Vitamin E Inadequacy in Humans: Causes and Consequences[1,2]

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ABSTRACT

It is estimated that >90% of Americans do not consume sufficient dietary vitamin E, as α-tocopherol, to meet estimated average requirements. What are the adverse consequences of inadequate dietary α-tocopherol intakes? This review discusses health aspects where inadequate vitamin E status is detrimental and additional vitamin E has reversed the symptoms. 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. When low dietary amounts of α-tocopherol are consumed, tissue α-tocopherol needs exceed amounts available, leading to increased damage to target tissues. Seemingly, adequacy of human vitamin E status cannot be assessed from circulating α-tocopherol concentrations, but inadequacy can be determined from "low" values. Circulating α-tocopherol concentrations are very difficult to interpret because, as a person ages, plasma lipid concentrations also increase and these elevations in lipids increase the plasma carriers for α-tocopherol, leading to higher circulating α-tocopherol concentrations. However, abnormal lipoprotein metabolism does not necessarily increase α-tocopherol delivery to tissues. Additional biomarkers of inadequate vitamin E status are needed. Urinary excretion of the vitamin E metabolite α-carboxy-ethyl-hydroxychromanol may fulfill this biomarker role, but it has not been widely studied with regard to vitamin E status in humans or with regard to health benefits. This review evaluated the information available on the adverse consequences of inadequate α-tocopherol status and provides suggestions for avenues for research. *Adv. Nutr. 5: 503–514, 2014.*

Introduction

The focus of this review is to evaluate the hypothesis that adequacy of human vitamin E status cannot be assessed from circulating α-tocopherol concentrations but that inadequacy can be determined from "low" values. Although α-tocopherol was discovered in 1922, it was not until the 1980s that α-tocopherol deficiency was described in humans (1). Moreover, it took nearly a decade longer to define the deficiency symptoms in humans, in cases in which the α-tocopherol deficiency disorder was not complicated by additional nutritional or metabolic defects. The question remains, however, as to what are the symptoms of vitamin E inadequacy; >90% of Americans do not consume sufficient dietary vitamin E to meet estimated average requirements (EARs)[3] (2,3), yet they appear to undergo no obvious ill effects and their circulating α-tocopherol concentrations are not obviously abnormal. This observation led to the idea that the EAR is too high and that this apparent low dietary α-tocopherol intake has no biologic significance. Thus, this review evaluates the consequences of low intakes in relation to both genetic and metabolic causes of inadequacy, as well as frank malnutrition.

Current Status of Knowledge

Vitamin E required amounts

The determination of how much of a nutrient is required daily is dependent on specifically assessing not only its function but also defining a biomarker that is indicative of inadequacy that changes with nutrient intakes (4). For vitamin E, results from the in vitro hydrogen peroxide–induced erythrocyte hemolysis test were chosen by the Institute of Medicine (IOM) in 2000 as a marker of vitamin E status because increased peroxide-induced erythrocyte hemolysis was correlated with increased erythrocyte fragility in vitamin E–deficient individuals. Additionally, anemia with increased erythrocyte turnover during vitamin E deficiency was observed in vitamin E–deficient children with cystic fibrosis (5). Moreover, anemia is a symptom of experimental vitamin E

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[3] Abbreviations used: α-CEHC, α-carboxy-ethyl-hydroxychromanol; α-TE, α-tocopherol equivalent; α-TTP, α-tocopherol transfer protein; AVED, ataxia with vitamin E deficiency; EAR, estimated average requirement; hpf, hours postfertilization; Elovl, dlongation of very long-chain fatty acid; IOM, Institute of Medicine; NASH, nonalcoholic steatohepatitis.

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deficiency in animals (6–8). The EAR for vitamin E 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 (4). The RDA of 15 mg α-tocopherol was extrapolated from that value (4). According to the IOM (4), only α-tocopherol meets human vitamin E requirements because it was the only form that was demonstrated to reverse vitamin E deficiency symptoms in humans, as well as being the only vitamin E form maintained in plasma and tissues, as discussed below.

Plants make 8 different forms of vitamin E, 4 (α-, β-, γ-, and δ-) tocopherols and 4 (α-, β-, γ-, and δ-) tocotrienols. The phytyl tail of natural α-tocopherol is in the *RRR*-conformation, whereas chemically synthesized α-tocopherol (all racemic) contains 8 stereoisomers [*RRR*-, *RSR*-, *RSS*-, and *RRS*- (the 2R- forms) and *SRR*-, *SRS*-, *SSR*-, *SSS*-] (4). In the diet, α-tocopherol is found in foods such as nuts and seeds and in vegetable oils, such as wheat germ, sunflower seed, safflower, and olive. Judicious food choices allow consumption of 15 mg α-tocopherol daily (9). However, most Americans require supplements to attain these recommended α-tocopherol intakes (10). In a study assessing a biomarker of vitamin E status, Lebold et al. (11) found that individuals who are highly motivated and interested in their diets consumed nearly the recommended α-tocopherol amounts (4). However, surveys of vitamin E intakes of the general public found that 90% of men and 96% of women do not consume the EAR of 12 mg α-tocopherol (2). The 2010 Dietary Guidelines (12) did not emphasize that vitamin E is a relatively difficult nutrient to obtain from the diet. For example, a report from the USDA covering the period from 2000 to 2006 states that vitamin E intakes, measured as α-tocopherol equivalents (α-TEs), were "21.1 mg alpha-TE per capita per day in 2006, up from 19.5 mg alpha-TE per capita per day in 2000. The level of vitamin E has generally increased over the series with the highest level in 2006" (13). These intakes apparently easily exceed the RDA. This α-TE value, however, includes intakes of non–α-tocopherol forms, largely γ-tocopherol, in addition to α-tocopherol. The α-TEs were defined in the 1989 RDA (14) but were not used in the 2000 DRI for vitamin E (4); instead milligrams of 2*R*-α-tocopherol was defined as the vitamin E unit. α-TEs fell out of favor because non–α-tocopherols are rapidly metabolized, do not substitute for α-tocopherol, and cannot be metabolically converted to α-tocopherol by humans and therefore should not be included in measures of vitamin E intake (4). Thus, a key controversy to be addressed by the nutrition community is "What are the adverse consequences of inadequate dietary α-tocopherol intakes?"

α-Tocopherol deficiency and inadequacy

Vitamin E deficiency is seldom found in adults but is more frequently found in children, likely because they have limited stores and are growing rapidly, thereby allowing deficiency symptoms to be readily apparent. This section, therefore, emphasizes findings in children. It should be noted that there are some reports of vitamin E deficiency in adults. For example, after decades of inadequate vitamin E absorption due to short bowel syndrome, a 71-y-old man complained of neurologic abnormalities that were consistent with vitamin E deficiency and responded to vitamin E supplementation (15). Thus, vitamin E is required throughout the life span.

Deficiency symptoms in humans

In humans, severe vitamin E deficiency occurs as a result of genetic defects in the α-tocopherol transfer protein (α-TTP), causing the disorder ataxia with vitamin E deficiency (AVED) (16). The lack of functional α-TTP results in the rapid depletion of plasma α-tocopherol (17,18), thereby demonstrating that α-TTP is needed to maintain plasma α-tocopherol concentrations.

Fat malabsorption also leads to vitamin E deficiency. Examples of fat malabsorption include genetic defects in the microsomal TG transfer protein or in apoB (abeta- and hypobeta-lipoproteinemia, respectively) and fat-malabsorption syndromes, such as cholestatic liver disease or cystic fibrosis (19).

Human vitamin E deficiency symptoms include a progressive neurologic disorder, spinocerebellar ataxia, which occurs as a result of a dying back of peripheral nerves, specifically sensory neurons (20). As the vitamin E deficiency continues over time, the neurologic defects become so severe such that they that result in ataxia (16). With progressing deficiency in humans, there is also muscle deterioration, and this deterioration can include the heart muscle. Vitamin E deficiency ultimately results in death. In severe vitamin E deficiency, cardiomyopathy was among the symptoms of a vitamin E–deficient child who died of hepatic and cardiac failure (21). Cardiomyopathy is also a symptom of vitamin E deficiency in some patients with AVED (16,20,22).

Vitamin E supplements in amounts well over 1000 mg/d have been prescribed for children with vitamin E deficiency. α-Tocopherol supplements are recommended because they prevent the further progression of the neurologic abnormalities, and in some cases reverse them. For example, when given before the onset of abnormalities, supplements prevented neurologic symptoms (23) and stopped the progression of myopathy in individuals with abetalipoproteinemia (24). Similarly, in children with AVED, vitamin E supplements improved symptoms and halted the disease progression (16).

Malnutrition. Vitamin E deficiency symptoms in humans have been sufficiently well characterized to allow detection of more subtle examples of vitamin E inadequacy. Examples of frank vitamin E deficiency due to low dietary intakes include children in India with severe malnutrition (25,26). In addition to general malnutrition, severe vitamin E deficiency was recognized in those children because the specific neurologic abnormalities associated with vitamin E deficiency were detected. Vitamin E supplementation was initiated and was found to reverse the symptoms (25,26),

thereby confirming that the neurologic abnormalities were dependent on α-tocopherol status.

Vitamin E inadequacy in children. Assessing normal plasma α-tocopherol concentrations in children is complicated because α-tocopherol is transported in plasma lipoproteins and concentrations of cholesterol and lipoproteins, as well as of α-tocopherol, increase with age (27). An example in which circulating α-tocopherol concentrations did not reflect dietary intakes was illustrated in a study in which these values in adolescents and their parent or grandparent were compared. Although the estimates of mean ± SEM vitamin E intakes in adolescents (9.2 ± 0.2 mg α-tocopherol/d) were higher than those of the adults (8.4 ± 0.2 mg α-tocopherol/d), plasma α-tocopherol concentrations were lower in adolescents (17 ± 0.4 μmol/L, corrected for cholesterol concentrations) compared with adults (26 ± 0.6 μmol/L) (28). The values for plasma α-tocopherol concentrations in these adolescents were similar to those reported for healthy children in Tunisia (29) or Germany (30). These data emphasize the well-accepted finding that circulating α-tocopherol concentrations do not correlate very highly with dietary α-tocopherol intakes.

Given the close relation between circulating lipids and α-tocopherol, it is important to recognize that both variables may be decreased in malnutrition. Squali Houssainï et al. (31) studied control children compared with severely malnourished children in Morocco. They reported, "In severely malnourished children, albumin, cholesterol and low density lipoprotein (LDL) cholesterol, plasma selenium, vitamin E and zinc were low, whereas inflammatory proteins and triglycerides were high. These features worsened with essential fatty acid deficiency." Their findings emphasize that malnutrition alters plasma lipid concentrations; thus, correction of plasma α-tocopherol for lipids may mask deficiency states. Decreased cholesterol concentrations were also observed in protein energy malnutrition (32). These cholesterol decreases were also associated with low circulating concentrations of α-tocopherol and increased inflammatory markers, such as IL-6. In contrast, Laryea et al. (33) suggested that the individuals they studied were active, normal Congolese village children and the low plasma α-tocopherol (mean ± SD: 7.3 ± 1.3 μmol/L) should be corrected for low lipids based on the observation that, when reported as tocopherol:lipid ratios, the vitamin E values were within the usual range for children. However, low plasma α-tocopherol concentrations (median: 7.33 μmol/L; range: 2.61–18.42 μmol/L) were also found in children with falciparum malaria infections compared with control children (median: 17.71 μmol/L; range: 6.48–28.08 μmol/L); both groups had similar α-tocopherol:cholesterol ratios [median (range): 4.61 (1.24–7.20) vs. 5.15 (1.80–8.92) μmol/mmol] because the children with malaria had depressed cholesterol concentrations (mean ± SD: 1.89 ± 0.62 vs. 3.47 ± 0.59 mmol/L in controls) (34). These data suggest that both malnutrition and infectious diseases can lower circulating cholesterol and its lipoprotein carriers. Thus, correction of plasma α-tocopherol concentrations for lipids is not appropriate in cases in which circulating lipids are below normal concentrations.

Circulating α-tocopherol concentrations <12 μmol/L were defined by the IOM to be in the deficient/inadequate range for healthy adults (4). For comparison, European children in the Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) study were reported to have mean ±SD circulating α-tocopherol concentrations of 23 ± 4.9 μmol/L (35), whereas pediatric reference intervals for circulating α-tocopherol from 1136 healthy U.S. children aged 7 to 17 y ranged from 11 to 30 μmol/L (36); and in U.S. children aged 7 mo to 9 y, values ranged from 12 to 40 μmol/L with a mean of ~20 μmol/L (37). Thus, the ranges of circulating α-tocopherol in healthy U.S. and European children were above the deficiency cutoff value of 12 μmol/L. However, there have been some reports of children in the United States with circulating α-tocopherol below this cutoff (38), suggesting low intakes. Indeed, some reports claim that dietary vitamin E intakes in U.S. children are generally below recommended values, except for those children taking supplements (39).

Taken together, these findings suggest that circulating α-tocopherol concentrations below the cutoff of 12 μmol/L are likely indicative of inadequacy if not frank vitamin E deficiency. Numerous reports worldwide have shown that such concentrations are frequently reported in children (**Fig. 1**). These low circulating α-tocopherol concentrations are caused by the combination of consumption of diets low in vitamin E, along with inadequate intakes of fat, protein, and calories. These latter dietary components are necessary for fat absorption and transport, which are required elements for vitamin E absorption and its lipoprotein transport, as reviewed elsewhere (40).

Obesity and metabolic syndrome. In contrast to malnourished children, many studies have shown that obese children

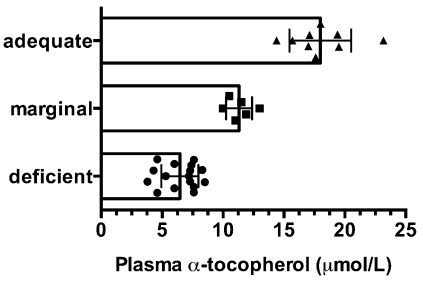

FIGURE 1 Plasma α-tocopherol concentrations are shown from various reports of children with potentially low vitamin E status. The bars and error bars indicate overall means ± SDs of all data points, and the individual data points represent mean or median values from specific research reports. Plasma α-tocopherol concentrations fell into 3 categories: deficient (<9 μmol/L), marginal (9–12 μmol/L), and adequate (>12 μmol/L). References for these reports are as follows: deficient (25,31–34,128–138), marginal (38,139–143), adequate (28–30,41–43,144–146).

do not have low plasma α-tocopherol concentrations; however, when their values were corrected for their circulating lipids, the α-tocopherol:lipid ratios were significantly lower than those in the control group because the obese children had elevated circulating cholesterol and TG concentrations (41–44). Vitamin E supplements in apparently adequately nourished obese children decrease oxidative stress markers (45), suggesting that obese children routinely consume inadequate amounts of antioxidants to prevent oxidative stress. Furthermore, it is likely that this increased oxidative stress is a consequence of chronic inflammation, which is seen secondary to obesity (46) and is a risk factor for other complications of obesity.

An extreme example is nonalcoholic fatty liver disease, which is a major cause of liver dysfunction and is increasing in children due to the increasing prevalence of obesity and type 2 diabetes. The severe negative effects associated with nonalcoholic fatty liver disease include progression to nonalcoholic steatohepatitis (NASH), liver cirrhosis, and ultimately liver cancer (47). Vitamin E supplementation decreases histologic evidence of NASH (48); therefore, supplementation has been tried in children with promising results (49). D'Adamo et al. (50) reported in obese children that 600 mg α-tocopherol daily doubled plasma concentrations from a mean (±SD) of 32.7 ± 1.5 μmol/L to 63 ± 14 μmol/L. The authors did not provide lipid-corrected values, but serum total cholesterol was, on average, 180 mg/dL (4.65 μmol/L) and TGs were 83 mg/dL (0.94 μmol/L). After 6 mo of vitamin E treatment, serum alanine aminotransferase decreased with vitamin E supplementation, indicating improved liver function. Supplementation also decreased urinary prostaglandin $F_{2\alpha}$, insulin, and fasting glucose concentrations, as well as their lipid profiles, and high-sensitivity C-reactive protein. It is unclear how many of these changes are due to the diet and behavior intervention rather than to vitamin E supplementation.

The findings in obese children raise the question as to whether the increased inflammation observed with obesity increases vitamin E requirements. Notably, interventions with vitamin E supplements in children (49) and in adults (48) with NASH had beneficial effects, especially with regard to serum alanine aminotransferase measures of liver dysfunction (51). In adults, Sanyal et al. (48) reported, "Vitamin E therapy, as compared with placebo, was associated with a significantly higher rate of improvement in nonalcoholic steatohepatitis (43% vs. 19%, $P = 0.001$)…." Taken together, these data suggest that obese children likely are consuming inadequate amounts of vitamin E, despite their apparently elevated circulating α-tocopherol concentrations.

To investigate whether antioxidant supplements could mitigate impaired inflammatory and antioxidant status, Murer et al. (45) studied overweight or obese children and adolescents ($n = 44$; mean ± SD age: 12.7 ± 1.5 y) participating in a lifestyle modification program, who were given daily antioxidants (vitamin E, 400 IU; vitamin C, 500 mg; selenium, 50 mg) or placebo for 4 mo. They then measured a number of variables, including the urinary vitamin E metabolite α-carboxy-ethyl-hydroxychromanol (α-CEHC). We previously proposed that the vitamin E metabolite could serve as biomarker of vitamin E adequacy because daily urinary α-CEHC excretion was reflective of adequacy when its excretion exceeded 1.39 μmol/g creatinine (11). Murer et al. (45) reported that the median urinary α-CEHC excretion in their obese and overweight children was low throughout the study in the placebo group [median (range) for baseline vs. postintervention:1.2 (0.01–2.9) vs. 1.2 (0.3–15.9) μmol/g creatinine], whereas in the antioxidant group it was low before supplementation but increased dramatically after antioxidant supplementation [1.8 (0.5–19.2) vs. 16.3 (0.01–81.2) μmol/g creatinine; $P < 0.001$ for intervention]. These data suggest that, despite apparently normal plasma α-tocopherol concentrations in the study participants, urinary α-CEHC excretion suggests inadequate vitamin E status. In support of this statement, vitamin E supplementation in these children also decreased F_2-isoprostanes but not markers of inflammation (45).

The findings in obese participants emphasize that obesity does not necessarily reflect adequate micronutrient intakes, and vitamin E status may be inadequate for normal liver function in these individuals because they have increased oxidative stress. These findings are especially important because lipid peroxidation has been shown to cause dysregulation of liver lipoprotein secretion, which was prevented by increases in vitamin E intake in experimental animal studies (52,53). Taken together, these data emphasize the importance of adequate vitamin E status in obese individuals to maintain healthy liver function and potentially prevent the progression of fatty liver to more serious forms of the disease. They further raise the question of whether liver dysfunction is thus a symptom of vitamin E inadequacy.

Circulating α-tocopherol concentrations as a biomarker of vitamin E status

As is apparent from the previous discussion, circulating α-tocopherol concentrations are very difficult to interpret. 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 (11). However, as a person ages, plasma lipid concentrations also increase, and these increases in lipids also increase the plasma carriers for α-tocopherol, leading to higher circulating concentrations. However, abnormal lipoprotein metabolism does not necessarily increase α-tocopherol delivery to tissues.

The experimental findings in obese children described above highlight the difficulty in assessing vitamin E status by measuring only circulating α-tocopherol. Another example is in individuals with cholestatic liver disease, who have high circulating lipids. Their plasma α-tocopherol concentrations are apparently within normal ranges; however, their α-tocopherol to lipid ratios are low, and most important, tissue α-tocopherol concentrations are at deficient levels (54). Thus, if plasma lipids are elevated, then correction of

α-tocopherol for lipid concentrations is appropriate to assess adequacy. In this case, adequate values for α-tocopherol: lipid ratios should be similar to those in individuals with normal circulating lipid concentrations (55).

This close relation of plasma α-tocopherol to lipids has led some investigators, who evaluated poorly nourished children with low circulating lipids, to postulate on the basis of these ratios that the children's α-tocopherol status was adequate. However, if both plasma lipids and α-tocopherol are abnormally low, then correction of circulating α-tocopherol concentrations for plasma lipids will yield a value indicating a normal α-tocopherol:lipid ratio. This assumption of adequate vitamin E status is likely invalid, because the low lipids reflect the inadequacy of the plasma carriers for delivery of vitamin E to tissues. Moreover, direct measurements of tissue α-tocopherol concentrations, or other surrogate markers of vitamin E status, have not been used to test the assumption that a normal circulating α-tocopherol:lipid ratio, which is caused by both low α-tocopherol and low lipids, reflects an adequate vitamin E status. The prevalence of stunting and anemia in malnourished children, who have limited intakes of both energy and micronutrients, suggests that these children lack important nutritional factors, including vitamin E (56).

Additional markers of inadequate vitamin E status are needed. Adipose tissue α-tocopherol concentrations have been used to assess vitamin E status (57–59). El-Sohemy et al. (58) reported that adipose tissue α-tocopherol concentrations were not well correlated with plasma α-tocopherol concentrations, but they did appear to reflect long-term vitamin E status. We found that in children suffering from severe burn injury, adipose tissue α-tocopherol concentrations rapidly (within 1 mo) become depleted, suggesting that this tissue can serve as an α-tocopherol storage site, releasing α-tocopherol upon increased metabolic demands (60). Additional studies are needed to evaluate intakes relative to tissue α-tocopherol concentrations and long-term health benefits.

Urinary α-CEHC may fulfill this biomarker role (11,61), but this marker of vitamin E status has not been widely studied with regard to vitamin E status in humans. Vitamin E metabolism is a hot topic and has been extensively studied with regard to non–α-tocopherol intakes in both humans and in experimental animals. Non–α-tocopherols are readily converted to their respective CEHC forms, even during α-tocopherol deficiency (62,63). Thus, hepatic vitamin E metabolism is a major regulator of the forms of vitamin E found in the body. The reader is directed to a recent review on this topic (64). Hypothetically, once liver α-tocopherol concentration reaches a threshold level, additional α-tocopherol will be metabolized; thus, plasma α-tocopherol reaches a "plateau," whereas α-CEHC excretion increases exponentially (**Fig. 2**).

Anemia has traditionally been a marker of poor vitamin E status, but anemia in pregnant women in Bangladesh was not only associated with decreased plasma α-tocopherol concentrations but also with deficiencies of some other micronutrients (65), emphasizing that anemia is not necessarily caused by the lack of just 1 micronutrient. Thus, the multifactorial nature of nutritional status must be taken into account when evaluating adequacy, adding to the complexity of evaluating vitamin E status in free-living individuals.

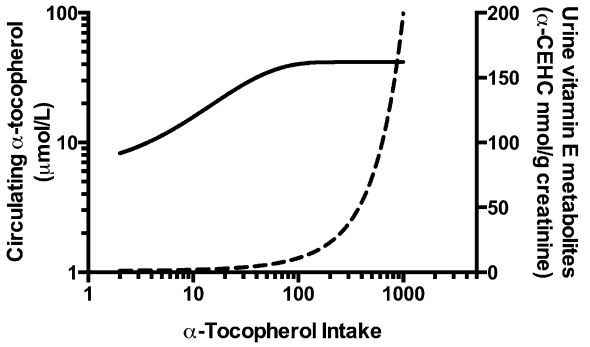

FIGURE 2 Hypothetical relation between dietary α-tocopherol intakes, plasma α-tocopherol concentrations, and urinary excretion of α-carboxy-ethyl-hydroxychromanol (α-CEHC). These hypothetical relations were based on data from published reports (11,45,61) with the assumption that as dietary α-tocopherol intakes increase, plasma α-tocopherol concentrations (solid line, left *y*-axis) reach a plateau. This nexus is the point at which the urinary α-CEHC excretion (dashed line, right *y*-axis) begins an exponential increase.

Consequences of low vitamin E status

It is notoriously difficult to show adverse consequences of vitamin E deficiency in experimental animals, as well as in humans. In patients with cystic fibrosis, anemia and decreased erythrocyte survival (5) were widely accepted as signs of vitamin E deficiency until reports of neurologic abnormalities that responded to vitamin E supplementation were discovered (66–68). The neurologic abnormalities at the outset of vitamin E deficiency are so subtle that they are difficult to assess, but with progression of the deficiency they become readily demonstrable (69,70). This section of the review will therefore address health aspects in which inadequate vitamin E status is detrimental and where supplemental vitamin E has been shown to be beneficial for health, including pregnancy and neurologic diseases.

Is vitamin E deficiency an important cause of spontaneous embryonic death? Vitamin E was discovered nearly 100 y ago because female rats fed a vitamin E–deficient diet resorbed their fetuses early in pregnancy (71); the cause of the embryonic failure has never been fully characterized. We investigated embryonic vitamin E deficiency in a vertebrate model, the zebrafish (*Danio rerio*), and discovered that α-tocopherol and α-TTP have critical roles in embryonic development. We based our research on the observation that α-TTP is expressed in the human yolk sac (72), that zebrafish embryos abundantly express α-TTP by 48 h postfertilization (hpf), and that α-TTP increases with oxidative stress in zebrafish embryos (73). We discovered that

α-tocopherol–deficient adult zebrafish could spawn and produce viable fertilized eggs, but within days the embryos suffered developmental impairment and increased mortality (74). The impaired brain formation in α-TTP knockdown zebrafish embryos raises the possibility that low vitamin E status has adverse events in early central nervous system development in other animals, including humans. Jishage et al. (75) showed that if the mother mouse did not express α-TTP and was not vitamin E supplemented, embryos (regardless of α-TTP status) developed neural tube defects and failed to come to term. Although the study by Jishage et al. focused on mouse maternal α-TTP deficiency, the embryonic phenotype and link to central nervous system development are similar to our findings in the zebrafish. In support of this notion, previous studies showed a clear association between maternal vitamin E status during gestation and cognitive function of the offspring in experimental animal models (76,77).

Importantly, we found that in the zebrafish embryo, α-TTP knockdown caused head malformation before 15 hpf (78). This phenomenon coincides with the timing for increased synthesis of highly peroxidizable lipids by the embryo, evidenced by increased gene expression in the head/brain of 2 FA elongase enzymes, elongation of very long-chain fatty acid (Elovl)4 (79) and Elovl5 (80). When we measured specific PUFA concentrations in zebrafish embryos between 24 and 72 hpf, we found that both α-tocopherol and DHA concentrations decreased in vitamin E–deficient embryos but not in control embryos. Moreover, arachidonic acid concentrations decreased 3 times faster in α-tocopherol–deficient embryos (21 pg/h) compared with vitamin E–sufficient E embryos (7 pg/h) ($P < 0.0001$) (81). At 36 hpf, vitamin E-deficient embryos contained double the 5-hydroxy-eicosatetraenoic acids and 7-hydroxy-DHA concentrations, whereas other detectable hydroxy-lipids remained unchanged (81). Thus, vitamin E deficiency during embryogenesis depleted both omega-3 and omega-6 FAs (DHA and arachidonic acid, respectively) and increased hydroxy-FAs derived from these PUFAs, suggesting that α-tocopherol is necessary to protect these critical FAs during development of the nervous system. Our studies show that the target zone that is most sensitive to α-tocopherol depletion is the head/brain/eye; without delivery of α-tocopherol, the brain fails to develop properly (78).

This absolute requirement for α-tocopherol by the zebrafish embryo takes place during a time analogous to the first 20 d of human embryonic gestation, a time during pregnancy before a woman knows she is pregnant. This time frame is 10–15 hpf for the zebrafish embryo (82), 9.5 d for rats (83), and 17–19 d for humans (84–86). Thus, the requirement for vitamin E very early in human pregnancy is analogous to situations of inadequate folic acid status.

The importance of α-tocopherol for preventing neural tube defects in humans can be surmised from studies in which multivitamins were compared with folic acid supplements. Specifically, folic acid supplements were not as effective in preventing neural tube defects as folic acid/multivitamin combinations, as shown in a review of 5 human trials (87). In a Hungarian trial to evaluate neural tube defects, the multivitamin contained 15 mg vitamin E along with other vitamins (88). The importance of vitamin E in preventing neural tube defects is emphasized by the findings from a study of neural tube defects and maternal micronutrient intakes, including 954 cases (300 with anencephaly, 654 with spina bifida) and 6268 controls (89). A decreased risk of spina bifida was associated with increased intakes of preconception supplements containing antioxidant vitamins E and C, as well as other micronutrients (89). The importance of vitamin E in the nervous system was also supported by a study in China that showed that higher maternal and cord blood α-tocopherol concentrations at birth were associated with improved cognitive function when the child was assessed at age 2 y (90). And conversely, low plasma α-tocopherol concentrations were associated with poorer cognitive function in patients with cystic fibrosis at diagnosis (91,92).

Vitamin E in pregnancy. The role of vitamin E in pregnancy is of increasing concern because it is clear that adequate nutritional status for the first 1000 d of life is necessary for subsequent adult health and well-being, given that stunting cannot be reversed after this critical window (93). Moreover, a study in Egypt emphasized that vitamin E is a key missing micronutrient in stunted children (56). The authors showed that 78.2% of stunted children were vitamin E deficient, with plasma α-tocopherol concentrations of 7.7 μmol/L compared with 14.1 μmol/L in control children (56). Fares et al. (94) reported that vitamin A, E, and D deficiencies were very common in very-low-birth-weight Tunisian neonates and were associated with pre-eclampsia (94). However, pre-eclampsia risk was not changed by vitamin E and C supplements in a number of studies in Western countries (95–99). The lack of benefit of vitamin E supplements in pre-eclampsia may be a result of the relative adequacy of vitamin E status of the women studied. For example, Poston et al. (99) reported that the circulating α-tocopherol:cholesterol ratios were >6 μmol/mmol in the placebo group and >9 μmol/mmol in the vitamin E and C supplement group; these ratios indicate that even the placebo group was well nourished with respect to vitamin E. Taken together, these data indicate that low vitamin E status may increase pre-eclampsia risk, but women with adequate vitamin E status do not benefit further from vitamin E supplements. The definitions of what is "low" and "adequate" vitamin E status are not clearly delineated and merit further research.

Worldwide, the adequacy of α-tocopherol status during pregnancy is unclear and not frequently measured, and thus the utility of vitamin E supplements in improving outcomes has been variable. In situations in which α-tocopherol status was documented to be low, vitamin E supplements had beneficial outcomes. For example, multivitamin supplements containing vitamin E reduced adverse pregnancy outcomes in HIV-positive women in Tanzania (100,101). However, by using a cutoff of <11.6 μmol/L for plasma α-tocopherol concentrations, the prevalence of low vitamin E

status was 5.9% of nonpregnant women of reproductive age in the northern Persian Gulf region, leading the authors to conclude that most women had an adequate vitamin E status. Additionally, in a study in the United States (n = 9968; n = 4992 in the vitamin group and n = 4976 in the placebo group), where at baseline pregnant women were taking 22 IU vitamin E in a daily multivitamin (equal to the RDA), additional vitamin E supplements (400 IU) were not beneficial in reducing the risk of preterm births (102). By contrast, vitamin E supplements were associated with a decreased incidence of preterm births in a Hungarian population study (103). Although it is apparent that the vitamin E status of pregnant women must be adequate to successfully bear a child, these findings suggest that vitamin E supplements in excess of the RDA to adequately nourished women do not provide additional benefits.

Neurologic disease and cognitive impairment with age. Given the importance of vitamin E in the developing nervous system and for the protection of peripheral nerves, as supported by studies in vitamin E–deficient humans and in experimental animals, it seems likely that vitamin E would also protect the nervous system with aging. There are some experimental data to support this hypothesis, especially with regard to Alzheimer disease. Vitamin E supplements were found to have benefit in slowing Alzheimer disease progression (104,105), but they did not seem to prevent Alzheimer disease occurrence (106). A recent meta-analysis found that patients with Alzheimer disease compared with cognitively intact elderly controls had significantly lower plasma α-tocopherol concentrations ($P < 0.001$) (107). Moreover, higher ventricular cerebrospinal fluid α-tocopherol concentrations, measured postmortem in 230 participants from the Religious Orders Study, were associated with a lower density of neuritic plaques and with higher performance on tests of perceptual speed measured before death (108). Furthermore, compared with cognitively normal individuals, patients with either Alzheimer disease or mild cognitive impairment had lower circulating concentrations of all forms of vitamin E and both disorders were associated with increased oxidized vitamin E (109).

In experimental vitamin E deficiency in mice, axonal degeneration was observed in the hippocampus, an important area for memory and cognition (110). The combination of vitamin E deficiency and α-TTP deficiency in mice caused atrophy and decreased branching of Purkinje neurons, which was associated with deficits in motor coordination and cognitive functions that were normalized upon vitamin E supplementation (111). Additionally in mice, impaired vitamin E delivery to the brain resulting from a knockout of the phospholipid transfer protein also resulted in increased memory impairment 1 wk after abeta$_{25–35}$ peptide injection (112). This impairment could be prevented by vitamin E supplementation (112). These experimental findings are consistent with a report in elderly humans showing that a lifelong dietary pattern that results in nutrient intakes that provide increased circulating concentrations of vitamins B, C, D, and E is associated with a larger brain size (as assessed by MRI) and higher cognitive function (113).

Given the importance of vitamin E in protecting unsaturated FAs, it is not surprising that patients with Alzheimer disease have increased concentrations of circulating lipid peroxidation products (114). Importantly, phosphatidylcholine 16:0/22:6 (DHA-PC 38:6), which contains the highly oxidizable FA DHA, was identified as 1 of 10 phospholipids that were depleted in the plasma of human participants who went on to develop Alzheimer disease (115). By contrast, individuals who were in the top quartile of plasma DHA-PC concentrations among the Framingham Heart Study participants had a significant 47% reduction in the risk of developing all-cause dementia (116). Taken together, these findings suggest that vitamin E protects critical FAs in the brain from lipid peroxidation and that improved brain vitamin E status is protective for cognitive function. Interestingly, vitamin E supplements (300 mg daily for 615 d compared with 30 mg for 361 d) were found to double brain α-tocopherol concentrations in a study carried out in 2 terminally ill patients (117).

Conclusions and Speculations

This review evaluated the information available on the adverse consequences of inadequate α-tocopherol status. 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. When low dietary amounts of α-tocopherol are consumed, tissue α-tocopherol needs exceed amounts available, leading to increased damage to target tissues. Hypothetically, these low α-tocopherol intakes in humans lead first to anemia because of the relatively rapid turnover of erythrocytes and their exposure to oxygen and their high iron contents. Further damage might be expected in other tissues with rapid turnover. Potentially, intestinal cells are spared because they are exposed to other dietary antioxidants, as well as to low oxygen concentrations. The nervous system is a special case because α-tocopherol is retained in the brain, likely as a result of brain expression of α-TTP (111). With continued extrahepatic tissue α-tocopherol depletion, peripheral nerves are at risk (5), likely due to their high PUFA contents compared with surrounding tissues. Sensory compared with motor neurons are likely more at risk because the information flow in sensory neurons is from the periphery to the brain, whereas in motor neurons the flow is in the opposite direction, potentially moving α-tocopherol toward the periphery. Severe, or perhaps chronic, vitamin E depletion ultimately decreases brain α-tocopherol, leading to damage and, in the elderly, cognitive impairment.

The adequacy of the middle range of α-tocopherol intakes is difficult to define. Plasma α-tocopherol concentrations between 12 and 20 μmol/L can be raised with increases in dietary intake, suggesting that hepatic α-TTP is not saturated. Studies in experimental animals suggested that hepatic α-TTP maintains circulating α-tocopherol, redistributing it

and potentially allowing tissue α-tocopherol depletion (118). In this case, α-tocopherol returning from the periphery to the liver is not metabolized but is salvaged by hepatic α-TTP and returned to the plasma (119), where it could be taken up by tissues with lipoprotein receptors. This process tends to increase circulating α-tocopherol concentrations and normalize them at the expense of depletion of tissue α-tocopherol.

Hepatic α-tocopherol trafficking, disposition, and metabolism are not well understood or characterized. The well-known lack of correlation between dietary vitamin E intakes and circulating α-tocopherol [for examples, see (58,120,121)] in this middle range of intakes speaks to the efficiency of the regulatory controls governing circulating α-tocopherol concentrations. These processes serve to protect circulating lipids, which are readily oxidized and potentially exposed to higher oxygen concentrations, as well as reactive oxygen species and free metals. Here the special case of fatty liver disease is of interest because the progression of this disorder to more serious forms of the disease is dependent on oxidative damage to lipids (122), suggesting that inadequate vitamin E intakes may promote disease progression.

Supplements providing vitamin E intakes in excess of 100-fold dietary intakes increase plasma concentrations by ~2- to 4-fold above baseline values (123–126). The limitation on plasma concentrations appears to be a result of increased hepatic vitamin E metabolism and excretion, as discussed previously (40). Intakes of 12–15 mg α-tocopherol/d are sufficient in normal healthy adult individuals to provide adequate vitamin E status on the basis of the health benefits associated with these intakes (127).

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