Suboptimal Serum α-Tocopherol Concentrations Observed among Younger Adults and Those Depending Exclusively upon Food Sources, NHANES 2003-2006

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Abstract

Vitamin E is an essential nutrient for human health, with an established function as a lipid-soluble antioxidant that protects cell membranes from free radical damage. Low vitamin E status has been linked to multiple health outcomes, including total mortality. With vitamin E being identified as a ‘shortfall nutrient’ because >90% of American adults are not consuming recommended amounts of vitamin E, we aimed to determine the prevalence of both clinical vitamin E deficiency (serum α-tocopherol concentration < 12 μmol/L) and failure to meet a criterion of vitamin E adequacy, serum α-tocopherol concentration of 30 μmol/L, based on the Estimated Average Requirement (EAR) and lowest mortality rate in the Alpha-Tocopherol Beta-Carotene (ATBC) study. The most recent nationally-representative cross-sectional data (2003–2006) among non-institutionalized US citizens with available serum concentrations of α-tocopherol from the National Health and Nutrition Examination Survey (NHANES); Centers for Disease Control and Prevention were analyzed. Serum α-tocopherol distributions were compared between those reporting consumption of food without supplement use (FOOD) and food and supplement use (FOOD+DS) by sex, age, and race/ethnicity. Only 1% of the US population is clinically deficient. FOOD consumers have lower average α-tocopherol levels (24.9 ± 0.2 μmol/L) than FOOD+DS users (33.7 ± 0.3 μmol/L), even when adjusted for total cholesterol. Using a criterion of adequacy of 30 μmol/L, based on the Estimated Average Requirement (EAR) and lowest mortality rate in the Alpha-Tocopherol Beta-Carotene (ATBC) study. The most recent nationally-representative cross-sectional data (2003–2006) among non-institutionalized US citizens with available serum concentrations of α-tocopherol from the National Health and Nutrition Examination Survey (NHANES); Centers for Disease Control and Prevention were analyzed. Serum α-tocopherol distributions were compared between those reporting consumption of food without supplement use (FOOD) and food and supplement use (FOOD+DS) by sex, age, and race/ethnicity. Only 1% of the US population is clinically deficient. FOOD consumers have lower average α-tocopherol levels (24.9 ± 0.2 μmol/L) than FOOD+DS users (33.7 ± 0.3 μmol/L), even when adjusted for total cholesterol. Using a criterion of adequacy of 30 μmol/L, based on the Estimated Average Requirement (EAR) and lowest mortality rate in the Alpha-Tocopherol Beta-Carotene (ATBC) study.
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Introduction

Vitamin E (α-tocopherol) is an antioxidant nutrient essential for health, first discovered in 1922 for its role in maintaining pregnancy in rodents [1]. The term vitamin E used in this publication refers to the 2R stereoisomer of α-tocopherol used to establish recommended intakes [2]. As a fat-soluble antioxidant, the primary recognized function of vitamin E is to serve as a free radical scavenger in lipid components of the cell, such as cell membranes and plasma lipoproteins [2]. Clinical signs of vitamin E deficiency include erythrocyte hemolysis and peripheral neuropathy. While overt clinical deficiencies of vitamin E are rare [3], inadequate vitamin E intake has been linked to outcomes such as heart disease [4,5], impaired immune function [6,7], infertility [8,9], and even overall mortality [10]. Dietary intake of vitamin E from food is below recommendations [11–13]. In the United States, 83% of children [14] and 91% of adults [15] fail to consume the Estimate Average Requirement (EAR) for vitamin E from food alone. Because of the prevalence of inadequate vitamin E intake, the 2015 Dietary Guidelines Advisory Committee identified vitamin E as a shortfall nutrient.

For vitamin E, the EAR (12 mg/day; expected to result in a serum α-tocopherol concentration of 27.9 μmol/L) is based on the α-tocopherol concentration limiting in vitro hydrogen peroxide-induced erythrocyte hemolysis to ≤12%. Vitamin E deficiency is defined as serum α-tocopherol concentration <12 μmol/L [2]. Given the prevalence of inadequate vitamin E intake and limitations of dietary intake assessments [16], especially for vitamin E [17], we used serum α-tocopherol data from the NHANES survey to assess vitamin E status according to the prevalence of vitamin E deficiency and the proportion of Americans failing to meet a criterion of vitamin E adequacy. Since functional markers for vitamin E adequacy are currently missing, the criterion of vitamin E adequacy was defined as 30 μmol/L. This cutoff was chosen because 30 μmol/L is the concentration associated with the EAR [18], the average serum concentration for American adults [11], the level at which urinary α-CEHC excretion increases [12,17], and the concentration associated with the lowest mortality risk in the Alpha-Tocopherol Beta-Carotene (ATBC) study [10].

Materials and Methods

This cross-sectional analysis included the most recent survey with nationally representative data for blood α-tocopherol concentrations conducted by the National Center for Health Statistics (NCHS) at the Center for Disease Control and Prevention (CDC), i.e. NHANES 2003–2006. Details regarding the complex survey design and multistage probability sampling of non-institutionalized United States (US) civilians have been previously documented [3]. Supplement use was according to self-report of any vitamin, mineral, herbal or other dietary supplement consumed in the past month. Participants were shown a card with examples of supplements.

Data collection included blood samples [19] and interviews.

Serum α-tocopherol concentrations were assessed by high performance liquid chromatography (HPLC) with multilength photodiode-array absorbance detection [20]. Quantification of samples was based on external standards, and corrected by tocol as an internal standard to account for post-run recovery [21]. Total cholesterol was measured in serum or plasma by enzymatic coupled reactions; a reaction by-product (H₂O₂) produced color and was quantified by assessing absorbance at 500 nm [22,23].

We considered a subsample of individuals with data available for α-tocopherol, cholesterol, and covariates (n = 7,922). NHANES (2003–2006) categorized race/ethnicity according to: 1) Mexican-American, 2) Other Hispanic, 3) Non-Hispanic White, 4) Non-Hispanic Black and 5) Other Race–Including Multi-Racial. Categories 2 and 5 were combined into a single “Other”
category. Age ranges were chosen to match Institute of Medicine groupings used for the Dietary Reference Intakes [2]. Exclusion criteria, i.e. pregnancy, lactation and age < 20 years, were modeled to facilitate comparisons with Ford et al. [11].

**Statistical analyses**

All reported values accounted for the multi-stage complex survey design through survey procedures in SAS (version 9.3, Durham, NC, USA), as well as the sampling weight, cluster, and strata variables provided by NCHS. This methodology adjusts for non-coverage as well as non-response, and allows for the oversampling of under-represented groups. Sampling weights from each two-year survey (2003–04, 2005–06) were combined according to NCHS recommendations and proportionally scaled by the respective subsamples of individuals with available data from each survey.

To account for differences attributable to circulating blood cholesterol concentrations, cholesterol-adjusted $\alpha$-tocopherol values (μmol /mmol) were calculated by dividing serum $\alpha$-tocopherol concentrations (μmol/L) by total cholesterol (mmol/L) [24]. Based on two cutoff values (<12, 30 μmol/L), serum $\alpha$-tocopherol concentration was dichotomized and low serum status was expressed as a percentage. In addition, a cholesterol-adjusted $\alpha$-tocopherol cutoff of 5.8 μmol/mmol was also derived by dividing the criterion of $\alpha$-tocopherol adequacy (serum concentration of 30 μmol/L) by the desirable total blood cholesterol concentration (5.17 mmol/L; 200 mg/dL) recommended by the National Heart, Lung and Blood Institute [25]. Observations with missing values for cholesterol (n = 2) were excluded from histograms and frequencies of cholesterol-adjusted $\alpha$-tocopherol.

Results for unadjusted- and cholesterol-adjusted $\alpha$-tocopherol concentrations were reported as totals and stratified by covariates, which were determined by *a priori* literature review. The proportions of the sample population with an $\alpha$-tocopherol concentration below 12 and 30 μmol/L and a cholesterol-adjusted $\alpha$-tocopherol status below 5.8 μmol/mmol were considered by subgroups. We stratified according to any self-reported supplement use, sex, race/ethnicity [non-Hispanic White, non-Hispanic Black, Mexican American, any other], and age groups (≥20–30, ≥31–50, 51+ years). Individuals who self-identified as multi-racial, any other Hispanic (excluding Mexican American), or other races/ethnicities were included in the “any other” category. Age as a continuous variable was categorized into three groups.

Differences in proportions below cutoffs, stratified by subgroups (by supplement use, sex, race/ethnicity, age), were compared by Rao-Scott chi squared tests, which account for the complex survey design [26]. Accounting for familywise error rate, Rao-Scott chi square tests were considered significant with a Bonferroni correction (alpha value of 0.05 divided by four hypotheses). We assessed the association between supplement use and low vitamin E status (30 μmol/L) through multivariate logistic regression which accounts for the complex survey design (SAS surveylogistic procedure). The initial model included known and suspected risk factors or correlates of the outcome (based on *a priori* literature search) and two-way interaction terms between independent variables. Only variables with a p-value ≤ 0.01 were retained using a step-by-step elimination approach to reach the final parsimonious model.

**Results**

A total of 7,922 participants with measurements of serum $\alpha$-tocopherol concentrations were included in this analysis. Distributions of serum concentrations of $\alpha$-tocopherol for the entire population were stratified by FOOD and FOOD+DS use (Fig 1). The mean (± SEM) of $\alpha$-tocopherol was 29.6 ± 0.2, 24.9 ± 0.2, 33.7 ± 0.3 μmol/L for the total population ≥ 20y, FOOD and FOOD+DS use, respectively. The mean cholesterol-adjusted $\alpha$-tocopherol value was 5.8 ±
The mean \( \alpha \)-tocopherol for men \( \geq 20 \) y was 25.0 ± 0.2 and 33.8 ± 0.4 \( \mu \text{mol} \)/L and for women \( \geq 20 \) y was 24.9 ± 0.2 and 33.7 ± 0.3 \( \mu \text{mol} \)/L by FOOD and FOOD+DS use, respectively (Fig 3). The mean \( \alpha \)-tocopherol was 25.7 ± 0.2, 35.7 ± 0.5 \( \mu \text{mol} \)/L for Caucasians, 23.5 ± 0.3, 30.5 ± 0.7 \( \mu \text{mol} \)/L African-Americans, 25.1 ± 0.2, 33.0 ± 0.5 \( \mu \text{mol} \)/L for Mexican-Americans, and 23.5 ± 0.4, 30.7 ± 0.6 \( \mu \text{mol} \)/L for all others \( \geq 20 \) y by FOOD and FOOD+DS use, respectively (Fig 4).

Using the Institute of Medicine [2] definition of vitamin E deficiency, 12 \( \mu \text{mol} \)/L, corresponding to \(<12\%\) hydrogen peroxide-induced in vitro erythrocyte lysis, only 0.6\% of Americans are clinically deficient (Table 1). The prevalence of vitamin E deficiency did not differ with age, sex, or race/ethnicity. We further examined this dataset seeking to identify the prevalence of the population below the criterion of vitamin E adequacy, 30 \( \mu \text{mol} \)/L, which was selected because it represents the concentration associated with the EAR [18], the average serum concentration for American adults [11], and the level at which urinary \( \alpha \)-CEHC excretion increases [12,17], and the concentration associated with the lowest mortality risk in the Alpha-Tocopherol Beta-Carotene (ATBC) study [10]. A greater prevalence of individuals not reporting supplement use had serum \( \alpha \)-tocopherol concentrations below 30 \( \mu \text{mol} \)/L (p<0.01; Table 1). The prevalence of not meeting the criterion of vitamin E adequacy was higher among younger populations (Fig 5A). Over 87\% of individuals between 20-30y had serum \( \alpha \)-tocopherol concentrations below 30 \( \mu \text{mol} \)/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). Significantly more males (64.1\%)...
than females (61.0%) have serum α-tocopherol levels below 30 μmol/L (p<0.01; Table 1). There are race/ethnicity differences in vitamin E status with a higher prevalence of inadequate vitamin E status among non-Hispanic Blacks (77.5%) than Mexican Americans (62.2%), other races (71.9%) and the lowest prevalence in non-Hispanic whites (57.2%) (p<0.01; Table 1). In all cases (sex, race/ethnicity, age), a smaller proportion of FOOD+DS users failed to meet the criterion of vitamin E adequacy (Table 1). These trends remained similar when serum α-tocopherol was adjusted for total cholesterol (Table 2). Use of supplements was associated with a reduced odds of low vitamin E status (<30 μmol/L; OR 0.17 [95% CI: 0.13–0.21]), adjusting for age, sex, race/ethnicity, and two-way interaction terms (supplement use * age, sex * age).

Discussion

Using NHANES data collected between 2003–2006, we find the prevalence of clinical vitamin E deficiency to be low, which was similar to observations from the 1999–2000 NHANES dataset [11]. In this nationally representative survey of adults, serum α-tocopherol concentrations ranged between greater than 0 and 84 μmol/L. Cholesterol-adjusted α-tocopherol values ranged between greater than 0 and 23 μmol/mmol. Serum α-tocopherol concentrations increased with age and supplement use, which has been observed by others [11,13,27]. Serum α-tocopherol concentrations are significantly lower in adolescent girls (16 μmol/L) than premenopausal women (31 μmol/L) [28]. Higher vitamin E status in persons 51+y may partially be explained by increased dietary supplement usage [29]. A low vitamin E concentration is more prevalent
among African Americans and Mexican-Americans than in non-Hispanic Whites. This may be at least partly due to lower use of dietary supplements overall in ethnic minorities [30].

While overt deficiency was rare in this nationally representative population, the prevalence of not meeting the criterion of vitamin E adequacy was significantly higher among those reporting exclusive dependence upon food sources. This finding is consistent with reports that >90% of children [14,31] and adults [13,15,31] consume less than the EAR for vitamin E from food sources.
Dietary consumption of antioxidant rich foods is positively associated with increasing α-tocopherol concentrations [32]. We and others [33] report that serum α-tocopherol levels are lower in people depending exclusively upon dietary sources. Clearly, there is an opportunity for increased consumption of vitamin E rich foods such as nuts, oils, and whole grains or dietary supplement use. The conclusion of a 2005 meta-analysis was that vitamin E supplements should be avoided [34]. Although this meta-analysis reported higher mortality only for higher doses, data from the Nurses’ Health Study and Health Professionals Follow-up Study reported the use of vitamin E supplements has declined ~50% from 2002 to 2006 and to the lowest level (19.8% and 24.5%, respectively) since the early 1990s [35]. We propose that for many Americans, especially those relying exclusively upon food sources, that serum α-tocopherol concentrations may not be adequate.

Vitamin E was first discovered for its role in supporting healthy pregnancy and development [1], the high prevalence of inadequate vitamin E status among men and women of reproductive age is concerning. According to global statistics, approximately 20–25% of couples have fertility problems [36]. Vitamin E insufficiency has been associated with impairments in spermatogenesis [37] and sperm competitiveness [38]. Men with higher dietary and supplement intakes of vitamin E have less sperm DNA damage [39]. Epidemiological data [8] has shown that infertile men had lower α-tocopherol concentrations in both the sperm (1.48 vs 1.68 μmol/L) and serum (17.8 vs 22.0 μmol/L) compared to fertile males. In normospermic males with low fertilization rates, vitamin E supplementation decreased lipid peroxidation.

Fig 4. Distribution of serum α-tocopherol concentrations among individuals ≥20y, excluding pregnant or lactating women, stratified by race-ethnicity and supplement. A. Non-Hispanic White. B. Non-Hispanic Black. C. Mexican American. D. Other. Lines represent density (as a percentage) through non-parametric kernel density estimation.

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levels in sperm from 12.6 to 7.8 nmol malondiadehyde per 10^8 spermatozoa [40], implying improvements in sperm viability. In women, low vitamin E status may contribute to the rising use of in vitro fertilization to become pregnant [9]. Increased production of biomarkers of oxidative stress have been associated with acute pregnancy complications or spontaneous abortion [41]. Vitamin E supplementation (400 IU daily) improved endometrial response during controlled ovarian stimulation in women with unexplained infertility [42]. Finally, pre-eclampsia is associated with significantly lower serum vitamin E levels [43]. More research is needed to understand the role of vitamin E status on reproductive success in men and women.

Low vitamin E status has been associated with age-related changes in brain function [44,45]. Beydoun et al. [46] examined the relationship of antioxidant status with depressive symptoms in US adults 20-85y and found lower serum levels of vitamins E (26 vs 30 µmol/L) and C (43 vs 60 µmol/L) as well as all carotenoids in depressed vs non-depressed counterparts. Maes et al. [47] reported lower vitamin E concentrations (~23 µmol/L) in American individuals with major depression vs normal volunteers (~32 µmol/L), as did a separate Australian cohort [48]. Healthy controls had α-tocopherol levels > 30 µmol/L whereas those with Alzheimer’s had an average < 30 µmol/L [44]. These studies suggest that suboptimal vitamin E status may negatively affect health.

In elderly men with comparable average serum α-tocopherol concentrations (~30 µmol/L), each standard deviation decrease in serum α-tocopherol concentration was associated with increased risks of hip fracture and any fracture [49]. Men with baseline serum α-tocopherol levels averaging 30 µmol/L had a 10% lower risk of developing prostate cancer compared to those at 18.6 µmol/L [50].

A 2008 review by Traber et al. [51] notes that male smokers from the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study with the lowest serum α-tocopherol quintile had significantly higher risk of total and cause-specific mortality than those in the highest quintile [52]. The median baseline α-tocopherol level was 26.7 µmol/L in the ATBC Study with

| Total | Sex | Race/Ethnicity | Age (y) |
|-------|-----|----------------|---------|
|       |     | Male Female    | 20–30   | 31–50 | 51+  | p  |
|       |     | n = 7922 a n = 4084 n = 3838 | n = 2864 | n = 929 | n = 2795 | n = 1334 | n = 1529 | n = 2785 | n = 3608 | p  |
| Total | 12  | 0.6 0.7 0.5 0.30 | 0.7 0.8 0.5 0.6 | 0.6 0.77 | 1.4 0.5 0.4 0.04 | p  |
|       | 30  | 62.5 64.1 61.0 | <0.01 | 57.2 77.5 62.2 | 71.9 | <0.01 | 87.4 67.9 43.1 | <0.01 | p  |
|       | n = 3873 | n = 1781 | n = 2092 | n = 1484 | n = 347 | n = 1481 | n = 561 | n = 521 | n = 1182 | n = 2170 | p  |
| FOOD + DS | 12  | 0.4 0.2 0.5 0.07 | 0.6 0.0 0.3 | <0.1 | 0.8 0.5 0.2 0.23 | p  |
|       | 30  | 45.9 45.7 46.0 | 0.89 | 39.6 61.0 47.3 | 57.4 | <0.01 | 79.2 54.0 28.5 | <0.01 | p  |
|       | n = 4049 | n = 2303 | n = 1746 | n = 1380 | n = 582 | n = 1314 | n = 773 | n = 1008 | n = 1603 | n = 1438 | p  |
| FOOD  | 12  | 0.9 1.2 0.6 0.02 | 0.8 1.2 0.8 | 1.1 0.82 | 1.8 0.5 0.7 0.10 | p  |
|       | 30  | 81.3 80.4 82.3 | 0.13 | 78.7 87.2 81.2 | 84.3 | <0.01 | 92.7 80.8 71.2 | <0.01 | p  |

Table 1. Prevalences of serum α-tocopherol concentrations below cut-offs (12 and 30 µmol/L) among individuals a in the United States (NHANES 2003–2006) (TOTAL), stratified by reported food use (FOOD), food and supplement use (FOOD+DS) and demographic characteristics (%).

a Sample size (n = 7,922) excludes individuals: <20 years; who are pregnant lactating; with α-tocopherol concentrations >99 percentile; or unavailable data for α-tocopherol concentration, age, sex, race-ethnicity. To account for complex survey design, SAS survey procedures (surveymeans) as well as cluster, strata, and sampling weights (proportionally scaled to included sample) were used.

b Includes multi-racial and any other Hispanic individuals

c Based on Rao-Scott chi-square p-value

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20th and 80th percentiles of 21.6 and 33 μmol/L, respectively. Basically, three quarters of ATBC volunteers had α-tocopherol concentrations below 30 μmol/L and lower than the average baseline value reported in reviews of vitamin E supplementation RCTS [34,53,54] (S1 Appendix). This indicates that most vitamin E intervention trials tested the effects of supplementation in persons with baseline serum α-tocopherol concentrations, i.e. 30 μmol/L that are higher than concentrations associated with the EAR. In summary, >80% of adult FOOD and 61% of FOOD+DS consumers fail to reach the criterion of vitamin E adequacy associated with the EAR. Similar concentrations have been reported in Irish adults [13].

A strength of this study is that it is a nationally representative sampling of serum α-tocopherol concentrations of Americans. A weakness is that a single time point blood sample does not necessarily reflect long-term vitamin E status any more than it does for vitamin D. For decades,
health professionals denied the need to investigate the role of vitamin D and health outcomes because the prevalence of rickets, i.e. vitamin D deficiency, was low. It is unlikely that vitamin D supplementation will benefit individuals with optimal vitamin D levels [55,56]. Similarly, the role of vitamin E status in maintaining health should not be judged by supplementation studies in individuals with optimal (>30 μmol/L) baseline α-tocopherol levels [34,53,54](S1 Appendix). Research is needed that correlates serum α-tocopherol concentrations with functional outcomes.

**Conclusion**

Our findings provide evidence that most Americans have serum α-tocopherol levels below 30 μmol/L. The EAR, epidemiological and randomized controlled studies all indicate that maintaining a serum α-tocopherol concentration of 30 μmol/L may have beneficial effects on mortality, cognitive function and reproduction. Given the prevalence of inadequate vitamin E status among those exclusively dependent upon food and decreasing use of vitamin E supplements since these samples were obtained (NHANES 2003–2006), it will be important to continue monitoring vitamin E status in Americans. This paper corroborates the need for research regarding to assessing serum α-tocopherol concentrations with respect to functional markers and health outcomes.

**Supporting Information**

S1 Appendix. Baseline vitamin E (α-tocopherol, μmol/L) concentrations reported in research summarized in 3 published meta-analyses.

(DOCX)

**Acknowledgments**

The choice of variables (α-tocopherol concentrations, sex, age, ethnicity/race) to be analyzed from the CDC dataset was jointly decided by all authors. EY and SM downloaded the CDC
dataset, conducted independent statistical analysis, and prepared the tables and figures. MM wrote the manuscript with input from co-authors. All authors read and approved the final manuscript. A special thank you to Elisabeth Stoecklin for helping MM collate the serum α-tocopherol concentrations reported in S1 Appendix.

**Author Contributions**
Conceived and designed the experiments: MM SM. Analyzed the data: EY SM. Wrote the paper: MM EY EC JB ME SM.

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