Infants discriminate between natural and synthetic vitamin E\textsuperscript{1–4}

William L Stone, Irene LeClair, Terry Ponder, Geraldine Baggs, and Bridget Barrett Reis

ABSTRACT

**Background:** In adults, \textit{RRR}-\textalpha-tocopheryl acetate (natural vitamin E) has approximately twice the biological activity of \textit{all-rac-\textalpha-tocopherol} (synthetic vitamin E). Similar studies have not been done in term infants.

**Objective:** We evaluated the vitamin E and antioxidant status of term infants fed formulas differing in the amount and form of vitamin E.

**Design:** A controlled, blinded, multisite study was completed with 77 term infants randomly assigned to 1 of 3 different infant-formula groups. The HIGHNAT-E formula (\(n = 26\)) contained 20 IU \textit{RRR}-\textalpha-tocopheryl acetate/L (14.5 mg/L), the LOWNAT-E formula (\(n = 25\)) contained 10 IU \textit{RRR}-\textalpha-tocopheryl acetate/L (7.3 mg/L), and the SYN-E formula (\(n = 26\)) contained 13.5 IU synthetic \textit{all-rac-\textalpha-tocopherol} acetate/L (13.5 mg/L). A human milk–fed group (\(n = 29\)) served as a reference.

**Results:** Although the LOWNAT-E formula contained only one-half the concentration of \textit{\alpha-tocopherol} that the SYN-E formula did (7.3 compared with 13.5 mg/L), the infants fed the LOWNAT-E formula had plasma \textit{\alpha-tocopherol} concentrations that were not significantly different from those of the infants fed the SYN-E formula. However, \textit{\alpha-tocopherol} intakes in the study population, when expressed as mg \textit{2R-tocopherol} isomers consumed/d, correlated with plasma \textit{\alpha-tocopherol} (\(r = 0.20, P = 0.02\)) and the ratio of plasma \textit{\alpha-tocopherol} to lipids (\(r = 0.19, P = 0.03\)). There were no significant differences in antioxidant status between the 3 groups, but the LOWNAT-E group showed a trend toward lower plasma isoprostanes.

**Conclusions:** This study supports the new definition for vitamin E given in the 2000 Dietary Reference Intakes and suggests that infants discriminate between \textit{RRR-\textalpha-tocopheryl acetate} and \textit{all-rac-\textalpha-tocopheryl acetate}. All 3 infant formulas supported adequate vitamin E status. Am J Clin Nutr 2003;77:899–906.

**KEY WORDS** Infants, biological activity, \textit{RRR-\textalpha-tocopherol}, \textit{all-rac-\textalpha-tocopherol}, \(\gamma\)-tocopherol, plasma tocopherols, red blood cell tocopherols, 8-\textit{epi}-prostaglandin \(F_{2\alpha}\), isoprostanes

INTRODUCTION

Animal and human studies show that the biological activity of tocopherols is dependent on their particular stereochernistry and chemical form (1–8). Tocopherols have a saturated phytyl group attached to the 2 position of the chromanol ring. Natural vitamin E (2\textit{R},4\textit{R},8\textit{R}-\textalpha-tocopherol or more simply, \textit{RRR-\textalpha-tocopherol}) has a particular stereochernistry in which the methyl groups in the 2, 4\textsuperscript{\textbeta}, and 8\textsuperscript{\textbeta} positions are all in the \(R\) configuration. For synthetic vitamin E (\textit{all-rac-\textalpha-tocopherol}), the configuration at the 2, 4\textsuperscript{\textbeta}, and 8\textsuperscript{\textbeta} positions can be \(R\) or \(S\). Natural vitamin E is a single stereoisomer, whereas synthetic vitamin E contains equimolar amounts of 8 isomers, half of which are \(2R\) isomers.

Discrimination between synthetic and natural forms of vitamin E appears not to occur during absorption but rather as a postabsorptive phenomenon in the liver (1, 7). A hepatic \textalpha-tocopherol transfer protein (\textalpha-TTP) selectively transfers \textit{RRR-\textalpha-tocopherol} from the liver to newly secreted VLDL (9, 10). Research in adults shows that VLDL secreted by the liver contains an amount of \textit{RRR-\textalpha-tocopherol} that is 4-fold that of 2\textit{S},4\textit{R},8\textit{R}-\textalpha-tocopherol (or \textit{SRR-\textalpha-tocopherol}) (7). \textit{RRR-\textalpha-tocopherol} does not have natural (ie, \textit{RRR}) stereochernistry but only differs at the 2 position where the phytyl tail and ring meet. Natural vitamin E is also preferentially taken up by plasma and red blood cells (RBCs) (1). Traber et al (11) also found preferential urinary excretion of \textit{all-rac-\textalpha-tocopherol} over that of \textit{RRR-\textalpha-tocopherol}.

In recent years, it has become necessary to evaluate the relative biological potencies of different forms of vitamin E. This is largely because of the elucidation of the role of \textalpha-TTP. The plasma concentrations of different forms of vitamin E are critically dependent on their affinity for \textalpha-TTP (9, 10, 12, 13). Based on these affinities, the biological activity of \textit{all-rac-\textalpha-tocopherol} is one-half that of \textit{RRR-\textalpha-tocopherol} (2, 14). This has been confirmed in adults, but similar studies have not been done in infants.

The new definition for vitamin E provided in the 2000 Dietary Reference Intakes (DRIs) (15) is primarily based on kinetic studies showing that although \textit{RRR-\textalpha-tocopherol} concentrations are maintained in human plasma, the same is not true for synthetic \textit{SRR-\textalpha-tocopherol} (7, 15). Traber (12) reviewed the determinants of plasma vitamin E concentrations. \textalpha-TTP requires the \(R\) configuration at the 2 position of tocopherol. The 2\textit{R} stereoisomeric forms of \textalpha-tocopherol (ie, \textit{RRR-}, \textit{RSR-}, \textit{RRS-}, and \textit{SSS-\textalpha-tocopherol}) are now used to estimate the vitamin E requirement, whereas the 2\textit{S} isomers (\textit{SRR-}, \textit{SSR-}, \textit{SRS-}, and \textit{SSS-\textalpha-tocopherol}) are not.

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The official United States Pharmacopoeia (16, 17) states that 1 IU vitamin E has the activity of 1 mg all-rac-α-tocopherol acetate or 0.74 mg RRR-α-tocopherol acetate. This definition is primarily based on rat fetal resorption assays conducted in the 1940s (3, 18). The United States Pharmacopoeia unit was, however, defined before the α-TTP and kinetic studies cited above. The 2000 DRI report (15) suggests that the United States Pharmacopoeia system be redefined on the basis of what actually contributes to the active form of vitamin E in humans, ie, the 2R isomers. In the present article, when doses are expressed as mg 2R isomers, they were calculated by using this new definition, ie, 1 IU RRR-α-tocopherol acetate is equivalent to 0.67 mg 2R isomers, and 1 IU all-rac-α-tocopherol acetate is equivalent to 0.47 mg 2R isomers.

It is well established that vitamin E functions in vivo as an antioxidant protecting lipids against peroxidative damage (19, 20). Work by Shahal et al (21) showed that RBCs from newborns are more sensitive to in vitro oxidative stress than are RBCs from adults. Most of the intracellular hemoglobin in neonatal RBCs is hemoglobin F, which has a greater tendency to denature and oxidize than does hemoglobin A. Denatured, oxidized hemoglobin is a potent catalyst for lipid peroxidation.

The detection and quantification of lipid peroxidation in vivo remain imprecise. However, a substantial body of evidence suggests that quantification of F2-isoprostanes, in particular 8-epi-prostaglandin F2α, represents a reliable and useful approach for assessing lipid peroxidation and oxidant stress in vivo (22–24). These unique prostaglandin-like compounds are produced in vivo by a non-cyclooxygenase mechanism involving peroxidation of arachidonic acid.

A lower concentration of plasma isoprostanes and lower vitamin E levels in plasma are thought to indicate better antioxidant status. Oxidation of α-tocopherol homologs and lower vitamin E levels in plasma contribute to the redox status (plasma ratio of α-tocopherol quinone to α-tocopherol) indicating better antioxidant status. Oxidation of α-tocopherol results in the formation of α-tocopherol quinone, which has been used to assess oxidative stress (25, 26).

The primary purpose of this research was to determine whether term infants also discriminate between RRR-α-tocopherol acetate and all-rac-α-tocopherol acetate. In this study, the effect of feeding infant formulas containing different amounts and forms of vitamin E on vitamin E and antioxidant status was assessed.

**SUBJECTS AND METHODS**

A randomized, controlled, blinded, multisite study was conducted. One hundred sixteen infants were enrolled at a total of 3 sites. Infants at each site were randomly assigned to 1 of 3 formula groups. Forty breast-fed infants from the same 3 sites were studied concurrently as a reference group.

Written informed consent was obtained from the parent or guardian of each infant before any study formulas were fed or any data were collected. The study was performed in accord with the Helsinki Declaration of 1975 as revised in 1983. Infants were in good health, were full term at birth (ie, had a gestational age of 37–42 wk), and had weight and length that were appropriate for their gestational age (ie, weight and length between the 5th and 95th percentiles for data from the National Center for Health Statistics). All infants had Apgar scores ≥ 5 at 1 min, had no signs of birth asphyxia, and showed no evidence of cardiac, gastrointestinal, or respiratory disease. Infants with any systemic disease or a family blood group incompatibility were excluded from the study. Infants in formula groups did not receive any human milk feeding.

Weight and length were recorded (at 1 and 2 mo of age), and blood samples were obtained for biochemical measurements.

Infants exited the study if they were no longer consuming the study product as their sole source of nutrition. Thus, only infants who were adhering to the feeding protocol at the time of the visit had their blood drawn. Biochemical data were analyzed from all study subjects who completed ≥ 1 mo of the feeding protocol and had ≥ 1 blood draw at either month 1 or month 2.

A parent or guardian agreed to provide the infant with the assigned study formula ad libitum as the sole source of nutrition for ≥ 2 mo. Water was given ad libitum as well. Formula consumption was recorded on the daily intake records by the parents for the 3 d preceding the study visits at 1 and 2 mo of age. Daily intake of vitamin E (expressed per mg) was calculated from the daily volume of formula intake by using the new definition for vitamin E forms specified by the DRIs (15).

**Study formulas**

Infants (n = 116) were randomly assigned to 1 of the following 3 formula groups within 8 d after birth: 1) Similac with Iron Ready to Feed (Ross Laboratories, Columbus, OH) containing 20 IU natural vitamin E (RRR-α-tocopheryl acetate)/L (14.5 mg RRR-α-tocopherol acetate/L, equivalent to 13.4 mg 2R-tocopherol isomers/L) (HIGHNAT-E group), 2) Similac with Iron Ready to Feed containing 10 IU RRR-α-tocopherol acetate/L (7.3 mg RRR-α-tocopheryl acetate/L, equivalent to 6.7 mg 2R-tocopherol isomers/L) (LOWNAT-E group), or 3) Enfamil with Iron Ready to Feed (Mead Johnson, Evansville, IN) containing 13.5 IU of the synthetic form of vitamin E (all-rac-α-tocopheryl acetate)/L (13.5 mg all-rac-α-tocopherol acetate/L, equivalent to 6.1 mg 2R-tocopherol isomers/L) (SYN-E group). At the time the study was conducted, all commercially available infant formulas contained only the synthetic form of vitamin E. Thus, the infants fed the SYN-E formula were considered the control group. All 3 study formulas were clinically labeled and identified with a 6-digit alphanumeric code. The primary investigator, the study staff, and the families were not informed of the identity of the study formulas during the clinical study. Infants were not to receive any vitamin or mineral supplementation except fluoride, if prescribed.

The compositions of the 3 study formulas were very similar, ie, 15 g protein/L, 36 g fat/L, and 73 g carbohydrate/L (Table 1). The Similac formulas contained by wt 40% monounsaturated fatty acids, 24% polyunsaturated fatty acids, and 35% saturated fatty acids, and the Enfamil formula contained by wt 37% monounsaturated fatty acids, 20% polyunsaturated fatty acids, and 42% saturated fatty acids. The amount of iron in the Enfamil formula was identical to that in the Similac formulas. All study formulas met or exceeded the nutrient levels recommended by the Committee on Nutrition of the American Academy of Pediatrics and required by the Infant Formula Act.

**Biochemical measurements**

All biochemical measurements in this study, except those for cholesterol and triacylglycerol, were performed at a single laboratory.

**Isolation of red blood cells and plasma**

Approximately 2 mL blood was drawn into an evacuated tube containing 3.6 mg K3-EDTA. Samples were centrifuged at 2600 × g and 4 °C for 15 min. Plasma (≈ 0.8 mL) was removed, and 8 μL propyl gallate (1 mg/mL) was added to the plasma samples as a preservative before they were frozen at −70 °C. For
RBCs, theuffy coat was removed and the remaining cells were washed 2 times with phosphate-buffered saline (pH 7.4). The final RBC pellet was suspended in 1 volume of phosphate-buffered saline to give a 50% suspension (total volume = 0.6 mL). A 6-μL aliquot of propyl gallate (1 mg/mL) was added to the RBC suspension. The RBC hemocrit was determined with a microcapillary centrifuge. The RBC suspension was stored at −70°C.

Plasma tocopherols and α-tocopheryl quinone

Tocopherols (α-tocopherol and γ-tocopherol) and α-tocopheryl quinone were measured by a modification of the HPLC–electrochemical detection technique described by Murphy et al (27) with the use of a 3-μm C18 reversed-phase column (80 mm × 4.6 mm inside diameter) and an ESA Coulochem II Model 5200A electrochemical detector (ESA, Chelmsford, MA). The reducing electrode was set to −600 mV (to reduce any tocopheryl quinone), and the downstream detection electrode was set at 400 mV. The mobile phase consisted of 20 mmol ammonium acetate/L and 1 mmol EDTA/L in a methanol:water (90:10, vol:vol) solvent (all HPLC grade). Tocol was used as an internal standard. A PE Nelson 900 Series (Perkin-Elmer, San Jose, CA) analog-to-digital interface was used to acquire data that were then analyzed with the Turbochrom Chromatography Workshop Software (Perkin-Elmer) installed in a Dell OptiPlex 133 MHZ Pentium computer (Dell, Austin, TX).

Red blood cell tocopherols

RBC tocopherols were extracted and analyzed by the HPLC method reported by Mino et al (28) with the use of ascorbate and pyrogallol as preservatives. Tocol was used as an internal standard. The tocopherols were eluted on a reversed-phase Altex-Ultraphase-ODS column (4.6 mm inside diameter × 25 cm; Beckman Instruments, Inc, Fullerton, CA) with methanol:water (99.5:0.5, vol:vol). A McPherson Model FL-750 fluorescence detector (McPherson Instrument, Chelmsford, MA) was used with 294 nm for excitation and 324 nm for emission.

Plasma cholesterol and triacylglycerol

Plasma cholesterol and triacylglycerol concentrations were measured enzymatically by the Clinical Chemical Department at Ross Products Division, Abbott Laboratories, with the use of a clinical chemistry analyzer (Abbott Spectrum Epx; Abbott Laboratories, Abbott Park, IL). These values were analyzed to normalize plasma tocopherol concentrations to lipid concentrations (cholesterol + triacylglycerol) as suggested by Van Zoeren-Grobben et al (29).

Isoprostanes

Plasma isoprostane concentrations were used to assess oxidative stress. A competitive enzyme immunoassay kit from Cayman Chemical Co (catalog number 516351; Ann Arbor, MI) was used to measure plasma 8-epi-prostaglandin F2α concentrations.

Statistical analysis

All statistical analyses were done for the 3 formula groups; the data for the breast-fed group are shown as reference data. The null hypothesis of no difference between the 3 formula groups was tested by using repeated-measures analysis of variance with SAS PROC MIXED (version 6.09e; SAS Institute Inc, Cary, NC) over the entire study period. All tests were carried out at a 0.05 significance level with research site included as a blocking factor. When a main effect or an interaction was found to be significant, pairwise multiple comparisons were made by using Tukey-Kramer adjustment of the P values. Pearson’s chi-square or Fisher’s exact test was used for categorical variables. The Pearson’s product-moment correlation coefficient between the 2R stereoisomer intake and plasma concentrations was calculated by using the combined data obtained from both study visits.

RESULTS

Study population

There were 116 infants enrolled and randomly assigned: 37 to the HIGHNAT-E group, 40 to the LOWNAT-E group, and 39 to


The SYN-E group. Of the 116 infants, 39 exited the study early and thus were not included in the analyses. Among the HIGHNAT-E, LOWNAT-E, and SYN-E groups, 26, 25, and 26 infants, respectively, adhered to the feeding protocol and had ≥1 blood draw during the study. There were no differences between the formula groups in the number of infants who exited the study. An additional group of human milk–fed infants (n = 29) served as a reference group.

Entrance, demographic, anthropometric, and formula-intake data

No significant differences between the HIGHNAT-E, LOWNAT-E, and SYN-E groups in sex, race, gestational age, birth weight, birth length, and age at study entry were detected at enrollment (Table 2). No significant differences in formula intake were observed between the groups during the study. The HIGHNAT-E group had a higher mean (± SD) intake of 2R-tocopherol isomers (10.6 ± 0.30 mg/d) than did the LOWNAT-E group (5.5 ± 0.20 mg/d) and the SYN-E group (5.1 ± 0.14 mg/d). The intake of 2R-tocopherol isomers correlated with plasma α-tocopherol concentration (r = 0.20, P = 0.02) and the plasma ratio of α-tocopherol to lipid (r = 0.19, P = 0.03). Plots of individual plasma α-tocopherol concentrations versus daily intakes are shown in Figures 1 and 2.

Vitamin E status in term infants

Consistent differences between the groups were found with respect to vitamin E status (Table 3). Plasma α-tocopherol concentrations in the HIGHNAT-E group were significantly higher than those in the SYN-E group (least-squares x: 30 compared with 25 μmol/L; P = 0.010). When total plasma lipid concentrations (ie, the sum of plasma cholesterol and triacylglycerol concentrations) were used to normalize plasma vitamin E concentrations, plasma ratios of α-tocopherol to lipids in the HIGHNAT-E group were significantly higher than those in the LOWNAT-E and SYN-E groups (least-squares x: 6.3 compared with 5.3 and 5.3 μmol/mmol,

![FIGURE 1. Dietary intakes of 2R-tocopherol isomers versus plasma α-tocopherol concentrations.](#)

![FIGURE 2. Dietary intakes of 2R-tocopherol isomers versus plasma ratios of α-tocopherol to lipids.](#)
TABLE 3
**α-Tocopherol status of term infants**

<table>
<thead>
<tr>
<th></th>
<th>Vitamin E formula groups</th>
<th>Breast-fed reference group</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>HIGHNAT-E</td>
<td>LOWNAT-E</td>
</tr>
<tr>
<td>Plasma α-tocopherol (μmol/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 mo</td>
<td>29 ± 2.0 [26]</td>
<td>25 ± 1.8 [25]</td>
</tr>
<tr>
<td>2 mo</td>
<td>33 ± 1.7 [21]</td>
<td>29 ± 1.5 [23]</td>
</tr>
<tr>
<td>Plasma α-tocopherol:lipo (μmol/mmol)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 mo</td>
<td>6.45 ± 0.5 [22]</td>
<td>5.43 ± 0.3 [22]</td>
</tr>
<tr>
<td>2 mo</td>
<td>6.15 ± 0.3 [20]</td>
<td>5.30 ± 0.3 [23]</td>
</tr>
<tr>
<td>RBC α-tocopherol (μg/dL)</td>
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</table>

*α ± SEM; n in brackets. The primary analysis was a repeated-measures ANOVA controlled for site. The breast-fed reference group was not included in the statistical analyses. HIGHNAT-E, Similac with Iron (SWI; Ross Laboratories, Columbus, OH) formula containing 20 IU natural vitamin E/L; LOWNAT-E, SWI formula containing 10 IU natural vitamin E/L; SYN-E, Enfamil with Iron (Mead Johnson, Evansville, IN) formula containing 13.5 IU synthetic vitamin E/L; RBC, red blood cell.

**Antioxidant status in term infants**

There were no significant differences in antioxidant status between the 3 groups as assessed by isoprostane concentrations (8-epi-prostaglandin F$_2$-x) or vitamin E redox status (plasma ratio of α-tocopheryl quinone to α-tocopherol) (Table 5). However, there was a trend for lower isoprostane concentrations in the LOWNAT-E group than in the SYN-E group (least-squares $\bar{x}$: 331 compared with 415 pg/mL; $P = 0.09$).

DISCUSSION

In this study, natural vitamin E (RRR-α-tocopheryl acetate) administered at 7.3 mg/L resulted in a plasma α-tocopherol concentration equal to that obtained with synthetic vitamin E (all-rac-α-tocopherol acetate) administered at 13.5 mg/L. However, the amounts of 2R-tocopherol isomers delivered were very similar, ie, 6.7 and 6.1 mg/L.
for natural and synthetic vitamin E, respectively. Our study, therefore confirms the new definition for vitamin E given in the 2000 DRI report (15) as well as previous work showing that the relative activity of natural vitamin E to synthetic vitamin E is higher than the accepted 1.36, which was based on rat fetal resorption assays (18).

Human studies using equimolar concentrations of deuterated natural and synthetic vitamin E acetate showed that the biological activity of natural vitamin E is roughly twice that of synthetic vitamin E (2, 14, 30). For example, in one study, pregnant women were given different amounts of vitamin E in a capsule that contained both the natural and synthetic forms for 5 d before giving birth (30). At delivery, the researchers found nearly twice the concentration of natural vitamin E than of synthetic vitamin E in the mothers’ own blood and nearly 3.5 times the concentration of natural vitamin E than of synthetic vitamin E in their placental cord blood, regardless of the amount given. This study suggested that human placenta could deliver natural vitamin E to the fetus significantly more efficiently than it could synthetic vitamin E.

For infants in all 3 formula groups and the breast-fed group, vitamin E status was within normal reference ranges. A plasma tocopherol concentration of < 11.6 μmol/L is suggestive of vitamin E deficiency, and a concentration > 80 μmol/L is associated with toxicity (31). Mean plasma α-tocopherol concentrations in infants in all 3 formula groups ranged from 25 to 33 μmol/L. The breast-fed group had somewhat lower mean plasma α-tocopherol concentrations (18–20 μmol/L) than did the formula-fed groups. In addition, mean plasma ratios of α-tocopherol to lipids in the 3 formula groups and the breast-fed group were > 1.68 μmol/mmol, a value considered to indicate deficiency in adults (26).

The breast-fed group had lower RBC α-tocopherol concentrations (110–143 μg/dL) than did the formula-fed groups (230–288 μg/dL). The mean RBC α-tocopherol concentration in the breast-fed group remained below the normal adult concentration of < 200 μg/dL (12, 21). We speculate that the secretion of vitamin E into breast milk further discriminates between natural and synthetic vitamin E carried in plasma lipoproteins.

The HIGHNAT-E and LOWNAT-E formulas contained approximately the same concentration of γ-tocopherol, which was in the vegetable oils that were in both formulas. The calculated concentrations of γ-tocopherol from the vegetable oils in the NAT-E and SYN-E formulas were ~7.9 and ~5.6 mg/L, respectively. Although the γ-tocopherol concentrations in the 2 NAT-E formulas were the same, the LOWNAT-E group had higher concentrations of γ-tocopherol in plasma (expressed per liter or per millimoles lipid.

### Table 5

<table>
<thead>
<tr>
<th>Plasma γ-tocopherol:α-tocopherol&lt;sup&gt;2&lt;/sup&gt;</th>
<th>HIGHNAT-E</th>
<th>LOWNAT-E</th>
<th>SYN-E</th>
<th>Breast-fed reference group</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mo</td>
<td>0.091 [26]</td>
<td>0.144 [25]</td>
<td>0.111 [24]</td>
<td>0.145 [29]</td>
</tr>
<tr>
<td>2 mo</td>
<td>0.079 [21]</td>
<td>0.108 [23]</td>
<td>0.080 [21]</td>
<td>0.128 [25]</td>
</tr>
</tbody>
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<tr>
<th>RBC γ-tocopherol:α-tocopherol&lt;sup&gt;3&lt;/sup&gt;</th>
<th>HIGHNAT-E</th>
<th>LOWNAT-E</th>
<th>SYN-E</th>
<th>Breast-fed reference group</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mo</td>
<td>0.061 [21]</td>
<td>0.101 [25]</td>
<td>0.098 [21]</td>
<td>0.154 [17]</td>
</tr>
<tr>
<td>2 mo</td>
<td>0.054 [17]</td>
<td>0.072 [21]</td>
<td>0.067 [18]</td>
<td>0.147 [14]</td>
</tr>
</tbody>
</table>

<sup>1</sup> SE; <sup>2</sup>n in brackets. The breast-fed reference group was not included in the statistical analyses. HIGHNAT-E, Similac with Iron (SWI; Ross Laboratories, Columbus, OH) formula containing 20 IU natural vitamin E/L; LOWNAT-E, SWI formula containing 10 IU natural vitamin E/L; SYN-E, Enfamil with Iron (Mead Johnson, Evansville, IN) formula containing 13.5 IU synthetic vitamin E/L; RBC, red blood cell.

<sup>3</sup> Repeated-measures ANOVA indicated an effect over the entire study period: LOWNAT-E > HIGHNAT-E and SYN-E; least-squares means, 0.125 compared with 0.084 and 0.096, respectively; <i>P</i> = 0.0001.

### Table 6

Antioxidant status of term infants: α-tocopherol quinone (α-TQ) and isoprostanes at 1 and 2 mo<sup>4</sup>

<table>
<thead>
<tr>
<th>Vitamin E formula groups</th>
<th>HIGHNAT-E</th>
<th>LOWNAT-E</th>
<th>SYN-E</th>
<th>Breast-fed reference group</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-TQ:α-tocopherol&lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>1 mo</td>
<td>0.0074 ± 0.001 [25]</td>
<td>0.0072 ± 0.001 [25]</td>
<td>0.0056 ± 0.001 [23]</td>
<td>0.0077 ± 0.001 [29]</td>
</tr>
<tr>
<td>2 mo</td>
<td>0.0046 ± 0.0001 [21]</td>
<td>0.0041 ± 0.0004 [23]</td>
<td>0.0046 ± 0.001 [21]</td>
<td>0.0050 ± 0.001 [25]</td>
</tr>
<tr>
<td>Isoprostanes (pg/mL)&lt;sup&gt;3,4&lt;/sup&gt;</td>
<td></td>
<td></td>
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</tbody>
</table>

<sup>1</sup> ± SEM; <sup>2</sup>n in brackets. The primary analysis was a repeated-measures ANOVA controlled for site. The breast-fed reference group was not included in the statistical analyses. HIGHNAT-E, Similac with Iron (SWI; Ross Laboratories, Columbus, OH) formula containing 20 IU natural vitamin E/L; LOWNAT-E, SWI formula containing 10 IU natural vitamin E/L; SYN-E, Enfamil with Iron (Mead Johnson, Evansville, IN) formula containing 13.5 IU synthetic vitamin E/L.

<sup>2</sup> α-TQ is an oxidized product of vitamin E. The plasma ratio of α-TQ to α-tocopherol is an index of the relative amount of oxidized α-tocopherol. This ratio is referred to as the vitamin E redox status.

<sup>3</sup> Formed in vivo by free radical oxidation of arachidonyl-containing lipids; plasma isoprostane concentrations have been suggested to be indicative of in vivo oxidative stress.

<sup>4</sup> Repeated-measures ANOVA indicated that LOWNAT-E tended to be < SYN-E over the entire study period; least-squares means, 329 compared with 406 pg/mL; <i>P</i> = 0.09.
and RBCs than did the HIGHNAT-E group. Although these differences were not significant, the lower \( \gamma \)-tocopherol concentrations in the HIGHNAT-E group may be explained by the higher intake of \( \alpha \)-tocopherol in this group. The results of this infant study agree with the results of adult studies showing that a high dietary intake of \( \alpha \)-tocopherol decreases the \( \gamma \)-tocopherol concentration in blood (32, 33). This is most likely due to the high affinity of \( \alpha \)-TTP for \( \alpha \)-tocopherol (13).

Compared with the SYN-E formula, the LOWNAT-E formula contained a similar concentration of \( 2R \)-tocopherol isomers but a higher concentration of \( \gamma \)-tocopherol. Conversely, compared with the HIGHNAT-E formula, the LOWNAT-E formula contained the same concentration of \( \gamma \)-tocopherol but only one-half the concentration of \( 2R \)-tocopherol isomers. Note that the HIGHNAT-E group had significantly higher ratios of \( \gamma \)-tocopherol to \( \alpha \)-tocopherol and significantly lower isoprostane concentrations than did the other 2 formula groups. This suggests that the relation between \( \gamma \)-tocopherol and \( \alpha \)-tocopherol (expressed as a ratio) may play an important role in antioxidant activity; however, this suggestion is highly speculative.

Although \( \gamma \)-tocopherol has not been considered an important in vivo antioxidant, evidence suggests that it has some unique biochemical properties not shared with \( \alpha \)-tocopherol (20, 32, 34, 35). For example, Jiang et al (36) found that \( \gamma \)-tocopherol, in contrast to \( \alpha \)-tocopherol, inhibits cyclooxygenase activity in macrophages and epithelial cells. At physiologic levels, \( \gamma \)-tocopherol may, therefore, have an antiinflammatory role and be important in disease prevention (36). It has been suggested that under in vivo conditions in animals, in which the vitamin E in biological membranes and lipoproteins exists almost entirely as \( \alpha \)-tocopherol and \( \gamma \)-tocopherol, the total vitamin E antioxidant activity can be accounted for by \( \alpha \)-tocopherol and \( \gamma \)-tocopherol (31).

The findings in this study relative to biomarkers of antioxidant status were unexpected. We had hypothesized that infants fed the formula containing the higher dose of natural vitamin E would have better antioxidant status; however, this was not the case. Infants fed the formula containing the lower dose of natural vitamin E showed a trend toward less lipid oxidation damage as measured by plasma isoprostane concentrations. Although this result was not significant, the LOWNAT-E group had lower plasma isoprostane concentrations than did the other groups, suggesting lower oxidative stress.

The natural vitamin E study products differed slightly from the synthetic vitamin E study product in nutrient composition (Table 1). However, it is unlikely that the compositions of the study products affected the outcomes (vitamin E and antioxidant status) measured in this clinical study. The evaluation of vitamin E status most likely reflected \( \alpha \)-tocopherol intakes, because the amounts of iron in all 3 products were the same and plasma \( \alpha \)-tocopherol concentrations were adjusted for plasma lipid concentrations. In addition, compared with the infants in the SYN-E group, the infants in the LOWNAT-E group consumed a product that was higher in polyunsaturated fatty acids and lower in antioxidant nutrients (ie, vitamin C and selenium) but had significantly lower plasma isoprostane concentrations. The LOWNAT-E group would not be expected to perform as well on biomarkers used to assess antioxidant status. Moreover, none of the 3 formulas studied contained long-chain polyunsaturated fatty acids (ie, no added docosahexaenoic or arachidonic acids); thus, dietary lipid intakes would not have been expected to affect plasma isoprostane concentrations in the formula-fed groups. Conversely, human milk contains long-chain polyunsaturated fatty acids, including arachidonic acid, which may have contributed to the somewhat higher isoprostane concentrations observed in the breast-fed group.

The clinical relevance of improving antioxidant status in healthy term infants is not clearly understood. It is well known, however, that vitamin E functions as an in vivo antioxidant. Vitamin E protects against oxidative damage, which has been linked to the onset of many degenerative conditions and diseases, including atherosclerosis, cancer, cataracts, arthritis, and Alzheimer disease. The long-term benefits of improving the antioxidant status of healthy infants by nutritional interventions need further research to be elucidated.

This is the first study to suggest that infants discriminate between the \( R R R \)-\( \alpha \)-tocopherol and \( all-rac-\alpha \)-tocopherol forms of vitamin E. Although the LOWNAT-E formula contained only one-half the concentration of \( \alpha \)-tocopherol that the SYN-E formula did (7.3 compared with 13.5 mg/L), the infants fed the LOWNAT-E formula had plasma \( \alpha \)-tocopherol concentrations that were not significantly different from those of the infants fed the SYN-E formula. This suggests that the \( 2S \) stereoisomers in synthetic vitamin E are not maintained in plasma, which supports the new definition for vitamin E given in the 2000 DRI report (15). All 3 infant formulas supported adequate vitamin E status.

REFERENCES

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