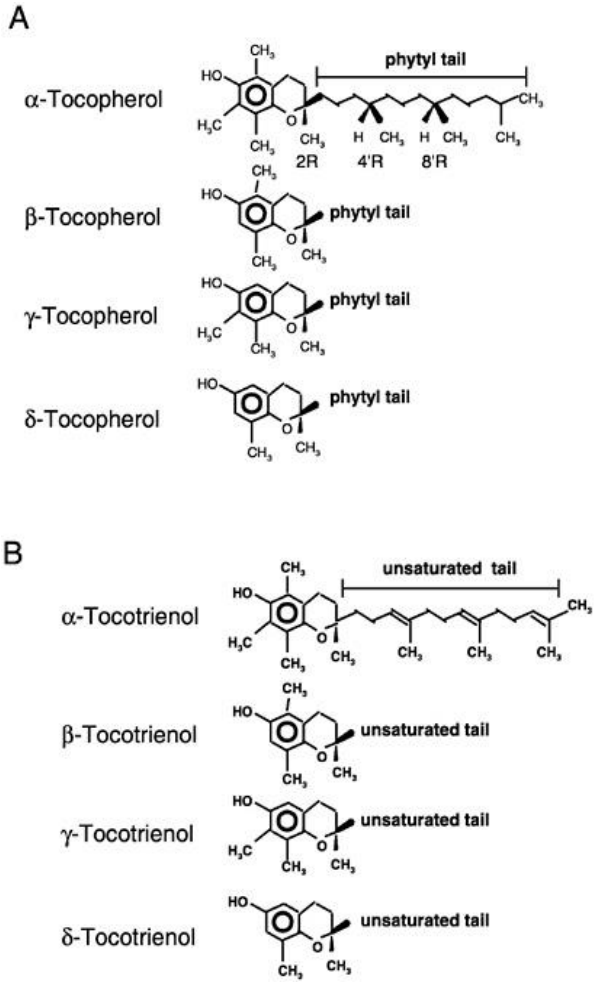
CHAPTER 2: BACKGROUND & REVIEW OF LITERATURE

OVERVIEW OF VITAMIN E

Structure, Metabolism and Storage:

The term Vitamin E refers to a group of eight lipid-soluble compounds: the α-, β-, γ-, and δ-tocopherols and the α-, β-, γ-, and δ-tocotrienols.[4] These different forms, often referred to as "tocochromanols or tocols," vary in their biological availability and in their physiological and chemical activities. As shown in figure 2, tocopherols and tocotrienols consist of a chromanol head group linked to an isoprenoid-derived hydrophobic tail. The aliphatic tail of tocopherols is fully saturated, while the side-

Figure 2: Tocopherol and Tocotrienol Molecular Structures

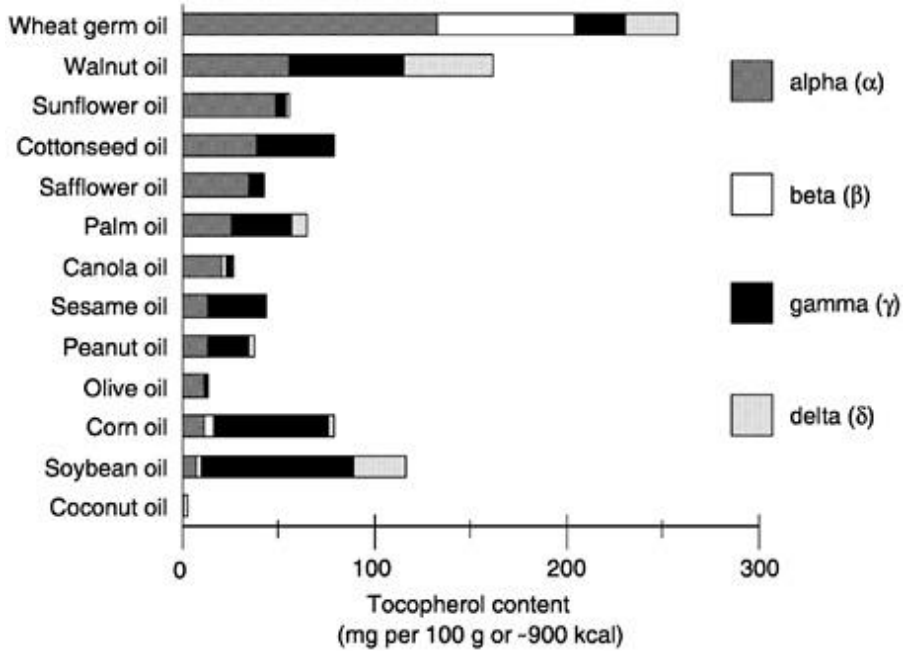


chain of tocotrienols contains three, trans double bonds. All of these molecules possess antioxidant activity, but α-tocopherol is thought to be chemically and biologically the most active.[15] The other naturally occurring forms of vitamin E (β-, γ-, and δ- tocopherols and α-, β, γ, and δ-tocotrienols) do not contribute toward meeting the vitamin E requirement because, although absorbed, they are not converted to α-tocopherol by humans, and they are poorly recognized by α-TTP in the liver.[16] The biological activity of vitamin E is normally expressed as international units relative to that of all-rac-α-tocopherol acetate, or the synthetic form. The relative values in International Units (IU) per mg are 1.00 for all- rac-α-tocopherol acetate, 1.36 for *RRR*-α-tocopherol acetate, 1.49 for *RRR*-β-tocopherol, 0.12-0.28 for *RRR*-γ-tocopherol, and 0.01-0.04 for RRR-δ-tocopherol according to the resorption-gestation test.[17]

Vitamin E was first recognized as a lipid-soluble substance necessary for the prevention of fetal death and resorption in rats that had been fed a rancid lard diet.[18] It is a major chain-breaking antioxidant thought to function to prevent the propagation of lipid peroxidation.[1] Tocopherols and tocotrienols play important roles in the oxidative stability of vegetable oils and in the nutritional quality of crop plants for human and livestock diets.[2] Vitamin E is involved in immune function, cell signaling, regulation of gene expression, and other metabolic processes.[19] In addition, it also increases the expression of two enzymes that suppress arachidonic acid metabolism, thereby increasing the release of prostacyclin from the endothelium, which dilates blood vessels and inhibits platelet aggregation.[20]

The α- and γ-tocopherol isoforms are the most abundant isoforms in diet and tissues, with α-tocopherol being the predominant form in mammalian plasma and the only form present in vitamin supplements.[21,22] The main source for dietary uptake of vitamin E is vegetables, plants, and plant oils. Plants synthesize the natural isoforms, or *RRR*-tocopherol (formerly called *d*-alpha tocopherol), of vitamin E from tyrosine and chlorophyll and are consumed in the diet from plant lipids.[2] In the United States diet, γ-Tocopherol is the principle vitamin E isoform consumed, being about 2.5 times as abundant in food as α-tocopherol.[23] Main sources of γ-tocopherol include walnuts, pecans, peanuts, soybean oil,

Figure 3: Various forms of Vitamin E Isoforms in Edible Vegetable Oils[20]



corn oil, and cottonseed oil, while α-tocopherol is primarily found in olive, sunflower, and safflower oils in addition to sunflower seeds and almonds.[24,25] Soybean oil is the predominant vegetable oil in the American diet (76.4%) followed by corn oil and canola oil (both 7%).[26] The average plasma γ-tocopherol level in the United States and the Netherlands has been reported as approximately 2-6 times higher than that reported for 6 European countries including Italy that consume higher amounts of α-tocopherol rich foods.[26] It is presumed that as countries assume western lifestyles, diets change including increased consumption of soybean oil with corresponding increases in plasma γ-tocopherol.[27]

The Institute of Medicine (IOM) reported that the effect of 300 mg of supplemental vitamin E was to increase plasma α-tocopherol concentrations threefold and to at least double most tissue concentrations, while supplementation with 30 mg had little effect on either plasma or tissue α-tocopherol concentrations.[20] Synthetic forms of α-tocopherol are found in fortified foods and vitamin supplements. Dietary supplements generally contain 100 IU to 1,000 IU α-tocopherol and contain only *RRR*-α-tocopherol. Large quantities of vitamin E have been shown to lead to multiple metabolic and cellular changes in humans. Effects of vitamin E supplementation include inhibition of low density lipoprotein (LDL) oxidation both in vitro and in vivo, inhibition of smooth muscle cell proliferation through

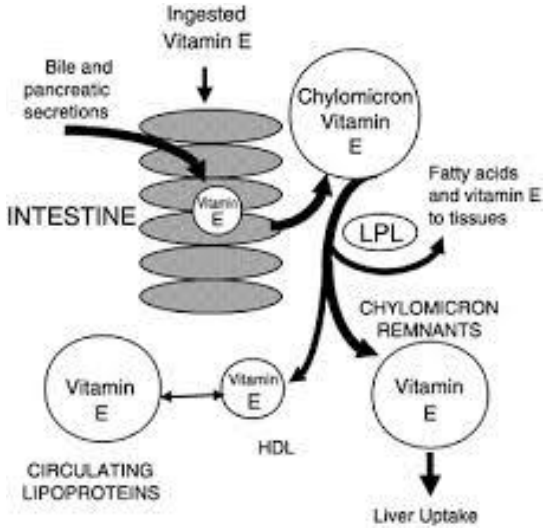
inhibition of protein kinase Cα (PKCα), inhibition of platelet aggregation,[28] and inhibition of plasma generation thrombin.[16]

Vitamin E is dependent on biliary and pancreatic secretions and is taken up as the free alcohol form by the intestine without discrimination of the individual isomers.[16] Prior to absorption, tocopherol esters are hydrolyzed in the intestine, making the bioavailability of free *RRR*-α-tocopherol and *RRR*-α-tocopherol acetate very similar.[29] After absorption, tocopherols are loaded in intestinal-formed chylomicrons which are then transported in the lymph to the liver where they are transferred to lipid particles.[30] Here, the natural *RRR*-α-tocopherol form has the highest biopotency as the α-TTP has a higher affinity for the natural isoform and enables the selective (but not exclusive) transfer of α-tocopherol across membranes and its incorporation into very low density lipoproteins (VLDLs).[31,32] After absorption, vitamin E isomers other than α-tocopherols are readily converted to their respective CEHC metabolite forms, even during α-tocopherol deficiency.[33] The secretory pathway via VLDL from the liver is critical in maintaining tocopherol concentrations in plasma, and discrimination among the isomers by α-TTP occurs during hepatic secretion of VLDL.[34,35] Therefore, hepatic vitamin E metabolism is a major regulator of the forms of vitamin E found in the body and once liver α-tocopherol concentration reaches a threshold level, additional α-tocopherol will be metabolized and α- carboxyethylchromans (CEHC) excretion increases exponentially.[33] However, some tocopherols in the VLDLs may end up in LDL by the action of lipoprotein lipase in plasma.[32] Finally, the tocopherols secreted are either rapidly returned from blood to the liver or excreted to feces. Based on recovery studies, 55-77% of radiolabeled α-tocopherol were absorbed.[36] By regulating the transfer and binding of tocopherols, α-TTP plays a key role in determining plasma concentrations and biological activities.

Vitamin E is taken up by several tissues, including liver, lung, heart, skeletal muscle, and adipose tissue.[37] In tissues, α-tocopherol is approximately 10 times higher than γ-tocopherol due to preferential transfer of α-tocopherol to lipid particles by liver α-TTP and a higher rate of production of γ-tocopherol metabolites (CEHC), for excretion.[21,38] Burton et al.[39] has reported that γ-tocopherol constitutes as much

as 30-50% of the total vitamin E in human skin, muscle, vein, and adipose tissue. However, although γ-tocopherol concentrations in these tissues appear to be 20-40 fold greater than those in plasma, the isomer has only about one-tenth of the vitamin E activity of α-tocopherol.[37] Plasma concentrations of vitamin E peak at 12-14 hours after administration: 13.5 hours for *RRR-α*-tocopherol, about 12 hours for *RRR-α*-tocopherol acetate, and 12.6 hours for synthetic tocopherol acetate,[40,41] while time to peak concentration of γ-tocopherol appear to be about 9 hours.[35] In North America, Schweldhelm, et al.[42] reported the serum concentrations of α- and γ-tocopherol are approximately 20.5 $\pm$ 6.6 μmol/L and 3.1 μmol/L respectively.

Figure 4: Vitamin E secretion in Chylomicrons and Distribution to Circulating Lipoproteins[20]



The antioxidant activity of tocopherols is rooted in their ability to donate electrons to lipid radicals.[25] Because of its lack of the electron-donating methyl groups on the chromanol ring, γ-tocopherol is somewhat less potent in donating electrons than is α-tocopherol, and is therefore thought to be a less powerful antioxidant.[43] Both α-tocopherol and γ-tocopherol have a similar ability to scavenge reactive oxygen species (ROS).[44] Excess generation of ROS may promote inflammation with increases in reactive nitrogen species and is associated with chronic inflammation-related diseases such as cancer, cardio-vascular disease (CVD), and neurodegenerative disorders.[45-48]

Cook-Mills et al. proposed that α- and γ-tocopherols have opposing regulatory effects on leukocyte recruitment and vascular cell adhesion molecule-1 (VCAM-1).[4,30] Leukocytes bind to these adhesion molecules and then the bound leukocytes are recruited into tissues by chemokines/chemoattractants.[49] VCAM-1 signals through low levels of ROS which are regulated by tocopherols.[6,50-52] During inflammation, VCAM-1 expression is induced on endothelial cells[30] and during allergic inflammation in the lung, eosinophil migration is dependent on VCAM-1.[50] VCAM-1 activates NADPH oxidase (NOX2) that generates ROS for the activation of PKCα, a serine/threonine kinase that utilizes the cofactors PS (phosphatidylserine), DAG (diacylglycerol) and calcium for activation.[53] McCary et al.[5] reported tocopherols directly bind and regulate PKCα. While α-tocopherol decreases activation, γ-tocopherol enhances activation of recombinant PKCα. Therefore, α-tocopherol is an antagonist and γ-tocopherol is an agonist of PS-dependent PKCα activity. In the same paper, both α- and γ-tocopherol inhibited the oxidative activation of recumbent PKCα, suggesting both tocopherols possess an antioxidant effect. However, α-tocopherol significantly decreased hydrogen peroxide activation of PKCα at a 10-fold lower dose compared with γ-tocopherol. McCary, et al. concluded α-tocopherol may inhibit VCAM-1 signaling by functioning both as an antioxidant and as an antagonist of PKCα while γ-tocopherol elevates VCAM-1 activation of PKCα and ablates the inhibitory effects of α-tocopherol on VCAM-1 activation of PKCα in endothelial cells.

In 2009, Berdnikovs, et al.[6] studied the effect of α-tocopherol and γ-tocopherol alone or in combination on eosinophilic lung inflammation and airway hyperresponsiveness in female mice. I*n vitro*, α-tocopherol decreased whereas natural γ-tocopherol elevated VCAM-1-dependent lymphocyte transmigration at physiological concentrations by a direct regulatory function in endothelial cells. α-tocopherol decreased and γ-tocopherol increased endothelial cell signaling during leucocyte migration across endothelial cells *in vitro*.[54] Additionally, when γ-tocopherol and α-tocopherol were given simultaneously, γ-tocopherol blocked the inhibition of the leukocyte transendothelial migration. Additionally, These findings were reflected in the recent results of retrospective cohorts that have found

statistically significant inverse relationships between γ-tocopherol and FEV1 and FVC whereas higher α-tocopherol was associated with higher FVC.[22,55] Alternatively, a multi-center RCT in the United States showed long-term supplementation with vitamin E (400 IU/day *all rac*-α-tocopherol acetate) had no effect on FEV1.[56]

Yang, et al.[57] showed that serum γ-tocopherol was associated with increasing concentrations of inflammatory biomarkers, including high-sensitivity C-reactive protein (HS-CRP), serum amyloid A (SAA), and E-selectin (P_{trend} all < 0.0001), while α-tocopherol was associated with decreasing concentrations of IL-6 and hs-CRP (P_{trend} all < 0.001). Elisia et al.[58] researched the relative abilities of different tocopherol isoforms to modulate intestinal oxidative stress and inflammatory responses and found tocopherol isoform promotion of Interleukin 8 (IL8) production was isoform specific in the fetal-derived FHs 74 Int intestinal cell line challenged with interferon-gamma/phorbol myristate acetate (IFNγ/PMA). After treatment of cells with 100 μM tocopherol isoforms (α- and γ-tocopherol), γ-tocopherol produced a significant ($P < 0.05$) increase in IL8 (eightfold). That level was also higher than that induced by α-tocopherol (twofold increase) as compared to control cells that were incubated with IFNγ/PMA alone.[59] Results showed that γ-tocopherol can modulate a greater inflammatory response than α-tocopherol.

Studies exploring vitamin E on asthma have also focused on the opposing effects of α- and γ-tocopherol isoforms. Cook-Mills, et al.[60] found the relationship of α-tocopherol on asthma risk varied with γ-tocopherol tertile. In the highest γ-tocopherol tertile, low levels of α-tocopherol appear to be associated with a marked elevation in asthma risk, while the highest tertile α-tocopherol levels tended to be protective. Abdala-Valencia, et al.[7,8] studied the effect of α- and γ-tocopherol supplementation in allergic female mice, with opposing results reported. Allergic, female mice receiving γ-tocopherol supplementation had pups with twofold the number of eosinophils in the bronchoalveolar lavage and lungs after allergen challenge,[8] while mice supplemented with α-tocopherol[7] had pups with reduced allergen-induced lung mRNA expression of IL-4, IL-33, TSLP, CCL11, and CCL24.

It has been suggested that vitamin E tocopherols can reduce the risk of cancer due to their strong antioxidant properties.[61,62] Multiple recent studies have shown dietary vitamin E intake is inversely associated with cancer risk.[63-73] However, supplemental vitamin E continues to be debated due to previous studies such as the Selenium and Vitamin E Cancer Prevention Trial (SELECT)[74] and the Alpha-Tocopherol and Beta-Carotene (ATBC) trial,[75] which suggested vitamin E supplementation increased the risk of prostate cancer and renal-cell carcinoma, respectively. In the past, the relationship between dietary intake of tocopherols and lung cancer risk has been evaluated in several prospective studies, with the majority showing no significant association.[76-81] However, in a cohort of tin miners in China, Ratansinghe et al.[82] found a significant gradient of decreasing lung cancer risk in men less than 60 years old with increasing serum α-tocopherol levels (OR by tertile: 1.0, 0.9, 0.2; *P* for trend = 0.002). In another study, point estimates comparing 4th quartile to 1st quartile of males, Goodman et al.[83] found serum concentration of α-tocopherol were significantly inversely associated with ORs of lung cancer (OR, 95% CI; 0.49, 0.25-0.95). Wu, et al.[68] prospectively investigated the associations between tocopherol intake from diet and supplements with lung cancer risk among 77,829 Chinese, female, nonsmokers aged 40-70 years participating in the Shanghai Women's Health Study. Total dietary tocopherol was inversely associated with lung cancer risk among women meeting dietary guidelines for adequate intake of tocopherol (14 mg/day or more: HR: 0.78; 95% CI 0.60-0.99; compared with the category less than adequate intake). In contrast, vitamin E supplement use was associated with increased lung cancer risk (HR: 1.33; 95% CI: 1.01-1.73), more so for lung adenocarcinoma risk (HR: 1.79; 95% CI: 1.23-2.60). Sayin, et al.[84] studied the impact of antioxidant supplementation (DL-α-tocopheryl acetate at doses of 12.5 and 61.5 mg/kg body weight) on tumor progression, severity, and lethality in mouse models of endogenous lung cancer in a RCT. Vitamin E supplementation reduced ROS and DNA damage, prevented p53 activation, and markedly increased tumor cell proliferation and tumor growth in mice, suggesting tumor cells proliferate faster when oxidative stress is suppressed. The impact of vitamin E on tumor initiation or prevention was not analyzed by Sayin, et al. and only used vitamin E in the supplemental form was used to obtain results. A meta-analysis by Chen, et al.[85] summarized the evidence

from epidemiological studies of vitamin E intake with the risk of lung cancer and concluded high intake of vitamin E might have a protective effect against lung cancer. The combined relative risk of lung cancer associated with vitamin E intake was 0.858 (95% CI = 0.742-0.991). Significant protective associations were also found in American population (RR = 0.862, 95% CI: 0.715-0.996) and prospective studies (RR = 0.913, 95% CI=0.827-0.996).

Dietary Reference Intakes:

The determination of how much of a nutrient is required daily is dependent on specifically assessing a nutrient's function and also by defining a biomarker that is indicative of inadequacy that changes with nutrient intakes.[20] The IOM chose results from the *in-vitro* hydrogen peroxide-induced erythrocyte hemolysis test as a marker of vitamin E status because increased peroxide-induced erythrocyte hemolysis was correlated with increased erythrocyte fragility in vitamin E-deficient individuals.[9] The estimated average requirement (EAR) of 12 mg α-tocopherol per day, which is expected to result in a serum α-tocopherol concentration of 27.9 µmol/L (12,025 µg/L), was set in humans on the basis of vitamin E depletion and repletion studies in men with the use of erythrocyte hemolysis as the biomarker.[20,86] The recommended daily allowance (RDA) of 15 mg α-tocopherol per day was extrapolated from that value. The following are conversion rates for *D*-α-tocopherol:

- 1 mg/dL α-tocopherol = 23.22 µmol/L α-tocopherol
- 1 IU α-tocopherol = 0.67 mg α-tocopherol (670 µg α-tocopherol)
- 1 µg/ml α-tocopherol = 2.32 µmol/L α-tocopherol

According to the IOM, only α-tocopherol meets human vitamin E requirements because it was the only isoform that was demonstrated to reverse vitamin E deficiency symptoms in humans, and was the only form maintained in plasma and tissues.[20] The upper limit of tolerable intakes (UL) is 1000 mg/day, equivalent to 1100 IU synthetic or 1500 IU natural vitamin E.[20] The UL was based on the adverse effect of an increased bleeding tendency observed in rat studies.[87]

Table 1: Dietary Reference Intakes for Women and Infants[20]

Age Group	EAR	RDA	AI	UL
Infants 0-6 months			4	ND
Women ≥19 years	12	15		1000
Pregnant Women ≥19 years	12	15		1000
Lactation ≥19 years	12	19		1000

The Dietary Reference Intakes for vitamin E as reported by the Institute of Medicine.
Values are reported in mg/day of vitamin E

In normal, healthy adults who consume a variety of foods, including nuts, seeds, and whole grains, plasma α-tocopherol concentrations average ~20 μmol/L, whereas those individuals who consume supplements or fortified foods have concentrations that average ~30 μmol/L or more.[88] National Examination Survey III (NHANES III) data compiled from 1988 to 1994 was used to determine a median α-tocopherol intake (including supplements) of 10.3 mg/day α-tocopherol and a median dietary intake from food alone of 9.4 mg/day in the United States.[20,89] Assessing normal plasma α-tocopherol concentrations in children is complicated because α-tocopherol is transported in plasma lipoproteins and due to the fact that α-tocopherol increase with age.[90] Data from participants of the myocardial case-control study in Costa Rica showed estimates of mean $\pm$ SEM vitamin E intakes in adolescents (9.2 $\pm$ 0.2 mg α-tocopherol/day) were higher than those of adults (8.4 $\pm$ 0.2 mg α-tocopherol/day). However, when corrected for cholesterol concentration, plasma α-tocopherol concentrations were significantly lower ($P <$ 0.05) in adolescents (17 $\pm$ 0.4 μmol/L, corrected for cholesterol concentration) compared with adults (26 $\pm$ 0.6 μmol/L). Due to this discrepancy, researchers have proposed other laboratory methods, such as urinary α-CEHC, with regard to vitamin E status in humans.

Vitamin E has been regarded as generally safe when administered orally, even in very high doses,[91] but toxicity has occurred with parenteral administration and less commonly with high-dose oral administration. Adverse effects of vitamin E in adults include increased bleeding tendency and impaired immune function.[92]

VITAMIN E DEFICIENCY

Alpha-tocopherol is considered the most important lipid-soluble antioxidant in cell membranes.[93] It protects cell membranes from lipid peroxidation by breaking the lipid radical chain reaction and acts as a direct scavenger of superoxide and the hydroxyl radicals.[93] Circulating α-tocopherol concentrations <12 μmol/L (5168 μg/L) are defined by the IOM to be in the deficient/inadequate range for healthy adults with low levels defined as <20 μmol/L (8620.7 μg/L).[20] Symptoms of deficiency include a progressive neurologic disorder, spinocerebellar ataxia, which occurs as a result of a dying back of peripheral nerves, specifically the sensory neurons.[94] In humans, severe vitamin E deficiency often occurs due to a genetic defects in the α-TTP resulting in the rapid depletion of plasma α-tocopherol and causing ataxia with vitamin E deficiency (AVED).[95,96] Vitamin E deficiency also occurs with fat malabsorption due to genetic defects in the microsomal triglyceride transfer protein, fat-malabsorption syndromes,[87] or surgical procedures such as gastrectomy or gastric bypass.[97-101]

In 2015, McBurney, et al.[86] assessed serum concentrations of α-tocopherol in participants with data collected from NHANES 2003-2006. Distributions of serum concentrations for the entire population were stratified by consumption of food without supplement use (FOOD) and food and supplement use (FOOD+DS). The mean ($\pm$ SEM) of α-tocopherol was 29.6 $\pm$ 0.2, 33.7 $\pm$ 0.3 μmol/L for the population $\geq$ 20y for FOOD and FOOD+DS use, respectively. The mean serum α-tocopherol for men $\geq$ 20y was 25.0 $\pm$ 0.2 and 33.8 $\pm$ 0.4 μmol/L and for women $\geq$ 20y was 24.9 $\pm$ 0.2 and 33.7 $\pm$ 0.3 μmol/L by FOOD and FOOD+DS use, respectively. Only 0.6% of Americans were clinically deficient, but over 87% of individuals between 20-30y had serum α-tocopherol concentrations below 30 μmol/L, whereas 67.9% of those 31-50 and 43.1% of those 51+y were below the criterion of adequacy for vitamin E ($P < 0.01$).

Insufficiency in men has been associated with impairments in spermatogenesis and sperm competiveness.[102,103] Epidemiological data has shown that infertile men had a lower α-tocopherol concentration in both the sperm (1.48 vs 1.68 μmol/L) and serum (17.8 vs 22.0 μmol/L) compared to fertile males.[104] Additionally, men with higher dietary and supplement intakes of vitamin E have been

shown to have less sperm DNA damage[105] and recent studies have reported a positive association between vitamin E supplementation and increased sperm count and mobility in mice and humans.[106,107]

Deficiency is more frequently found in children, especially newborns, likely because they have limited stores and are growing rapidly.[9] Vitamin E supplements have been prescribed for children that are diagnosed with vitamin E deficiency. α-tocopherol supplements are recommended because they prevent the further progression of the neurologic abnormalities, and in some cases, can reverse them.[9]

MATERNAL AND INFANT SERUM VITAMIN E LEVELS

Maternal Serum Vitamin E Levels

Vitamin E was first discovered for its role in supporting healthy pregnancy and development.[15] In women, increased production of biomarkers of oxidative stress have been associated with acute pregnancy complications or spontaneous abortion. Prevalence of vitamin E deficiency in pregnancy and the effect of maternal deficiency on infants continues to be debated. Previously, vitamin E deficiency was thought to be relatively rare in pregnant women as it was detected in less than 10% of individuals in different studies without higher risk for maternal and fetal complications.[108-111] However, it is important to note that criteria for maternal deficiency is not standardized and may be identified at <16 μmol/L or <12μmol/L depending on the study. A 2014 cohort of HIV-infected mothers in Brazil[112] reported a mean serum α-tocopherol level of 17.1 $\pm$ 4.9 μmol/L with only 1 participant (1%) classified as being deficient, while a 2015 prospective cohort found 51% of women attending antenatal care in North-West Nigeria were clinically deficient in vitamin E (5.99 $\pm$ 3.95 μg/dL).[113] Serum vitamin E in the North-West Nigeria subjects significantly decreased with the progression of pregnancy (1st trimester: 10.27 $\pm$ 5.05; 2nd trimester: 5.23 $\pm$ 3.45; 3rd trimester: 2.49 $\pm$ 3.35; P = 0.01) with a corresponding increase in the level of malondialehyde, (MDA) indicating an increase in oxidative stress.[114] Low serum α-tocopherol may be a result of physiological hemodilution of pregnancy, inadequate intake, and increased oxidative stress in this population. Conversely, in Saudi Arabia, Al Senaidy et al.[115] reported that during pregnancy, α-tocopherol levels steadily increased, reaching maximum levels at late gestation (first trimester: 16.4 $\pm$ 1.3

μmol/L, third trimester: 19.2 ± 1.6 μmol/L, P <0.05), with a corresponding increase in cholesterol and triglycerides. The pronounced increase in the total plasma vitamin E concentration in the late stage of normal pregnancy is thought to be due to gestational secondary hyperlipidemia and to the accompanied increases in lipoperoxides. Here, correlation coefficients between vitamin E and plasma total cholesterol, triglycerides, and total lipids were 0.76 (P < 0.001), 0.53 (P < 0.05), and 0.62 (P < 0.001), respectively. Optimum levels of γ-tocopherol were at mid-gestation (second trimester: 2.1 ± 0.2 μmol/L), which was significantly higher than that of non-pregnant women (1.8 ± 0.2 μmol/l, P <0.05). The sharp decline that continued in the third trimester and after delivery may be explained by the increase in α-tocopherol, resulting in the displacement of γ-tocopherol. Scholl et al.[116] found pregnant women at 28 weeks gestation with the highest circulating concentrations of α-tocopherol were Asian (P_{trend} < 0.0001, ANOVA) and older than 16 years of age (>16YO: 11.12 ± 0.16 μg/mL; <16YO: 10.22 ± 33μg/mL; P for trend <0.01, ANOVA). Obese women (BMI > 29) had significantly lower concentrations of α-tocopherol (obese: 10.99 ± 0.15 μg/mL; non-obese: 11.51 ± 0.09 μg/mL; P < 0.01, t test) and significantly higher concentrations of γ-tocopherol (obese: 2.51 ± 0.04 μg/mL; non-obese: 1.84 ± 0.09 μg/mL; P < 0.0001) than did the non-obese women. Concentrations of both α- and γ-tocopherol isomers increased significantly (P < 0.0001) between entry to the study and week 28 gestation (entry α- and γ-tocopherol: 11.37, 1.92 μg/mL; 28-week gestation α- and γ-tocopherol: 13.70, 2.11 μg/mL). A prospective case-control study by Sen, et al.[117] studied micronutrient status in lean (BMI 18-25) and obese (BMI > 30) pregnant women and found vitamin E levels were significantly (P < 0.05) lower in the obese group than the lean group and correlated with maternal BMI (r = -0.19).

A case-control study of 1605 pregnant Bangladeshi women who participated in a placebo-controlled supplementation trial assessed odds ratios (ORs) of miscarriages in women with low serum α- and γ-tocopherol (<12 μmol/L and <0.81 μmol/L, respectively). Results showed low α-tocopherol was associated with an OR of 1.83 (95% CI: 1.04, 3.20) whereas a low γ-tocopherol concentration was associated with an OR of 0.62 (95% CI: 0.41, 0.93) for miscarriage.[118] These data suggest low α-

tocopherol may be associated with an increased risk of miscarriage while low γ-tocopherol is associated with a decreased risk. In 2015, a prospective, cross-sectional study of Turkish women found serum vitamin E levels were not significantly different between women with preterm premature rupture of membranes (PPROM) and those without ($P = 0.15$).[119] However, it is important note that the cases and controls of this trial had much higher levels of vitamin E (20.38 ± 6.47 and 18.15 ± 6.38 mg/L, respectively).

Impaired antioxidant activity and the reduction of antioxidant levels, which increase the level of lipid peroxidation products, may cause peroxidative damage of vascular endothelium and result in clinical symptoms of preeclampsia.[120] In 1999, Sagol et al.[120] found significant higher ($P = 0.03$) serum α-tocopherol levels in women with normal pregnancy (7.4 ± 2.72 µg/L) versus those with severe pre-eclampsia (5.6 ± 3.8 µg/L). These results were reflected by Siddiqui et al.,[121] who reported preeclampsia was associated with significantly lower serum levels of α-tocopherol (pre-eclamptic: 26.1 ± 9.15 µmol/L, normal: 34.64 ± 10.55 µmol/L, $P < 0.05$). In a Canadian case control study by Cohen, et al.,[122] median (IQR) of α-tocopherol was significantly lower in cases of preeclampsia than in controls ($P = 0.07$) without a significant difference found in γ-tocopherol levels ($P = 0.60$). The adjusted odds ratios (<34 weeks) for α-tocopherol (0.92; 95% CI: 0.72, 1.17) and α-tocopherol:cholesterol (0.82; 95% CI: 0.65, 1.04) suggest an inverse association between serum vitamin E and overall preeclampsia risk. Atiba, et al.[123] studied plasma MDA and vitamin E levels in the second and third trimesters in pre-eclamptic Nigerian women. As a group, mean plasma vitamin E levels were highest in the non-pregnant subjects (30.24 ± 14.09 µmol/L) and lowest in subjects with pre-eclampsia (26.9 ± 13.81 µmol/L). In the second trimester, mean plasma vitamin E was significantly lower in preeclamptic pregnancy (25.09 ± 12.79 µmol/L) than in comparison with the nonpregnant group (30.24 ± 14.09 µmol/L; $P < 0.005$). The lower value of vitamin E in the second trimester of pre-eclamptic women (25.09 ± 12.79 µmol/L) was lower than that of normal pregnancy (28.62 ± 13.86 µmol/L), but the difference was not considered statistically significant ($P = 0.068$).

Maternal body fat accumulation during early pregnancy allows the accumulation of an important store of long-chain polyunsaturated fatty acids (PUFAs) derived from the maternal diet and maternal metabolism.[124] During the first two-thirds of gestation, the maternal body accumulates fat, but stops during late gestation because maternal lipid metabolism changes to a catabolic condition.[125] At the end of gestation, there is an enhanced lipolytic activity in maternal adipose tissue in order to transfer long-chain polyunsaturated fatty acids (PUFAs) to the fetus that is at its maximal growth.[124] A study by Mino et al. showed that the ratio of plasma α-tocopherol concentration to plasma total lipid content remains unchanged during gestation, while the red blood cell (RBC) α-tocopherol levels decrease somewhat during the last trimester. RBC tocopherol levels remained unchanged (170-180 μg/100 mL packed cells) during the first trimester, but decreased gradually and reached a minimal level (138.4 $\pm$ 5.3 μg/dl packed cells) at 40 weeks. The difference in the level was significant between the first 15 weeks and 40 weeks ($P < 0.05$).[126] The result that RBC tocopherol levels decrease somewhat during the last trimester may indicate that decreases in tissue tocopherol result in less tocopherol available for biological function in the biomembrane.

Infant Serum Vitamin E Levels

Vitamin E is extremely important during the early stages of life, from the time of conception to the postnatal development of the infant. After fertilization, vitamin E has a positive impact on the development of early embryos which are susceptible to be damaged by ROS.[127] At birth, an adequate supply of vitamin E to the newborn is of utmost importance to protect the organism against oxygen toxicity and to stimulate the development of its immune system.[128,129] Brion et al.[13] proposed 24-hour α-tocopherol levels between 0.5 and 3.5 mg/dL to be both adequate and safe as serum levels $\geq$ 0.5 mg/dL (5004.3 μg/L) are required for protection against lipid peroxidation. However, as previously discussed, vitamin E is affected by total lipids, and therefore some experts advocate that the vitamin E/total lipids ratio is more suitable to reflect the true tissue level.[130,131] Supplemental dosing of vitamin E has been

suggested as a potential agent for the prevention of intracranial hemorrhage due to vitamin E's function to protect cell membranes against lipid peroxidation.[132-134]

Factors that may impact serum vitamin E levels of newborns include: nutrition status of the mother, birth order, intrauterine growth, and birth weight.[135] The relationship between gestational age and vitamin E continues to be debated. Earlier studies by Wright et al.,[136] Leonard et al.[108] and Haga et al.[137] found no significant difference in vitamin E levels between preterm and term babies. However, in the 1999 study by Chan et al.,[138] a weak, but significant correlation with gestational age and vitamin E (without distinction of tocopherol isoform) was found, ($r = 0.13$, $P = 0.046$). No significant correlation was found between vitamin E and birth weight ($r = 0.08$, $P = 0.2$).

Studies in Tunisia and Thailand found the prevalence of low α-tocopherol levels in term newborns to be 56% and 77%, respectively, while da Silva Ribeiro et al. found 90% of newborns from a study in Brazil.[139-141] Wu et al.[142] studied serum vitamin E levels in full-term neonates in order to establish a reference range and found mean α- and γ-tocopherol levels of 0.212 $\pm$ 0.127 mg/dL and 0.029 $\pm$ 0.019 mg/dL respectively with a vitamin E/total lipids ratio of 1.219 $\pm$ 0.740 mg/dL. Preterm infants are born with sparse body fat and low body stores of fat-soluble vitamins, including vitamin E as demonstrated by elevated erythrocyte hemolysis in the presence of hydrogen peroxide.[143,144] In the study by Wu, et al., preterm infants <32 weeks gestation were found to have mean α- and γ-tocopherol levels of 0.158 $\pm$ 0.105 mg/dL and 0.019 $\pm$ 0.015 mg/dL with a vitamin E/total lipids ratio of 0.847 $\pm$ 0.532 mg/dL. Here, the mean value of vitamin E level in preterm neonates was significantly lower than that of full-term infants ($P = 0.024$) but the mean ratio of vitamin E/total lipids did not significantly differ ($P = 0.276$).[142] Wu et al.[142] went on to separate subgroups of infants <32 weeks gestation and >32 weeks gestation and concluded that pre-term infants had lower α-tocopherol levels, but not at a level of significance (GA < 32 wk: 0.158 $\pm$ 0.105; GA > 32 wk: 0.176 $\pm$ 0.091, $P = 0.604$). The clinical assessment of vitamin E deficiency remains a challenge in preterm infants, because serum tocopherol levels may not reflect tissue levels and depend on serum lipid levels.[145]

Relationship of Serum Vitamin E Status in Mothers and Infants:

Pregnancy is a state of increased oxidative stress. Tocopherols are essential micronutrients that can react with ROS such as peroxyl radicals and singlet molecular oxygen, exerting a protective antioxidant effect.[146] α-TTP seems to play a role at earlier stages of pregnancy and has been identified in the secretory epithelium of the human uterine glands as well as in the secondary yolk sac during early pregnancy. Its importance is in supplying vitamin E to the fetus during that period.[147] The expression of α-TTP was observed in the uterus and its levels transiently increased after implantation, suggesting that the placental oxidative stress is protected by vitamin E which is essential for the formation of labyrinthine trophoblasts and is likely to play a role in the critical process of implantation.[148]

Although vitamin E has been shown to be essential to the fetus during pregnancy, the net placental transfer appears to be low and is not, or only slightly influenced by variations in maternal vitamin E intake or supplementation.[149] Neonatal vitamin E levels have been shown to be only 1/6 to 1/2 of their mothers and, in general, plasma α-tocopherol concentrations <12 μmol/L are associated with increased infection, anemia, stunting of growth, and poor outcomes during pregnancy for both the infant and the mother.[9,108] Without distinguishing which tocopherol isoform, Martinez et al.[150] found serum vitamin E levels in mothers, placenta, and newborns to be 1.23 $\pm$ 0.44 mg/dl, 1.28 $\pm$ 0.44, and 0.58 $\pm$ 0.38 mg/dL respectively. Again, in a 2014 cohort where only 1% of HIV-infected mothers were vitamin E deficient, 81.1% of infants were deficient with a mean serum α-tocopherol level of 8.5 $\pm$ 3.4 μmol/L.[112] Tocopherols are also significantly higher in maternal plasma than in cord blood ($P < 0.001$)[151] A cross-sectional study, da Silva Ribeiro et al.[141] explored the relationship between maternal α-tocopherol concentration levels and the association of α-tocopherol levels of the newborn and colostrum. Results showed the α-tocopherol level in umbilical cord was 20% of that in maternal serum (mean umbilical cord blood, CI: 6 mmol/L, 5-8); mean maternal serum α-tocopherol, CI: 28 mmol/L, 24-32; $P < 0.001$, Wilcoxon test). Researchers also found that newborns born to mothers with marginal levels of alpha-tocopherol at the end of pregnancy also had lower alpha-tocopherol levels (P <0.001), suggesting that

maternal vitamin E status during pregnancy protects against low vitamin E levels at birth.[141] Different mechanisms have been proposed to explain the low transfer of vitamin E to the placenta. Jauniaux, et al.[147] proposed the lack of lipids (triglycerides, total cholesterol, and phospholipids) in the circulation of fetuses and neonates and its inefficient trans-placental transfer may, among others, contribute to the low levels of α- and γ-tocopherol in neonate plasma. Cholesterol and triglyceride concentrations, which are strongly correlated to the levels of α-tocopherol have been found to be lower in cord blood as compared to maternal plasma.[151]

Although placental transfer of vitamin E is limited, α-TTP appears to play an important role in the placental transfer of vitamin E by maintaining adequate α-tocopherol levels in the fetus by facilitating the transfer of the hydrophobic nutrients between maternal and fetal circulation.[152] According to Kaempf, et al.,[11] fetal tissue concentrations are gestation-related and "stores" of vitamin E are not accumulated *in utero*. The placenta may also restrict vitamin E passage to prevent its accumulation by the fetus. An *in vitro* study by Schenker, et al.[153] investigating the transfer of vitamin E across the term, normal human placenta, has shown that vitamin E is transferred slowly, at a rate of only 10% of L-glucose. In contrast to the satisfactory level of aqueous-phase defenses, the primary lipid-phase defense, α-tocopherol, only crosses the placenta poorly and is presumed to be relatively deficient in the face of oxidative stress in neonates after delivery[108]

In relation to fetal growth, Scholl et al.[116] found a high plasma concentration of α-tocopherol at 28 weeks gestation was associated with a nearly 3-fold reduction in risk of bearing a small-for-gestational age (SGA) infant when highest and lowest quintiles were compared with the *P* for trend across quintiles ($P < 0.05$). Additionally, the concentration of α-tocopherol was significantly lower at week 28 when data from women who delivered an SGA infant were compared with women who did not deliver an SGA infant (SGA infant: 12.95 ± 0.35 μg α-tocopherol/mL; no SGA infant: 13.73 ± 0.09; $P < 0.05$, *t* test). These results reflect previous studies of the effects of maternal concentrations of Vitamin E on pregnancy. A prospective cohort by Dresfuss, et al.[154] reported low vitamin E concentrations (without discrimination

of tocopherol isoform) <8.0 mg/L were associated with a higher risk of SGA (Adjusted OR: 1.89; 95% CI: 1.10, 3.27) but was not a determinant of low birth weight (LBW).

Studies conducted on laboratory animals have allowed to show some aspects of the transfer of α-tocopherol that are impossible to investigate in humans. In rats, the transfer appears higher in lung and heart tissue as compared to liver which was very low, suggesting that there is some preferential incorporation into some tissues as compared to others.[155] In guinea pigs, which seem to have higher placental transfer of α-tocopherol than that reported by other species, it was shown that at late gestation, the fetal liver appears to act as a storage site for α-tocopherol, the majority of which are released immediately following birth.[156,157] In contrast to rats, lung and brain vitamin E levels in guinea pigs are relatively constant over the final period of gestation and during early neonatal life. Levels of α-tocopherol in the brain and lung appear to be similar to that of RBC, while plasma α-tocopherol content varies considerably and does not accurately reflect tissue α-tocopherol status.[158] Fetal and maternal lung α-tocopherol concentrations in these guinea pigs were similar at all time points during pregnancy, whereas fetal liver α-tocopherol status was always considerably greater than maternal liver α-tocopherol content.[157]

MATERNAL VITAMIN E INTAKE

Children born to mothers with sub-optimal calorie intake have been shown to have an increased risk of neurocognitive disorders.[159] Now known as the developmental origins of health and disease hypothesis (DOHAD), maternal health and quality of diet may impact fetal development, with long-term implications for the child's future health.[160] Brunst, et al.[161] analyzed participants from the Programming of Intergenerational Stress Mechanisms (PRISM) study, a prospective pregnancy cohort of 276 mother-child pairs in the United States. Vitamin E was one of two most common inadequacies identified with 51.5% of mothers consuming less than the EAR (12 mg/day). When compared with Caucasians, African Americans and Hispanics had greater OR's of inadequate intakes across all antioxidants and being foreign-born was a significant predictor of vitamin E inadequacy (OR = 3.73; 95% CI: 2.04, 6.82). The

OR of inadequacy increased more than twofold among those experiencing difficulty living on their total household income or those with difficulty in meeting monthly payments/bills.

A study by Turner et al.[162] found that estimates of vitamin E intake were positively associated with maternal plasma α-tocopherol levels. A prospective cohort of 1231 gravid women from Camden, New Jersey found that women consumed 7.19 ± 0.13 mg α-TEE per day when adjusted for energy intake. Those who consumed vitamin E at the recommended daily allowance had circulating concentrations of α-tocopherol 0.42 μg/mL higher than a gravid woman with mean vitamin E intake of the sample. Women using prenatal multivitamins daily by week 28 increased circulating concentrations of α-tocopherol by 0.966 μmol/mL as compared to women who did not use prenatal vitamins ($P < 0.0001$; 95% CI 0.468-1.464). Schulpis, et al.[163] studied α-tocopherol levels in the sera in Greek and Albanian mothers. Greek mothers had relatively high α-tocopherol levels (32.9 ± 9.5 μmol/L) with 0% of infants deficient. This result was thought to be due to the exclusively used vegetable oils and especially olive oil in their Mediterranean diet. In contrast, Albanians, accustomed to using animal fat in their diet, were found to have normal levels of serum alpha-tocopherol (20.0 ± 8.8 μmol/L), but 8% of their newborns were vitamin E deficient (<12.5 μmol/L).[163] When examining α-tocopherol/cholesterol in the cord blood, Albanian mean α-tocopherol was 14.5 ± 2.5 μmol/L with 15% of newborns being deficient (<7.5 μmol/L).

Léger et al.[164] supplemented ten pregnant women with a daily dose of 1 gram *dl*-α-tocopherol acetate for 3 days before caesarean section with all pregnancies ranging from 37-39 weeks gestation. The vitamin E plasma content of the mothers after supplementation was approximately twice as high as that before supplementation (after supplementation: 33.43 ± 3.26 mg/L; before supplementation: 18.57 ± 2.05 mg/L; $P < 0.01$). Plasma Vitamin E levels of neonates were not increased after short-term supplementation (3.51 ± 0.38) when compared to cord blood of fetuses at 20-40 weeks of non-supplemented women from a previous sample. Researchers concluded that a long period of supplementation in mothers is probably needed to increase the fetal vitamin E status. However, recent

animal models have shown supra-nutritional doses of α-tocopherol may lead to long-lasting effects in the adult brain, impacting spatial memory.[165]

It was suggested that a potential relationship exists between the contents of vitamin E in maternal diet and human milk.[166] This relationship was originally confirmed by a case report which has documented that high maternal intake of vitamin E (approximately 27 mg per day) was reflected by an elevated level of the vitamin in human milk.[167] A study of breastfeeding mothers from Bangladeshi revealed that individuals with extremely low socioeconomic status were characterized by very low breast milk levels of vitamin E (2.04 $\pm$ 0.11 mg/L) which was insufficient to cover requirements of their infants.[168] However, mean breast milk concentration of α-tocopherol from Bangladeshi women from a higher income group of Bangladeshi women did not differ significantly from that of a lower socio-economic group (higher: 1.86 $\pm$ 0.08 mg/l, lower: 2.18 $\pm$ 0.18 mg/l). It is important to note that the mean α-tocopherol of all Bangladeshi women (2.04 mg/L) was lower than the Western values (3-8 mg/L).[169] A case-control study by Garg et al.[170] did not find a significant difference in serum α-tocopherol concentrations in colostrum samples from well- and undernourished Indian women (well-nourished mothers: 1.272.8 $\pm$ 890.7 µg/dL; undernourished mothers: 870.2 $\pm$ 451.2 µg/dl) and a 2013 study by Martysiak-Zurowska et al.[171] found no significant correlation between dietary vitamin E intake and breast milk concentration (r = 0.034, P = 0.22). Here, dietary intake of vitamin E was determined directly before milk sampling based on a 3-day nutritional diary reported by mothers. Women who took vitamin supplements at the time of sampling did not differ significantly from non-supplemented individuals in terms of mean breast milk concentration of vitamin E (3.46 $\pm$ 1.36 TE/mg vs 3.35 $\pm$ 1.25 TE/mg; 95% CI: -0.56, 0.0; P = 0.33). These results further confirmed findings by Tokusoglu, et al.[172] who found no relation between maternal vitamin E intake and breast milk concentration. When mothers were separated by socioeconomic groups, participants in both categories in this study had low intakes of corn oil, soy oil and fast foods and high intakes of fish, olive oil, sunflower seed oil, nuts, grains, fruits, and vegetables based on a food frequency questionnaire.

In a 2015 RCT, a comparison of α-tocopherol concentrations in colostrum at 0 and 24 hours from women supplemented with natural and synthetic tocopherol increased colostrum levels by 57.6% and 39.0%, respectively ($P = 0.0000$).[173] Pires Medeiros, et al.[174] evaluated the effect of maternal vitamin E supplementation on the α-tocopherol concentrations of colostrum, transitional milk, and mature milk of women who had given birth prematurely by providing a single postpartum dose of 400 IU *RRR*-α-tocopheryl acetate to women in the experimental group. Women in the experimental and control group had similar baseline concentrations (1159.8 $\pm$ 292.4 and 1128.3 $\pm$ 407.2 µg/dL, respectively) with no cases of vitamin E deficiency. Breast milk α-tocopherol increased by 60% 24 hours after supplementation in the intervention group and did not increase at all in the control group. Concentrations of α-tocopherol in the transitional milk of the supplemented group was 35% higher compared to the control group but no difference was identified in mature milk. In the study by Barua, et al.,[168] concentration of α-tocopherol varied significantly for the variation of lactation period. Amongst the relatively lower income group, breast milk beyond 6 months *post-partum* contained a higher concentration of α-tocopherol as compared to those of earlier lactation period. Infants' calculated daily intake of α-tocopherol through breast milk varied from 1.25 mg at 1.5-3 months *post-partum* to 1.33 mg at $\geq$ 10 months *post-partum*.

INFANT VITAMIN E INTAKE

In newborns with vitamin E deficiency, oxidative stress may cause neonatal diseases. During delivery and right after birth, fetal tissues are exposed to oxidative damage caused by higher free radical production because the newborn passes from a low-oxygen to a high-oxygen environment.[175] Oxidative damage may also be caused by low antioxidant capacity because newborns have a low antioxidant-enzyme complex. Ostrea et al.[128] found serum concentrations of vitamin E in the term (0.31 $\pm$ 0.09 mg/dl) and preterm (0.29 $\pm$ 0.08 mg/dl) infants are one-third the concentration in the maternal serum (0.97 $\pm$0.16 mg/dL). These serum levels can be replenished postnatally through breastfeeding. Exclusive breastfeeding can be a strategy against vitamin E deficiency because the milk excreted until postnatal day

4 (colostrum) is particularly rich in α-tocopherol, supplying infants with their vitamin E requirement, essential for preventing deficiency.[176] Average α-tocopherol content of colostrum ranges from 6.8-23 mg/L with average γ-tocopherol levels of 1.5 ± 0.4 μg/mL and 1.0 ± 0.3 μg/mL for early samples.[158,169] Mean concentrations of vitamin E were highest in breast milk of 1 to 3 days, after which their concentration rapidly diminished (Table 2).

Table 2: Concentrations (mean ± SD) of Vitamin E in Human Milk[170]

Human Milk	Vitamin E (mg/dl)
Day 1	3.28 ± 2.93
Day 2	2.74 ± 2.30
Day 3	2.09 ± 1.44
Day 4	1.03 ± 0.50
Day 5	0.45 ± 0.33

Milk produced during the early weeks postpartum by mothers of preterm infants (preterm milk) more closely approximates the nutrition requirements of the preterm infant than does mature human milk.[177] In 1985, Gross et al.[178] reported preterm milk contains approximately two to three times more alpha-tocopherol than mature milk. Yet, the same year, Chappell et al.[169] showed the initial α-tocopherol content of preterm milk (11 ± 2.5 μg/ml) is not significantly different from full term milk (15 ± 2.5 μg/ml). However, on all subsequent days (7-35), the mean α-tocopherol concentration was significantly higher in preterm milk (P <0.05). Average γ-tocopherol levels for colostrum were 1.5 ± 0.4 μg/ml and 1.0 ± μg/ml for early milk.

Treating preterm infants with pharmacologic doses of Vitamin E (200 mg/day α-tocopherol) has been proposed to prevent or limit associated vitamin E deficiency conditions, and current

recommendations encourage routine vitamin E supplementation in preterm infants due to decreased reserves.[13] However, the 2003 Cochrane review on vitamin E supplementation of preterm infants concluded that high-dose supplementation, greater than 30 IU/kg/day α-tocopherol, resulting in concentrations >3.5 mg/dL (1507 µg/L), was associated with an increased incidence of sepsis (RR 1.57, CI 1.15, 2.14; RD 0.06, CI 0.02, 0.11).[13] In 2014, Bajčetić, et al.[179] conducted an open label study involving preterm neonates (control/sepsis) which were divided into two groups non-supplemented and supplemented with vitamin E (25 IU/day for 60 days). At 30 days, vitamin E provoked lower glutathione peroxidase (GPx), an enzyme that protects against oxidative damage, compared to untreated septic neonates (P = 0.014). Vitamin E also suppressed glutathione reductase (GR) in septic neonates (P = 0.025 and P = 0.017 at 30 and 60 days), while causing a significant increase in GPx activity in control neonates (P = 0.015 at 60 days). A meta-analysis by Brion et al.,[13] concluded that available data show vitamin E supplementation significantly reduced the risk of intraventricular hemorrhage (RR 0.85, CI 0.73, 0.99) and increased the risk of sepsis after study entry (RR 1.52, CI 1.13, 2.04). Subgroup analysis showed that serum tocopherol levels greater than 3.5 mg/dL in very low birth weight infants were associated with a significantly increased risk of sepsis after study entry (RR 1.72, CI 1.24, 2.40), with increased risk of necrotizing enterocolitis among those treated for more than one week (RR 1.60, CI 1.02, 2.52) and with reduced risk for severe retinopathy among those examined (RR 0.34, CI 0.13, 0.88); and intravenous, high-dose administration of vitamin E in very low birth weight was associated with a significant increased risk of sepsis after study entry (RR 1.56, CI 1.07, 2.27), with increased risk of parenchymal hemorrhage in one study and with increased risk of necrotizing enterocolitis among those treated for more than one week in another study.

Bell et al.[180] examined the impact of a single enteral dose of α-tocopherol acetate soon after birth on serum α- and γ-tocopherol levels of very preterm infants during the first week of life. Median serum α-tocopherol levels in experimental and control groups at baseline were 0.31 mg/dL and 0.33 mg/dL respectively. The experimental group received a 50-IU/kg enteral dose of vitamin E as *dl*-α-tocopherol

acetate within the first 4 hours after birth. The α-tocopherol levels were similar between the groups at baseline but higher in the vitamin E group at 24 hours (median 0.63 mg/dL vs 0.42 mg/dL, $P = 0.003$) and 7 days (2.21 mg/dL vs 1.86 mg/dL, $P = 0.04$) with no differences between groups in γ-tocopherol levels. Researchers concluded a higher dose or several doses of vitamin E may be needed to raise serum α-tocopherol levels and consistently achieve α-tocopherol levels >0.5 mg/dL. A prospective cohort by Kositamongkol, et al.[139] studied Vitamin E status in very low birth weight (VLBW) infants at different time points in relation to feeding. At time of birth, median plasma α-tocopherol was 283.7 µg/dL with 77.4% of the sample identified as having vitamin E deficiency (<500 µg/dL). Parenteral nutrition with multivitamin supplementation was started within 24 to 48 hours of life with enteral feeding initiated as soon as clinically stable with an increment of 15 to 25 mL/kg/day. At the time of full feeding, median plasma α-tocopherol was 871 µg/dL with only 16.1% of infants deficient. Again, this study concluded a higher dose of vitamin supplementation may need to be considered, especially for those who are at risk for deficiency.

VITAMIN E CONTENT OF INFANT FEEDING

In newborn infants, vitamin E from dietary sources prevents vitamin E deficiency. Elisia et al.[58] found that α-tocopherol was the dominant vitamin E isoform in human milk while γ-tocopherol was present at significantly ($p<0.05$) lower concentrations. Depending on the stage of lactation, human breast milk has been found to have 2.07-12.13 mg α-tocopherol/L and 0.22-0.60 mg/L γ-tocopherol. Infant formulas contained significantly ($p<0.05$) higher concentrations of γ-tocopherol (5.5 µg/mL-9.6 µg/mL) compared to human milk. Infant milk is a human milk substitute composed of de-fatted bovine milk and a blend of vegetable oils.[58] The use of vegetable oils as the lipid component of infant formula leads to the relatively high amount of γ-tocopherol in formula.[171]

A study by Anderson, et al.[167] revealed that the average content of vitamin E in the infant formulas intended for children under 6 months of age amounts to 4.34 mg (including 3.94 mg of α-tocopherol) per 1 liter of ready-to-feed solution, with the minimum level of vitamin E in infant formulas

being 0.5 mg/100 kcal. This concentration did not differ significantly from that of human milk from the 14th day of lactation and was higher than the breast milk concentrations determined on the 30th and 90th day of lactation. In contrast, mean concentrations of γ-tocopherols in analyzed infant formulas were approximately 5- to 18-times higher than in human milk. This disproportion in γ-tocopherol concentration results from the technological aspects of infant formula manufacturing.[171] During processing, components of the formula are exposed to high temperatures and aerobic conditions which promote oxidation of PUFAs included in formulas. γ-tocopherols are added to prevent the oxidative degradation and have been shown to promote pro-inflammatory responses in the fetal-derived small intestinal cell line.[59,181] The potentially distinct vitamin E profile between human milk and infant formula could be a contributing factor to the greater susceptibility of formula-fed infants to contract inflammatory conditions compared to breast-fed infants.[14]

Recommended doses of intravenous multivitamins in VLBW infants vary considerably. The optimal amount of parenteral vitamin E in preterm infants in uncertain, although the maximum recommended dose for any neonate is 7 IU per day.[13] In 2004 the AAP endorsed a dose of 2.8 IU/kg/day, which can be provided either by 2 ml/kg/day of MVI® Pediatric™ or by 1.6 ml/kg of Infuvite Pediatric™ in VLBW infants.[182] Intakes of IV vitamin E > 4IU/kg/day often yield potentially toxic serum tocopherol levels (>81μmol/L or 3.5 mg/dL) after 2 to 3 weeks of administration.[182] Toxic doses of vitamin E increase the risk of infection and hemorrhage by at least three mechanisms: decreased leukocyte function, a decrease in vitamin K-dependent factors in vitamin K-deficient individuals, and inhibition of platelet prostaglandin synthesis and platelet aggregation.[182]

CHAPTER 3: METHODS

STUDY DESIGN AND POPULATION

After obtaining University of Nebraska Medical Center Institutional Review Board approval, parents of infants admitted to the NICU who meet eligibility criteria were approached by study personnel and 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 19 years of age or if infants were made wards of the State of Nebraska.

DATA AND BLOOD COLLECTION

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 α- and γ-tocopherol 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, respiratory distress syndrome (RDS), necrotizing enterocolitis (NEC), intraventricular hemorrhage (IVH), positive blood cultures, and cord blood α- and γ-tocopherol 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.

Thirty-four mother-infant pairs were enrolled into the study. Maternal and umbilical cord blood samples were taken at the time of delivery. Samples were processed efficiently after collection, protected from heat and light, and stored in -80º F freezers until they were analyzed. Dietary intake throughout pregnancy was assessed by administering food frequency questionnaires (FFQ) to the mothers. The FFQ's 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.

Blood samples were collected from participating infants at one time point during the administration of each feeding modality (parenteral, breast milk, formula) after the feeding modality has been stable for 72 hours. The half-lives of α- and γ-tocopherol are 48 and 15 hours,[20] respectively, therefore 72 hours should allow adequate time for serum concentrations to reflect intake. Infants who are receiving parenteral nutrition will have one blood draw after 3 days of parenteral nutrition administration (in conjunction with trophic enteral feeding). As enteral feedings are initiated and advanced, a sample will be obtained after the infant has received full feedings of either human breast milk or formula for 72 hours. Another sample will be obtained if the infant transitions from breast milk to formula during NICU hospitalization and intake is stable for 72 hours.

Serum nutrient levels were assessed in the Biomarker Research Institute at Harvard University. Concentrations of α- and γ-tocopherol in plasma samples were measured as described by El-Sohemy et al.[183] Plasma samples (250 µl) are mixed with 250 µl ethanol containing 10 µg/ml *rac*-Tocopherol (Tocol) as an internal standard, extracted with 4 ml hexane, evaporated to dryness under nitrogen, and reconstituted in 100 µl ethanol-dioxane (1:1 v/v) and 150 µl acetonitrile. Samples were quantitated by a high-performance liquid chromatography (HPLC) on a Restek Ultra C18 150mm x 4.6mm column, 3 mm 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) is used as 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 (300nm and 445nm), and a Hitachi L-2200 auto-sampler with water-chilled tray. The Hitachi System Manager software (D-2000 Elite, Version 3.0) is used for peak integration and data acquisition. Internal quality control is monitored with four control samples analyzed within each run. These samples consist of two identical high-level plasmas and two identical low-level plasmas. Comparison of data from these samples allows within-run and between-run variation estimates.

In addition, external quality control is monitored by participation in the standardization program for carotenoid analysis from the National Institute of Standards and Technology U.S.A.

Ongoing clinical data was collected on all infants during NICU hospitalization. Infant vitamin E intake was calculated based on published infant formula composition and recorded daily. Sample of maternal milk was analyzed for tocopherol content in the Harvard Biomarker Research Institute. As misclassification of serum tocopherol isomers can occur if serum lipid levels are abnormal, all infants at risk for hypertriglyceridemia (including those receiving parenteral nutrition) will have monitoring of serum lipid levels performed according to clinical protocol. Otherwise, serum lipid levels will be assumed to be normal in an infant population, consistent with other studies of infant serum tocopherol levels.[180]

STATISTICAL ANALYSIS

Descriptive statistics were used for continuous and categorical variables. Continuous variables (maternal BMI, serum α- and γ-tocopherol 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 α- and γ-tocopherol levels) were described using means $\pm$ standard deviations, medians, and ranges. Ratios of α- to γ-tocopherol levels were also included. For categorical variables (maternal race, delivery mode, birth month, gestational diabetes mellitus, preeclampsia, and placental chorioamnionitis and infant sex, supplemental oxygen use, intubation on admission, BPD, RDS, NEC, IVH, and positive blood cultures), frequencies and percentages were reported.

Spearman correlation coefficients were used to describe associations between maternal and cord blood α- and γ-tocopherol levels, maternal dietary intake and maternal and cord blood α- and γ-tocopherol levels, gestational age and maternal and cord blood α- and γ-tocopherol levels, and birth weight and maternal and cord blood α- and γ-tocopherol levels.

Continuous variables, specifically serum γ-tocopherol levels, were compared between the groups using a Mann-Whitney test. Kruskal-Wallis test was used to compare mean ranks of serum α- and γ-tocopherol levels of infants on different feeding modalities with post-hoc tests performed to examine between-group differences.

All tests were two-sided and a *P*-value <0.05 was considered statistically significant. All statistical analysis were carried out using the Statistical Package for the Social Sciences software (IBM SPSS Statistics; Version 24.0).

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 3 and 4.

Maternal Characteristics

Of 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 occurred 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, 2015).

Table 3: Maternal Baseline Characteristics

Categorical Demographics (n=34)		**n(%)**		
Race				
	Caucasian	22 (64.7)		
	Hispanic	6 (17.6)		
	American 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 (n=34)		**Mean ± SD**	**Median**	**Range**
BMI (kg/m2)		30.7 ± 6.3	29.62	18.01-41.6
Serum Vitamin E Levels				
	Alpha-tocopherol (mcg/L)	7910.8 ± 5627.0	8326.9	1150.9-23233.9
	Gamma-tocopherol (mcg/L)	1305.9 ± 1178.2	1142.7	63.75-4303.3
	Delta-tocopherol (mcg/L)	237.9 ± 260.4	107.9	0-1019.9
Dietary Intake (n=20)				
	Calorie Intake (kcals)	2600.3 ± 991.3	2323.7	1314.0-4415.59
	Protein Intake (gm)	104 ± 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
	Alpha-tocopherol (mg)	10.9 ± 9.0	9.1	3.67-35.77
	Beta-tocopherol (mg)	0.4 ± 0.3	0.3	0.1-0.79
	Gamma-tocopherol (mg)	11.2 ± 6.0	10.9	4.1-24.1
	Delta-tocopherol (mg)	2.9 ± 1.7	2.7	0.7-6.73
	Alpha-tocotrienol (mg)	1.1 ± 0.7	1.0	0.4-3.7
	Beta-tocotrienol (mg)	1.0 ± 0.6	1.0	0.2-2.1
	Gamma-tocotrienol (mg)	1.0 ± 0.6	1.0	0.2-2.1
	Delta-tocotrienol (mg)	0.05 ± 0.04	0.05	0.0-0.18
	Total Tocopherol (mg)	33.9 ± 17.0	28.4	11.7-65.4

Infant Characteristics

The mean gestational age of infants in the study was 36.7 ± 3.4 weeks and was comprised of 58.8% males and 41.2% females. 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 4: 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 (0.0)		
Intraventricular Hemorrhage (IVH)	0 (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 ± 34.4	47.0	35.0-55.0
Birth Length Percentile	47.2 ± 34.4	48.5	0.0-99.7
Days of Ventilator (n=9)	2.0 ± 2.3	1.0	1.0-8.0
Cord Blood Vitamin E Levels (n=32)			
Alpha-tocopherol (mcg/L)	6093.6 ± 5218.2	3959.1	1030.1-19478.3
Gamma-tocopherol (mcg/L)	758.8 ± 732.1	552.3	103.2-2873.0
Delta-tocopherol (mcg/L)	203.7 ± 179.8	140.9	22.5-699.2

VITAMIN E STATUS

Maternal and cord blood samples were collected and analyzed for 31 mothers and 32 infants. Maternal and cord blood α-, γ, and δ-tocopherol serum levels are displayed in Tables 3 and 4.

Maternal Status

The mean serum α-, γ-, and δ-tocopherols in µg/L were 7910.8 ± 5627.0, 1305.9 ± 1178.2, and 237.9 ± 260.4, respectively. The ratio of mean α- to γ-tocopherol was 7910.8:1305.9 or 6.1. Of the 31 maternal blood samples, 25.8% had serum levels of α-tocopherol greater than 12015.5 µg/L (27.9 µmol/L), which is the serum level expected to result from an intake of 12 mg α-tocopherol/day. 45.2% of samples had adequate α-tocopherol concentrations >20 µmol/L. Low α-tocopherol concentration, < 20 µmol/L (8620.7 µg/L) and >12 µmol/L, was present in 12.9% of maternal samples (Table 5). 41.9% of mothers were considered α-tocopherol deficient with serum levels <12 µmol/L (5172.0 µg/L).

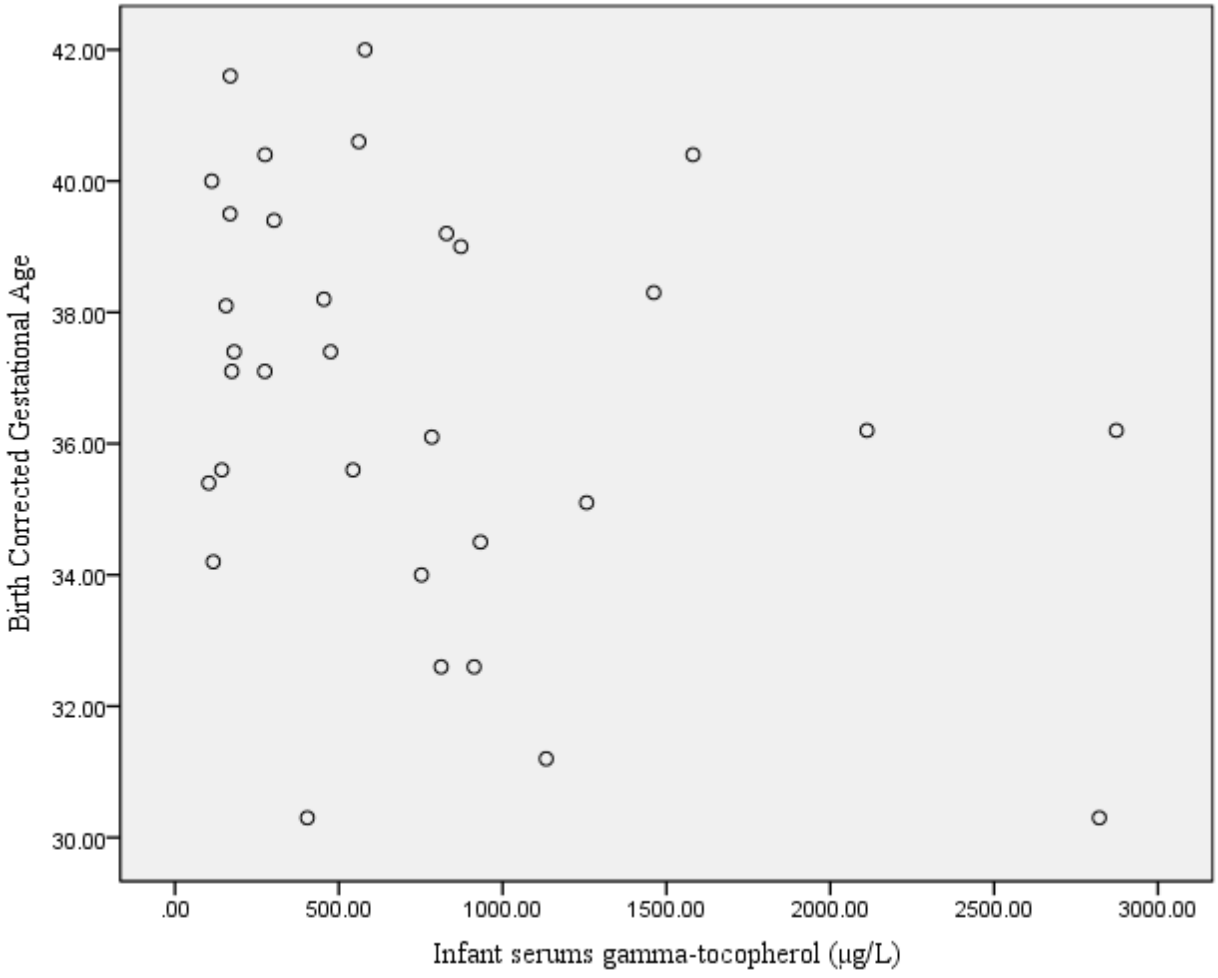
Infant Status

At birth, mean serum α-tocopherol levels were 6093.6 ± 5218 µg/L (14.1 ± 12.1 µmol/L). 20 infants, or 64.5% of the sample, were considered to be deficient with serum α-tocopherol levels <5000 µg/L. 12 infants (38.7%) had adequate serum α-tocopherol levels with 0% of infants with a serum level >3.5 mg/dL (Table 5). Mean serum γ-tocopherol at birth was 758 ± 732.1 µg/L (1.8 ± 1.7 µmol/L). No significant correlations were found between gestational age and infant serum α-tocopherol ($r = -0.024$, $P = 0.898$) or birth weight and infant serum α-tocopherol ($r = 0.005$, $P = 0.977$). As shown in Figure 5, correlation between corrected gestational age and infant γ-tocopherol show a slightly higher correlation but remain insignificant ($r = -0.312$, $P = 0.82$). No significant correlation was found between birth weight and infant serum γ-tocopherol ($r = -0.230$, $P = 0.205$).

Table 5: Prevalence of α-Tocopherol deficiency in Mothers and Infants at Birth

Mothers (n=31)	**n (%)**
Deficient (<12 µmol/L [<5172.0 µg/L])	13 (41.9)
Low (12 - 20 µmol/L [5172.0-8620.7 µg/L])	4 (12.9)
Healthy (>20 µmol/L [>8620.7 µg/L])	14 (45.2)
Infants (n=32)	
Deficient (<0.5 mg/dL [<5004.3 µg/L])	20 (64.5)
Adequate (0.5 - 3.5 mg/dL [5004.3-35,196.0 µg/L)	12 (38.7)
High (>3.5 mg/dL [>35,196.0])	0 (0.0)

Figure 5: Relationship of Birth Corrected Gestational Age and Infant Serum γ-Tocopherol Concentrations



Relationship between Serum Vitamin E Levels of Mothers and Infants:

Associations between maternal and cord blood tocopherols were determined and are displayed in Table 6. Maternal serum α-tocopherol had a low correlation to infant serum α-tocopherol concentrations (r = 0.024, *P* = 0.899). Maternal serum γ-tocopherol also has a low correlation to infant serum γ-tocopherol concentrations, but appears to have a stronger correlation than does maternal and infant α-tocopherol concentrations. Figures 6 and 7 display correlations of maternal and cord blood α- and γ-tocopherol.

Table 6: Correlations of Maternal and Infant α- and γ-Tocopherol Levels

	Correlation Coefficient (r)	*P*-Value
Alpha-Tocopherol	0.024	0.899
Gamma-Tocopherol	-0.204	0.271

Figure 6: Relationship of Maternal and Infant Serum α-Tocopherol Levels

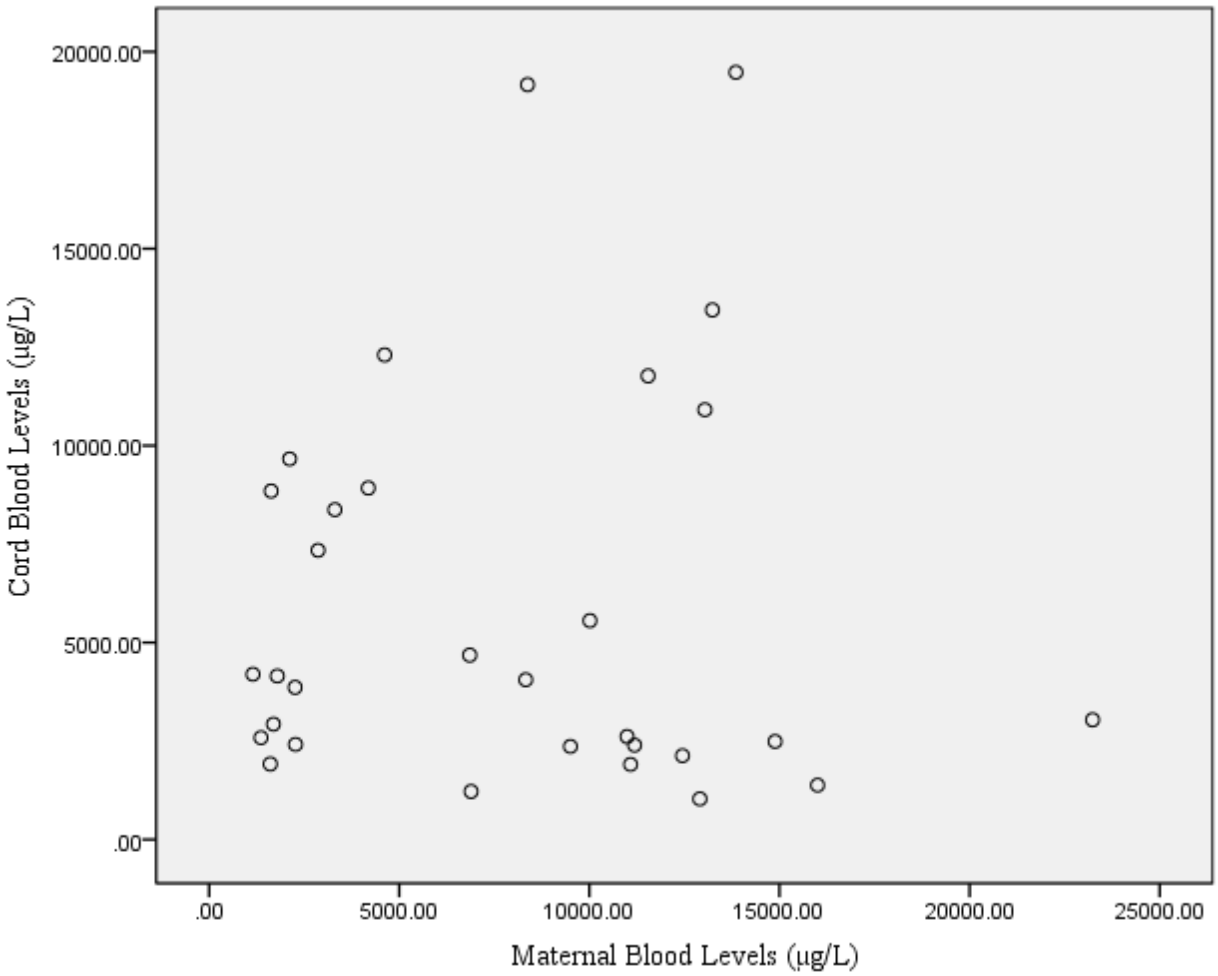
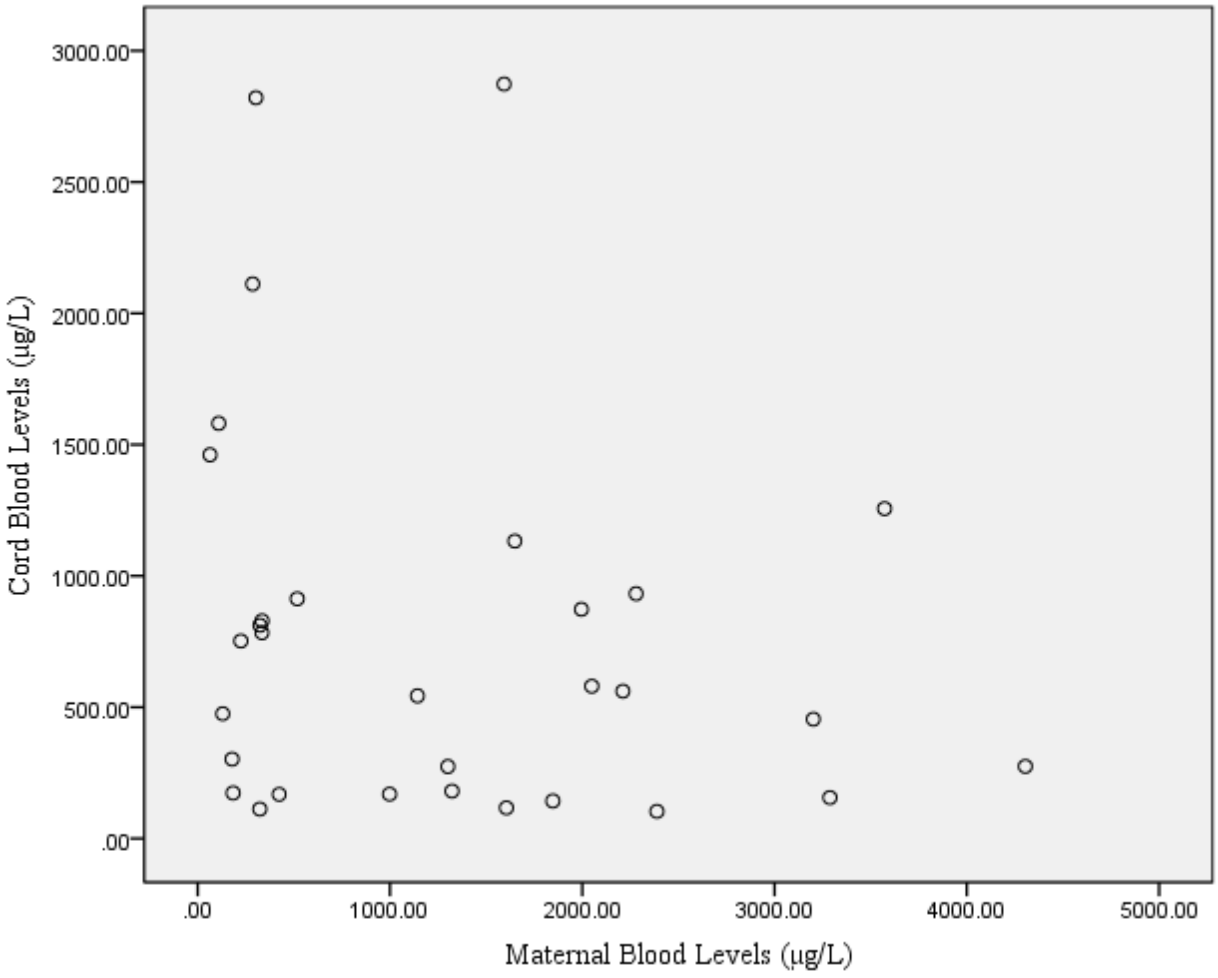
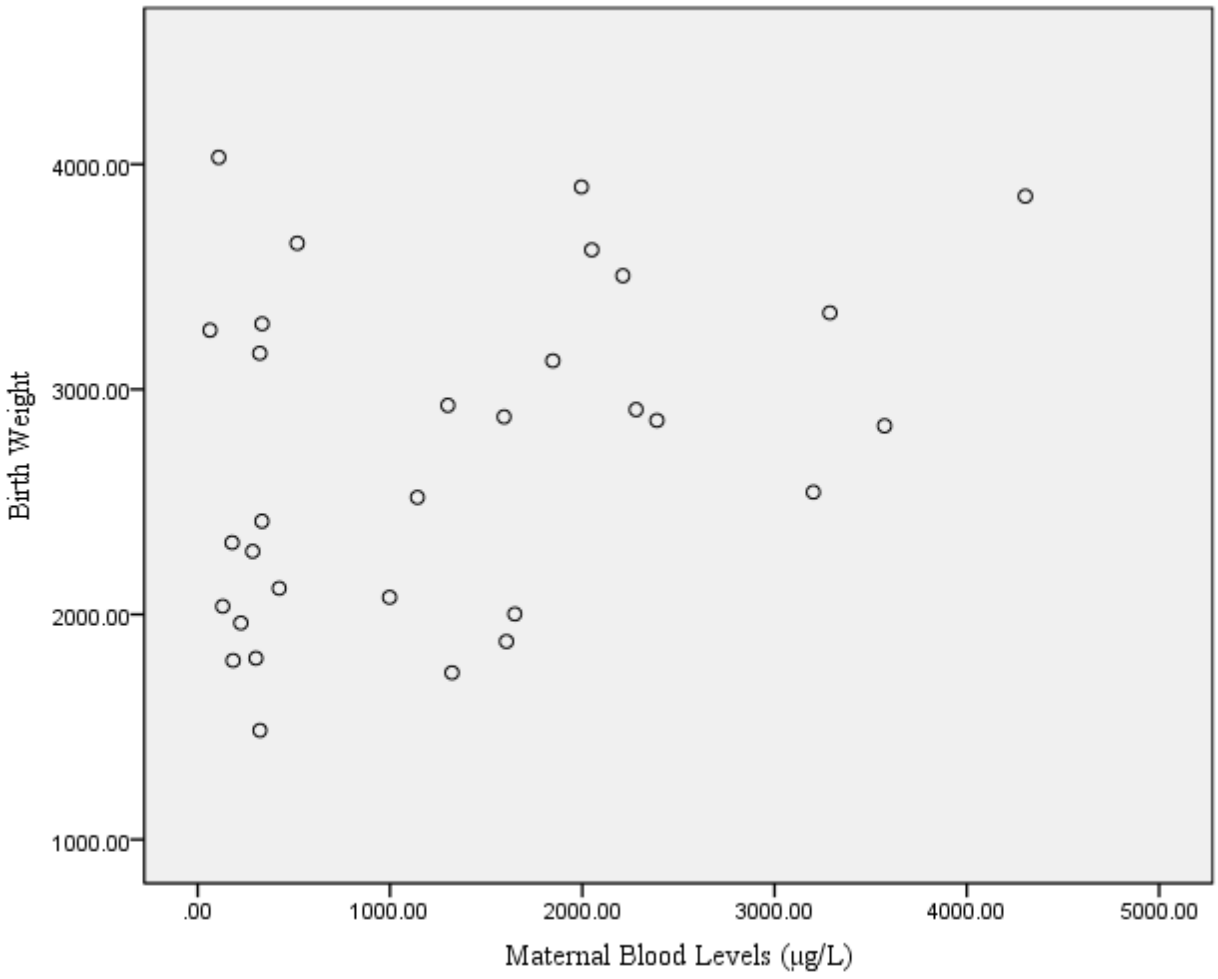


Figure 7: Relationship of Maternal and Infant Serum γ-Tocopherol Concentrations



There was no significant correlation between gestational age and maternal serum α-tocopherol (r = 0.17, *P* = 0.360) or γ-tocopherol (r = 0.263, *P* = 0.152). A positive significant correlation was found between birth weight and maternal serum γ-tocopherol (r = 0.374, *P* = 0.038), as shown in Figure 8. This correlation was not observed between birth weight and maternal serum α-tocopherol (r = 0.201, *P* = 0.278).

Figure 8: Relationship of Birth Weight and Maternal Serum γ-Tocopherol Concentrations



MATERNAL VITAMIN E INTAKE

FFQs were completed for 21 mothers, but data from 20 FFQs were used for analysis (Table 3). FFQ data from one mother was excluded due to results of very high calorie intake (31727 calories), exceeding the expected amount. The median daily calorie intake was 2323.7 $\pm$ 991.3 calories per day the median protein, fat, and carbohydrate intake being 108.7 $\pm$ 32.3, 89.9 $\pm$ 35.4, and 299.0 $\pm$ 157.5 grams per day respectively. Median α- and γ-tocopherol intake without supplementation was 9.1 $\pm$ 7.8 and 10.9 $\pm$ 5.9 mg per day, respectively.

Maternal α- and γ-tocopherol intake was not associated with maternal serum α- and γ-tocopherol levels (Table 7) or cord blood α- and γ-tocopherol levels (Table 8). Additionally, no significant correlations were found when comparing calorie or fat intake with maternal or cord blood α- and γ-tocopherol levels as seen in Tables 7 and 8. Correlations of maternal intake and maternal serum levels and maternal breast milk samples were also evaluated without any significant results (Tables 9 and 10). However, when maternal intake was separated into groups of meeting the EAR (12 mg/day or over), median α- γ-tocopherol concentrations in mothers' breast milk were significantly correlated (Table 11). In the subgroup, median α- and γ-tocopherol levels of mothers meeting the EAR were 9578.0 and 1790.4 μg/L, respectively, while those not meeting the EAR had levels of 2789.9 and 378.8 μg/L, respectively.

Table 7: Correlations of Maternal Intake and Maternal Serum Levels

Dietary Intake vs Serum Level	Correlation Coefficient	P-Value
total calories (kcal) vs α-tocopherol (μg/L)	0.023	0.944
total calories (kcal) vs γ-tocopherol (μg/L)	0.404	0.192
total fat (g) vs α-tocopherol (μg/L)	0.412	0.183
total fat (g) vs γ-tocopherol (μg/L)	0.022	0.946
α-tocopherol (μg) vs α-tocopherol (μg/L)	-0.455	0.137
α-tocopherol (μg) vs γ-tocopherol (μg/L)	0.078	0.809
γ-tocopherol (μg) vs α-tocopherol (μg/L)	0.068	0.834
γ-tocopherol (μg) vs γ-tocopherol (μg/L)	0.49	0.106

Table 8: Correlations of Maternal Intake and Cord Blood Levels

Dietary Intake vs Serum Level	Correlation Coefficient	P-Value
total calories (kcal) vs α-tocopherol (μg/L)	0.424	0.07
total calories (kcal) vs γ-tocopherol (μg/L)	-0.23	0.926
total fat (g) vs α-tocopherol (μg/L)	0.402	0.088
total fat (g) vs γ-tocopherol (μg/L)	-0.05	0.838
α-tocopherol (μg) vs α-tocopherol (μg/L)	0.022	0.928
α-tocopherol (μg) vs γ-tocopherol (μg/L)	0.07	0.775
γ-tocopherol (μg) vs α-tocopherol (μg/L)	0.372	0.117
γ-tocopherol (μg) vs γ-tocopherol (μg/L)	0.049	0.843

Table 9: Correlations of Maternal Serum Levels and Mothers Breast Milk Samples

Maternal Serum Level vs Breast Milk Samples	Correlation Coefficient	P-Value
α-tocopherol (µg/L) vs α-tocopherol (µg/L)	0.199	0.534
α-tocopherol (µg/L) vs γ-tocopherol (µg/L)	0.244	0.446
γ-tocopherol (µg/L) vs α-tocopherol (µg/L)	0.246	0.44
γ-tocopherol (µg/L) vs γ-tocopherol (µg/L)	0.138	0.668

Table 10: Correlations of Maternal Dietary Intake and Breast Milk Samples

Dietary Intake vs Breast Milk Samples	Correlation Coefficient	P-Value
α-tocopherol (µg) vs α-tocopherol (µg/L)	-0.455	0.137
α-tocopherol (µg) vs γ-tocopherol (µg/L)	0.078	0.809
γ-tocopherol (µg) vs α-tocoperhol (µg/L)	0.068	0.834
γ-tocopherol (µg) vs γ-tocopherol (µg/L)	0.49	0.106

Table 11: Correlations of Maternal Dietary Intake and Breast Milk Samples meeting EAR (n=6)

Dietary Intake (≥12 mg/day) vs MBM and Cord Blood	Correlation Coefficient	P-Value
α-tocopherol (mg) vs MBM α-tocopherol	-0.880	0.021
α-tocopherol (mg) vs MBM γ-tocopherol	-0.857	0.029
α-tocopherol (mg) vs CBM α-tocopherol	-0.052	0.494
α-tocopherol (mg) vs CBM γ-tocopherol	-0.515	0.296

Table 12: Correlations of Maternal Dietary Intake and Breast Milk Samples not meeting EAR

Dietary Intake (<12 mg/day) vs MBM and Cord Blood	Correlation Coefficient	P-Value
α-tocopherol (mg) vs MBM α-tocopherol	-0.279	0.380
α-tocopherol (mg) vs MBM γ-tocopherol	-0.273	0.390
α-tocopherol (mg) vs CBM α-tocopherol	-0.246	0.419
α-tocopherol (mg) vs CBM γ-tocopherol	-0.062	0.841

INFANT VITAMIN E INTAKE

Table 13 shows serum α- and γ-tocopherol levels after an infant had been stable on a feeding modality for 72 hours. Median α- and γ-tocopherol concentrations in 12 samples of mothers' breast milk were 8215.8 ± 4883.2 and 840.5 ± 458.8 µg/L, respectively. Samples of infants stable on breastmilk, infant formula, and mixed feeding modalities resulted in median α- and γ-tocopherol levels of 2853.4 and 370.6 µg/L, 10670.1 and 1107.8 µg/L, and 11185.3 and 1203.0 µg/L respectively. No infants were stable on only total parenteral nutrition for 72 hours; therefore, no data are available. No significant difference was found between groups (α-tocopherol chi-square: 2.653, *P*=0.27; *γ*-tocopherol chi-square: 1.962, *P*=0.375).

Table 13: Median serum α- and γ-tocopherol levels after 3 days on feeding modality

	α-Tocopherol (µg/L)	γ-tocopherol (µg/L)	Ratio (α/γ)
Breastmilk (n=13)	2853.4	370.6	7.7
Formula (n=7)	10670.1	1107.8	9.6
Mixed (n=17)	11185.3	1203.0	9.3

CHAPTER 5: DISCUSSION

VITAMIN E STATUS IN MOTHERS AND INFANTS

Circulating α-tocopherol concentrations <20 μmol/L (8620.7 μg/L) were defined by the IOM to be in the low range for healthy adults and concentrations <12 μmol/L (5172 μg/L) considered deficient.[20] We found a mean serum α-tocopherol concentration of 7910.8 ± 5627.0 with 4% of the sample having low serum concentrations and 41.9% of the sample with levels defined as deficient. While prevalence of α-tocopherol deficiency during pregnancy continues to be debated world-wide, it is concerning that a high percentage of the sample had mean levels of α-tocopherol are considered low (12.9%) or deficient (41.9%) in a predominantly Caucasian sample in an urban setting of a developed country. Maternal mean γ-tocopherol was 1305.9 ± 1178.2 μg/L with an α-tocopherol to γ-tocopherol ratio of 6.1. γ-tocopherol levels were similar to those reported by Wu, et al.[142] who found a mean maternal γ-tocopherol to be 0.214 ± 0.122 mg/dL with an α-tocopherol to γ-tocopherol ratio of 5.4.

Mean cord blood α- and γ-tocopherol levels were 6093.6 ± 5218.2 μg/L and 758.8 ± 732.1 μg/L respectively with 64.5% of the sample having serum α-tocopherol levels <0.5 mg/dL (5004.3 μg/L), qualifying as deficient. Wu et al.[142] found 32 of 34 (94.1%) full-term infants had α-tocopherol levels less than 0.5 mg/dL with a mean α-tocopherol level at birth of 0.212 ± 0.127 mg/dL (2121.8 ± 1271 μg/L). In the same study, preterm infants (GA >32 wk) had mean serum α-tocopherol levels of 0.176 ± 0.091 mg/dL (1761.5 ± 910.8 μg/L).

As discussed previously, the exact mechanism of placental vitamin E transfer remains unknown and neonatal vitamin E levels are generally only 1/6 to 1/2 those of their mothers.[108,150] Conversely, our data show neonatal α-tocopherol levels, when comparing cord blood to maternal serum levels, to be 77% while neonatal γ-tocopherol was approximately 1/2 that of maternal serum at 58%. However, when comparing medians of maternal serum and cord blood α- and γ-tocopherol, percentages drop to 47.5% and 48.3% respectively. As expected, all correlation coefficients showed no significant association between mother and infant plasma levels. However, these levels were not adjusted for total lipids.

In our study, we did not find a significant correlation between gestational age and serum α-tocopherol levels as opposed to other findings from Chang and Wu.[138,142] who found weak, but significant correlations. In agreeance with Chan et al.,[138] a lack of association was seen between maternal weight and α-tocopherol, but a significant association was seen between maternal weight and γ-tocopherol.

MATERNAL VITAMIN E INTAKE

Median maternal α-tocopherol intake without supplementation was 9.1 ± 7.8 mg/day. This value is less than the EAR (12 mg/day) and RDA (19 mg/day) for pregnant women. Our sample had higher intakes of vitamin E than Scholl, et al.[116] found in women in Camden, NJ (7.19 ± 0.13 mg α-tocopherol per day) but less than Chappell, et al.[169] found in Ontario, Canada (15.0 ± 6 mg/day). Low levels of α-tocopherol intake in the United States may be explained, in part, by the increased intake of γ-tocopherol, which is the predominant isoform of vitamin E in soy oil and processed foods. Median intakes of α-tocopherol less than the EAR is concerning due to association between maternal concentration and fetal growth and the potential relationship of maternal intake and level of α-tocopherol in human milk. Breastmilk can increase oxidative stress index to newborns if serum vitamin E levels are low.[184] Therefore, it is critical for women to have adequate α-tocopherol levels in breast milk to protect infants from deficiency and the effects of low antioxidant capacity.

Median α- and γ-tocopherol levels from a sample of mothers' breast milk samples were 8216.9 ± 4883.2 μg/L and 840.4 ± 458.8 μg/L, respectively. These values were lower than that reported by Chappell et al.[169] in preterm mothers' breast milk (α-tocopherol: 11 ± 2.5 μg/ml; γ-tocopherol: 1.5 ± 0.4 μg/L). Median α-tocopherol levels in colostrum were also lower than reported by da Silva, et al.[141] (16810 μg/L) but were consistent with reported Western levels (3-8 mg/L). However, when only sampling women who had met the EAR for v α-tocopherol in the FFQ, median α, and γ-tocopherol levels increase to 9578.0 and 1790.4 μg/L, respectively.

IMPROVING VITAMIN E STATUS

Adequate vitamin E intake is required in infants to prevent vitamin E deficiency, which is characterized by hemolytic anemia, edema, thrombocytosis, and rarely spinocerellar degeneration.[12] Prior to our study, multiple studies have shown pre-term and term infants to be clinically deficient in α-tocopherol at birth. Exclusive breastfeeding can be a strategy against vitamin E deficiency as studies have shown milk excreted until postnatal day 4 (colostrum) is particularly rich in α-tocopherol, supplying infants with their vitamin E requirement, essential for preventing deficiency.[176]

In our study, 64.5% of the infant population had deficient serum levels of vitamin E. Interestingly, median serum levels of α-tocopherol did not rise above 0.5 mg/dL after 3 days stable on infant feeds with only breast milk. However, both infant formula and mixed modality feeds reached that level. Multiple explanations could be offered to explain the result such as increased nutrient provision and vitamin E supplementation in formula and mixed modality feeds. Of note, with 3 days stable on breastmilk, mean serum γ-tocopherol was found to be 370.6 µg/L while mean levels of infants stable on formula or mixed modality feeds were 1107.8 and 1203.0 µg/L respectively. Further research regarding the effect of serum levels of γ-tocopherol on inflammation are needed to evaluate indications of use in the NICU. Additionally, further research is needed to re-evaluate the recommended dose of supplemental vitamin E for infants on breastmilk alone as a feeding modality to achieve serum levels above 0.5 mg/dL.

Based on the available evidence from a systematic review, the vitamin E dose recommended by the American Society for Clinical Nutrition (2.8-3.5 IU/kg/day) should remain the current standard, However, researchers have concluded that the current "best" pharmacologic dose of vitamin E supplementation is unclear.[182] Bell et al.[180] examined a single enteral dose of α-tocopherol while Kositamongkol et al.[139] studied infants with parenteral nutrition with multivitamin supplementation started within 24-48 hours of life with enteral feeding initiated as soon as clinically stable. Both studies

concluded a higher dose of α-tocopherol supplementation may need to be considered, especially for those who are at risk for deficiency.

LIMITATIONS

There are limitations to this study that should be taken into consideration. The overall sample size of 34 mother-infant pairs was relatively small and a larger sample size would have been beneficial for analysis. Of the 34 mother-infant pairs enrolled, serum samples from three mothers and cord blood samples from two infants were not collected. 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. In addition, it has been proposed that the best indicator of vitamin E status is the ratio of tocopherol to circulating lipids.[131] This study did not adjust for lipids, which may have produced slightly different results.

21 of 34 mothers completed FFQ with only 20 analyzed. Almost half of the mothers did not complete a FFQ, making it difficult to draw solid conclusions related to the intake of vitamin E intake of the population. As with other intake assessment forms, self-reporting can lead to both under or overestimations of intake.

CHAPTER 6: CONCLUSION

This is one of the first studies to evaluate the effect of feeding modality on infant serum α- and γ-tocopherol levels. Previously, studies have studied the effect of colostrum and maternal breast milk on infant vitamin E tocopherols with a main focus on serum α-tocopherol levels post-partum and the difference between pre-term and term infant levels. This prospective study found results consistent with previous research as maternal serum levels, maternal intake, gestational age, and birth weight were not significantly correlated with infant serum α- and γ-tocopherol levels. Additionally, prevalence of vitamin E deficiency was high in mothers and infants which is concerning due to the detrimental side effects of low levels. Further research is needed to detect the mechanism of transport of serum tocopherols to the fetus and to determine safe supplementation dosages of infants at risk of vitamin E deficiency.

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