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Less than adequate vitamin E status observed in a group of preschool boys and girls living in the United States☆

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Abstract

In that data were not available on the vitamin E status of young children, the aim of the study was to evaluate the vitamin E status of preschool children by three commonly used criteria: vitamin E intakes, plasma α-tocopherol concentrations and plasma α-tocopherol/total lipid ratios. Twenty-two ethnically diverse preschool children (13 males and 9 females), aged 2 to 5 years, living in Lincoln, NE, served as subjects. The subjects were in two groups: 2–3 and 4–5 years old. Energy, fat, and α- and γ-tocopherol intakes of the subjects were estimated utilizing two 24-h food recalls. Plasma α- and γ-tocopherol and total lipid concentrations were ascertained. No significant differences by age grouping or gender were observed for vitamin E intakes, plasma α-tocopherol concentrations, plasma γ-tocopherol concentrations and plasma α-tocopherol/total lipid ratios of subjects. Plasma α-tocopherol concentrations indicative of less than adequate status (<12 μmol/L) were observed in 91% of the children, and values <7 μmol/L (proposed cutoff for pediatric populations) in 68%. Sixty-eight percent of the subjects had plasma α-tocopherol/total lipid values <0.8 mg/g. The majority of the 2- to 5-year-old children included in the study had less than adequate vitamin E status.

Keywords: Vitamin E intake; Plasma α-tocopherol; Plasma γ-tocopherol; Vitamin E status; Preschool children

1. Introduction

Vitamin E functions primarily as an antioxidant. α-Tocopherol is the major form of vitamin E and represents over 90% of the total tocopherols present in plasma [1,2]. Plasma vitamin E is transported in the lipoproteins, and, as a result, plasma vitamin E concentrations are related to the concentrations of plasma lipids [3].

Data were not available from apparently healthy children for vitamin E to set an estimated average requirement (EAR) or recommended dietary allowance (RDA). The EAR and RDA for children were extrapolated from adult data based on lean body mass and the need for growth [1]. The Institute of Medicine in 2000 used milligram α-tocopherol, rather than α-tocopherol equivalents (α-TE), as used in the 1989 recommendations [4], for the dietary reference intakes based on research taking into account the premise of α-tocopherol's activity and functioning in the body [1].

The EAR for vitamin E is based on studies in which plasma tocopherol concentrations that limited the hydrogen peroxide-induced hemolysis to ≤12% were determined in vitamin E-depleted adults repleted with supplemental α-tocopherol [5–7]; assumingly, their plasma tocopherol was α-tocopherol as they were supplemented with α-tocopherol. The corresponding plasma α-tocopherol concentration was found to be ≤12 μmol/L. The hemolysis test is critically dependent on the hydrogen peroxide concentration, the amount of catalase and antioxidants in the erythrocytes, and the precise incubation conditions [1], and, generally, is not currently used as a status criterion. The EAR is used in evaluating the possibility of inadequacy of nutrient intakes of individuals; the EAR for vitamin E is 5 and 6 mg/day for children aged 1–3 and 4–8 years, respectively [1].

Vitamin E deficiency has been defined in adults as plasma concentrations of either total tocopherol or α-tocopherol <11.6 μmol/L [2,3,5,8] (assumingly α-tocopherol, as this was the vitamer used in the repletion studies) or rounded to

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<12 μmol/L [1]. The adult reference range may not be appropriate for young children. Farrell et al. [9] suggested that the "lower limit of normal" of <11.6 μmol total tocopherol/L be revised downward for pediatric populations to <7 μmol total tocopherol/L; thus, <7 μmol total tocopherol/L plasma or serum has been used as a cutoff for inadequate α-tocopherol concentrations in pediatric populations [9,10]. A plasma tocopherol/total lipid ratio (assumingly α-tocopherol/total lipid ratio as repletion was with α-tocopherol) of 0.8 mg/g has been proposed as an indicator of vitamin E status, and individuals with higher concentrations of plasma α-tocopherol would be considered to be adequate in vitamin E [3,9,10], although most researchers utilize plasma α-tocopherol concentration as the status criterion.

Few studies have been published regarding the vitamin E status of preschool children, particularly using plasma or serum α-tocopherol concentrations as the status parameter. The researchers who have published serum α-tocopherol concentrations of children of various ages [10–12] did not estimate the α-tocopherol intakes of their subjects.

Most dietary vitamin E is present in fats and oils [13]. Therefore, changes in dietary consumption to decrease fat intake may have unfavorable effects on vitamin E intake [14]. Low-fat diets can decrease vitamin E intakes, and food choices should be monitored to improve vitamin E intakes [15].

The objectives of the current study were to determine vitamin E intakes, plasma concentrations and status of a group of apparently healthy children living in the United States. Plasma α-tocopherol concentrations, plasma total lipid concentrations, plasma α-tocopherol/total lipid ratios, and dietary and total intakes of α-tocopherol were measured as well as plasma concentrations and dietary intakes of γ-tocopherol. Two age groupings of children were selected for the study: 2–3- and 4–5-year-olds. These age groupings were selected based on the 1–3 and 4–8-year age groupings the Institute of Medicine [1] utilized in making their nutrient intake recommendations.

2. Subjects and methods

2.1. Subjects

The study was approved by the University's Institutional Review Board. Twenty-two apparently healthy boys and girls, 25–71 months of age (9 girls aged 25–71 months, 13 boys aged 28–70 months), participated in the research study. Subjects were recruited from various daycare centers throughout Lincoln, NE.

2.2. Demographic and anthropometric assessments

Participant's parents completed a preexperimental questionnaire providing information regarding their child's ethnicity, age, gender, present illnesses, vitamin or mineral supplements taken, special diets of any kind, medications taken, appetite, food allergies and exposure to cigarette smoke in the home as well as the country(s) in which the parents were born [16]. Participant's heights and weights were measured in light clothing and without shoes [16]. The body mass index (BMI), also called Quetelet's index, was calculated [17,18].

2.3. Dietary assessment

The nutrient intakes of the children were calculated using two nonconsecutive weekday 24-h food recalls, separated from each other by at least 2 weeks. The 24-h recall was conducted by a trained interviewer using cross-checking techniques and food models [16]. Daycare providers were interviewed with regard to foods eaten by the child at daycare, and parents were interviewed with regard to foods eaten otherwise. A computerized nutrient database developed in our laboratory, which utilized values of the USDA Nutrient Database release 17 [19] and other published labeling information, was used in calculating nutrient intakes. When α- and γ-tocopherol content values of a food were not available, their content values for these vitamers were extrapolated from those of similar foods as was done in the USDA nutrient database [19]. The reported food intakes were evaluated for food energy, total fat, saturated fat, monounsaturated fat (MUFA), polyunsaturated fat (PUFA), cholesterol, and α- and γ-tocopherol content. Estimated nutrient intakes from the recalls were averaged and expressed on a daily basis, and intake data for the fats expressed as a percentage of caloric intake.

2.4. Blood collection

Approximately 5–10 ml of venous blood was obtained from children following an overnight fast by antecubital venipuncture between the hours of 7:00 and 9:00 a.m. by a qualified phlebotomist. Blood samples were immediately centrifuged at 4000×*g* at 5°C for 15 min. Plasma was then collected and transferred to airtight vials and stored at −70°C for future analyses. Samples were always protected from light.

2.5. Biochemical analysis

Plasma tocopherol (α-, γ-) concentrations were quantified using the HPLC method of Nierenberg and Nann [20] as modified by Sun et al. [21]. The HPLC system consisted of the following Waters Associates (Milford, MA) equipment: 600E solvent delivery system, 484 Tunable Absorbance Detector at 290 nm, 745B data integrator and a Rheodyne injector (Model #7725). The tocopherols were separated isocratically using a Microsorb-MV (5 μm, 250×4.6 mm) C_{18} column (Rainin, Woburn, MA) with a guard column of C_{18} material (4×2.0 mm id) packed with sphere-5-C_{18} (5 μm particle size). The mobile phase consisted of 65 parts acetonitrile/25 parts tetrahydrofuran/6 parts methanol/4 parts of 1% ammonium acetate. Proofs of identities and preextraction spikings were conducted. Percent recoveries were

Table 1
Ethnic groups of children and countries in which their parents were born

Age group	Ethnic group	Gender	Country in which parents were born
2–3 years	Caucasian	3 males, 5 females	USA
	Asian	3 males	2, Korea; 1, India
	Black	1 male, 1 female	1, Ethiopia; 1, Uganda
	Mixed[a]	2 males	USA/Mexico
4–5 years	Caucasian	2 males, 2 females	USA
	Latino	1 male, 1 female	1, Mexico; 1, Mexico/Guatemala
	Mixed	1 male	Mexico/Czech Republic

[a] Mixed ethnic group: parents were from more than one ethnic group.

92–94% for α-tocopherol and 92–94% for γ-tocopherol. All injections (50 μl) were made in duplicate for each plasma sample. The coefficients of variation for α- and γ-tocopherol were 5.8% and 9.7%. Plasma total lipids were quantified using a colorimetric method [22].

2.6. Statistical analysis

Data were analyzed by age grouping (2–3 and 4–5 years) and gender using the general linear model (GLM) procedure and Pearson's correlation coefficient (SAS version 8.02, SAS Institute, Cary, NC). Differences were considered significant at $P<.05$. Values are expressed as mean±S.D.

3. Results

3.1. Age and diversity

An ethnically diverse group of 22 children, 2–5 years old, were recruited for the study. The ethnic groups of the children and the countries in which their parents were born are given in Table 1. The parents of the subjects reported their children having no illnesses within the last month, consumption of special diets, medications or food allergies that may influence the status parameters. Five children were exposed to cigarette smoke in the home; however, their plasma α- and γ-tocopherol concentrations were similar to those who were not exposed.

3.2. Demographic and nutrient intake measurements

Age, height, weight, BMI and nutrient intakes of the participants are given in Table 2. As expected, height and weight measurements were significantly different ($P<.05$) by age grouping, with the 4–5-year group being heavier and taller than the 2–3-year one. Body mass index was not significantly different ($P>.05$) between age groupings. Age, height, weight and BMI values of boys were similar to those of girls, and values of Caucasians were similar to those of non-Caucasians.

Over 90% of the parents reported that the two 24-h food recalls were representative of the children's usual daily nutrient intakes. Over 80% of the parents reported that their children had good appetites. The estimated food energy, total fat, saturated fat, MUFA, PUFA, cholesterol, dietary α-tocopherol, dietary plus supplemental α-tocopherol and γ-tocopherol intakes of the subjects by age grouping are given in Table 2.

Energy intakes were significantly different ($P<.05$) between the age groupings, although no differences were observed in saturated fat, MUFA, PUFA, cholesterol, dietary α-tocopherol, dietary plus supplemental α-tocopherol and γ-tocopherol intakes; this was true whether the intake values for the various fats were expressed as gram or as a percentage of kilocalorie. There was a positive correlation between age and energy intake ($r=.519$, $P<.05$). No significant differences ($P\geq.05$) in energy, total fat, saturated fat, MUFA, PUFA, cholesterol, dietary α-tocopherol, dietary plus supplemental α-tocopherol and γ-tocopherol intakes were observed by gender. Seven of the 22 subjects,

Table 2
Anthropometric and estimated nutrient intake values of apparently healthy children, 2 to 5 years of age[a]

	2–3 years ($n=15$)	4–5 years ($n=7$)
Age (months)	36.9±5.2	62.1±6.9[b]
Height (cm)	96.9±5.0	113.7±4.8[b]
Weight (kg)	14.4±1.9	21.6±4.4[b]
BMI (kg/m^2)	15.3±1.3	16.7±3.1
Energy intake (kJ)	4805±608	5932±693[b]
Total fat (g)	42.9±14.5	50.9±8.4
Saturated fat (g)	16.6±6.8	20.4±3.3
Monounsaturated fat (g)	16.5±5.4	19.6±3.3
Polyunsaturated fat (g)	6.5±2.3	7.3±2.6
Cholesterol (mg)	136±69	216±181
Vitamin E (mg α-tocopherol)		
Dietary	4.11±1.77	4.27±1.21
Dietary+supplemental[c]	6.76±3.98	10.01±9.66
γ-Tocopherol (mg)	1.20±1.49	1.86±1.56

[a] Mean±S.D. No significant differences were observed by gender.
[b] Values for the 4–5-year-old group were significantly higher ($P<.05$) than those for the 2–3-year-old.
[c] Five 2–3 year olds and two 4–5 year olds reported taking a dietary supplement containing α-tocopherol.

Table 3
Plasma α-tocopherol, γ-tocopherol, α-tocopherol/total lipid ratio and total lipid values of apparently healthy children, 2 to 5 years of age[a]

	2–3 years ($n=15$)	4–5 years ($n=7$)
α-Tocopherol (μmol/L)	7.82±5.44[b]	5.34±2.13[c]
γ-Tocopherol (μmol/L)	2.15±1.04	1.87±1.26
α-Tocopherol/total lipid (mg/g)	0.92±0.61	0.58±0.17
Total lipid (g/L)	3.60±0.45	3.80±0.40

[a] Mean±S.D. No significant differences were observed by gender.
[b] The mean±S.D. for the 10 subjects not taking supplements containing α-tocopherol was 5.60±2.13 μmol/L, while that for the five subjects taking supplements containing α-tocopherol was 12.24±7.52 μmol/L; the values of the nonsupplemented and supplemented groups were significantly different ($P<.05$).
[c] The mean±S.D. for the five subjects not taking supplements containing α-tocopherol was 5.25±2.55 μmol/L, while that for the two subjects taking supplements containing α-tocopherol was 5.55±1.06 μmol/L; no significant differences were observed between the values of the nonsupplemented and supplemented groups.

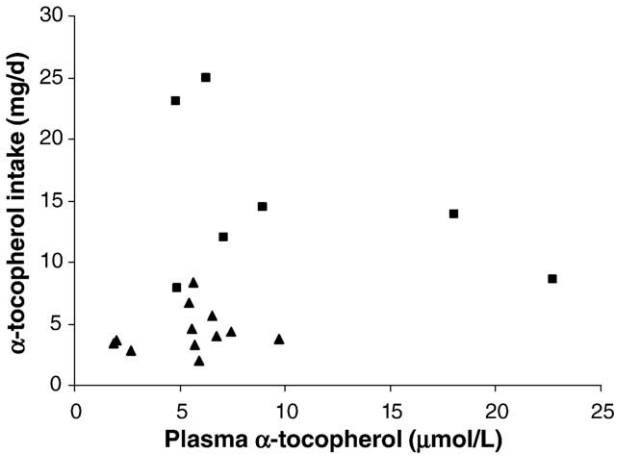

Fig. 1. Scattergram of individual α-tocopherol intakes of the children, 2–5 years old (n=22), vs. their plasma α-tocopherol concentrations. ▲=subject not taking supplement containing α-tocopherol; ■=subject taking supplement containing α-tocopherol.

5 in the 2–3-year group and 2 in the 4–5-year group, took dietary supplements containing α-tocopherol; the amount of α-tocopherol in the supplements ranged from 5 to 30 IU daily.

3.3. Plasma tocopherol and lipid measurements

Plasma α- and γ-tocopherol, total lipid and α-tocopherol/total lipid values of the subjects are given in Table 3. No significant differences (P>.05) were observed by age grouping or by gender. Plasma α-tocopherol concentrations of subjects who took dietary supplements containing α-tocopherol (9.94±7.31 μmol/L) were significantly higher (P<.05) than those not taking the supplement (5.33±1.99 μmol/L), but the plasma γ-tocopherol concentrations of those taking supplements and not taking supplements were similar (2.09±1.21 vs. 1.82±1.13 μmol/L, respectively). Individual estimated α-tocopherol intakes of the subjects plotted by their plasma α-tocopherol concentrations indicated that variation existed in the plasma α-tocopherol concentrations of subjects at specified intake levels of the vitamin (Fig. 1). Nonsignificant correlations were observed in relation to plasma concentrations of α- as well as γ-tocopherol and dietary α-tocopherol, dietary plus supplemental α-tocopherol and γ-tocopherol intakes of subjects not taking supplements, those taking supplements, and all subjects combined.

The percentages of subjects with less than adequate vitamin E status using various criteria are given in Fig. 2. Depending on which parameter values indicative of inadequacy are used, 50% to 91% of the subjects had inadequate vitamin E status.

4. Discussion

Mean weights, heights and BMI values of the children in the present study were similar to medians given in the Centers for Disease Control and Prevention revised growth charts [23] and an international survey [24]. Body mass index findings have been classified into four categories: (1) underweight, <5th percentile; (2) normal weight, 5–85th percentile; (3) at risk of being overweight, 85–95th percentile; and (4) overweight, >95th percentiles [25]. In the present study, 13.6% of the children were considered underweight; 72.7%, normal weight; 9.0%, at risk of being

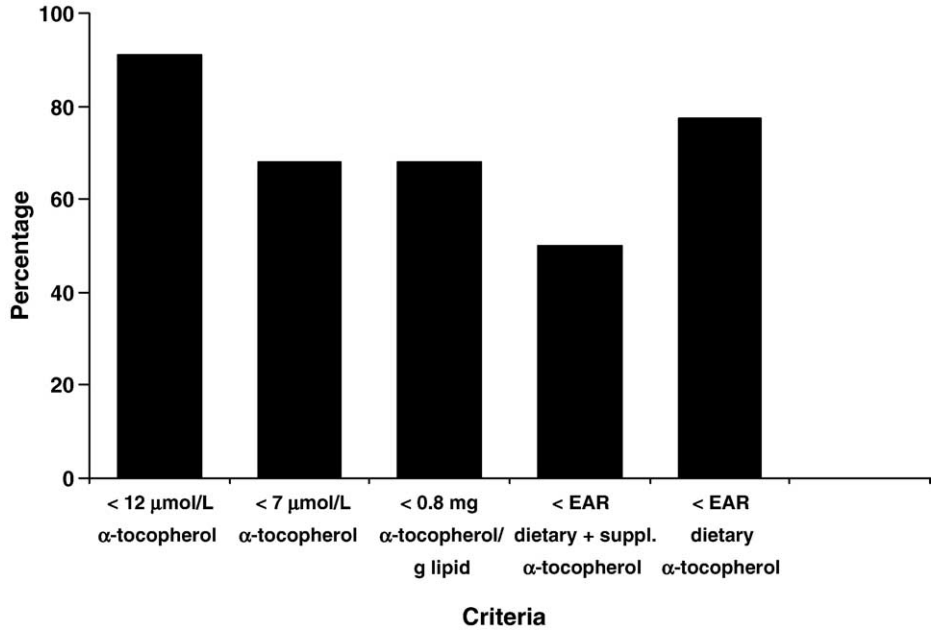

Fig. 2. Percentage of children, 2–5 years old (n=22), with less than adequate vitamin E status using various criteria. References for the criteria are as follows: <11.6 or rounded to <12 μmol/L α-tocopherol [1–3,5,8], <7 μmol/L α-tocopherol [9], <0.8 mg α-tocopherol/g lipid [3,9,10] and <EAR [1].

overweight; and 4.5%, overweight using these categories. In agreement with these two reports [23,24], distinct differences between heights and weights of the two age groupings in the current study were observed.

Mean food energy, total fat, saturated fat, MUFA, PUFA and cholesterol intakes of children in the present study were within the ranges of mean or median values reported by others [26–29]. The 2–3-year-old children in the present study had higher mean energy intakes than the estimated energy requirement (EER) [30] for 1–2-year-olds, while that for the 4–5-year group was lower than the EER for 3–8-year-olds. Mean fat contributions to food energy intakes of the 2–3- and 4–5-year-old children in the present study were 31.9% and 32.8%, respectively, and were within the acceptable macronutrient distribution range of 25% to 40% for young children [30].

Several studies have been published on the vitamin E intakes of children, generally as milligram α-TE. Since approximately 80% of the milligram α-TE from foods in the third National Health and Nutrition Examination Survey was reported to be contributed by foods containing α-tocopherol [1], 80% has been used as a correction factor in converting vitamin E intakes from milligram α-TE to milligram α-tocopherol, even though vitamin E intakes are more accurately estimated when nutrient databases are used that give the vitamin E content as α-tocopherol. Mean dietary α-tocopherol and dietary plus supplemental α-tocopherol intakes of children in the current study were in line with means or medians, usually estimated using the 80% factor, reported by others [26,31–35]. We are the first to report γ-tocopherol intakes of preschool children.

Half of the subjects in the current study had dietary plus supplemental vitamin E intakes less than the EAR, and 77% consumed less than the EAR for vitamin E with regard to dietary vitamin E (Fig. 2). The chief dietary sources of α-tocopherol of these subjects were canola oil, fortified cereals, peanut butter and chicken nuggets, while those of γ-tocopherol were corn oil, peanut butter, corn chips and corn tortillas. All seven of the subjects who took dietary supplements containing vitamin E were dependent on these dietary supplements for meeting their EAR. Fifty-eight percent of a group of toddlers aged 12–24 months reportedly had vitamin E intakes less than the EAR [32].

Although both α- and γ-tocopherols are absorbed, only α-tocopherol is preferentially secreted by the liver into the plasma for transport to the tissues, while γ-tocopherol is preferentially metabolized and excreted. This implies that the body requires α-tocopherol for some special need (yet to be elucidated), and other forms of vitamin E may not qualify [1]; however, some researchers [36] believe that measuring γ-tocopherol in vitamin E assessment may be of value. γ-Tocopherol has been proposed to be a powerful nucleophilic entity that traps electrophilic mutagens in lipophilic compartments. It may be that γ-tocopherol intake and plasma concentrations may be of importance with regard to health [37–39].

The plasma α-tocopherol concentrations of children in the current study are less than those reported for subjects 5 months–6 years [11], 0–14 years [12] and 4–5 years [10]. Reports of plasma γ-tocopherol concentrations of preschool children were not found; however, teenagers were reported to have a median plasma γ-tocopherol concentration of 0.72 μmol/L [40], which is lower than that observed in the present study.

Much individual variation was observed in plasma α-tocopherol concentrations at various intakes of α-tocopherol in preschool children in the present study. Much individual variation in α-tocopherol concentration has also been reported in rabbits fed a given quantity of α-tocopherol [21] and in young and middle-aged women and men who did not take supplements containing α-tocopherol [41].

As shown in Fig. 2, 91% of the children in the present study had plasma α-tocopherol values of <11.6 and <12 μmol/L; none of the subjects had concentrations between 11.6 and 12 μmol/L, so the results using either cutoff were the same. Substantial γ-tocopherol was detected in the plasma of preschool children in the present study. If plasma α- and γ-tocopherol concentrations were combined, 73% of the subjects had total tocopherol concentrations <11.6 and <12 μmol/L. Thirty-six percent of US children aged 1–12 years [9] were reported to have total tocopherol concentrations of <11.6 μmol/L. Sixty-eight percent of our subjects had plasma α-tocopherol values of <7 μmol/L (Fig. 2), but if plasma α- and γ-tocopherol concentrations were combined, 50% of the children had plasma total tocopherol concentrations <7 μmol/L.

The plasma tocopherol/total lipid ratio has been proposed and utilized as a vitamin E status criterion [3,9,10]. Over half of the subjects in the present study had plasma α-tocopherol/total lipid concentrations <0.8 mg/g (Fig. 2). Mean plasma total lipid concentrations of the 2–5-year-old children in the present study (3.6 g/L for the 2–3-year group and 3.8 g/L for the 4–5-year one) are slightly lower than the mean values (4.84 g/L) reported for 39 children, 1–12 years of age [9]. Children have lower plasma total lipid levels than adults [42]. The mean plasma α-tocopherol/total lipid values of the children in the current study are lower than that calculated using mean plasma total tocopherol and total lipid concentrations of inner city and suburban children, 1–12 years of age, in a study by Farrell et al. [9] in 1978. However, food consumption habits have changed since then [13,14].

The vitamin E intake and plasma values of the 15 ethnically diverse 2–3-year-olds in the present study may be useful in setting future dietary recommendations based on data from young children rather than being extrapolated from data from adults. Vitamin E intake and plasma data are needed on more young children; the findings of the current study delineate the need for conducting a much larger population-based study. Our data may also be useful in the setting of "norms" for young children. The median

vitamin intake of the 2–3-year-old subjects was 4.9 mg α-tocopherol/day, and 66.7% had plasma α-tocopherol concentrations <7 μmol/L (46.6% had plasma total tocopherol concentrations <7 μmol/L) and 53.3% had plasma α-tocopherol/lipid ratios <0.8 mg/g. Our data corroborate the current EAR of 5 mg α-tocopherol/day for 1–3-year-old children [1].

The majority of this group of preschool children had less than adequate vitamin E status as assessed using several status criteria. We would have liked to have had more subjects in the study; however, finding parents willing to subject their child to the blood draw was a challenge as has been noted by others. Further research involving a larger sample size is needed. However, the findings of the present study do suggest that the EAR and RDA set by the Institute of Medicine [1] by extrapolation from adult data are appropriate for young children. Preschool children need to eat more vitamin E-rich foods (vegetable oils — corn, cottonseed, soybean, safflower, sunflower, wheat germ; almonds and other nuts; sunflower seeds; wheat germ), including fortified foods.

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