Dietary Zinc Deficiency Decreases Plasma Concentrations of Vitamin E[1] (42876)

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Abstract. **Experiments were conducted to examine the effects of dietary zinc (Zn) upon plasma vitamin E (E) concentrations to test the hypothesis that there may be a significant dietary interaction between these two nutrients. Weanling female Sprague-Dawley rats were fed diets that were (i) Zn-deficient (<0.9 μg Zn/g diet) *ad libitum*; (ii) Zn-adequate (50.9 μg Zn/g diet), pair-fed to the Zn-deficient group; and (iii) Zn-adequate (50.9 μg Zn/g diet) *ad libitum*. Plasma E in Zn-deficient animals (4.02 ± 1.20 μg/ml) was significantly reduced ($P \leq 0.05$) compared with results in both Zn-adequate pair-fed (9.21 ± 0.70 μg/ml) and Zn-adequate *ad libitum*-fed (9.47 ± 0.90 μg/ml) animals. Zn deficiency in this model system also resulted in significant ($P \leq 0.05$) reductions in femur and plasma Zn concentrations as well as in plasma retinol, plasma triglyceride, and plasma cholesterol concentrations. Plasma albumin and total plasma protein concentrations were normal in Zn-deficient animals. With dietary Zn deficiency, the decrease in plasma E appeared to be out of proportion to associated decreases in plasma triglyceride and plasma cholesterol concentrations. Since E is associated with plasma lipoproteins, these data suggest that lipid and/or E malabsorption may be a consequence of Zn deficiency.**

In response to increased dietary intake of E, increments of plasma E were lower in Zn-depleted than in Zn-adequate, pair-fed animals. These findings suggest that dietary Zn deficiency possibly may increase the nutritional requirement for E necessary to maintain adequate plasma concentrations. [P.S.E.B.M. 1989, Vol 190]

Vitamin E (E) is widely recognized as a lipid-soluble antioxidant which protects cell membranes from damage caused by toxic by-products of normal cellular metabolism, radiation, or foreign chemicals (1–3). E also exhibits pronounced effects upon immune function (4) and has been shown to inhibit chemically induced cancers in rats, mice, and hamsters (5–8), as well as transplantable tumors (9). Moreover, E supplementation inhibits formation of nitrosamines both *in vivo* and *in vitro* (7), and recent epidemiologic evidence (10–12) suggests an association between low plasma levels of E and increased risk for developing breast, colon, and lung cancer in humans.

In addition to its role as a component of numerous enzyme systems, zinc (Zn) also affects immune function (13), the stability of cell membranes (14), and carcinogenesis (15). There is evidence that E acts to prevent peroxidation in red blood cell membranes, whereas Zn stabilizes these membranes against damaging events that occur following peroxidation (16) and that supplementation with either nutrient greatly reduces these effects (17). Skin and joint pathologies attributed to Zn deficiency, moreover, are also completely alleviated by the addition of 500 IU/kg of E to the diet (18). These and other observations (19, 20) suggest that a specific dietary interaction may exist between these two nutrients.

In order to provide further evidence for the existence of a significant dietary interaction between Zn and E, we have explored the effects of manipulating the dietary Zn concentration upon the plasma concentration of E, both under basal conditions and following increasing E intake.

Materials and Methods

Animals and Diets. Two experiments were conducted with weanling female Sprague-Dawley rats fed Zn-deficient diets shown in Table I. Rats were housed in stainless steel hanging wire cages in thermostatically controlled animal quarters with a 10-hr light:14-hr dark diurnal cycle. Extreme precautions were taken to prevent Zn contamination of cages, drinking water, and

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Table I. Composition of Basal Diets

Ingredient	EWS Diet (g/kg)	CAA Diet (g/kg)
Egg white, spray dried	200.0	—
Amino acid mix[a]	—	182.9
Dextrose, monohydrate	630.7	636.4
Cottonseed oil	100.0	100.0
Cellulose	30.0	30.0
Vitamin mix[b,c,d]	10.0	10.0
Mineral mix, EWS diet	29.3[e]	—
Mineral mix, CAA diet	—	40.7[f]
	1000.0	1000.0

[a] Amino acid mix (g/kg): L-alanine, 3.5; L-arginine HCl, 12.1; L-asparagine, 9.33; L-aspartic acid, 3.5; L-cystine, 3.5; L-glutamic acid, 40.0; glycine, 23.3; L-histidine HCl-H_2O, 4.5; L-isoleucine, 8.2; L-leucine, 11.1; L-lysine HCl, 18.0; L-methionine, 8.2; L-phenylalanine, 7.5; L-proline, 3.5; L-serine, 3.5; L-threonine, 8.2; L-tryptophan, 1.8; L-tyrosine, 5.0; L-Valine, 8.2.

[b] Vitamin mix (g/kg): Corn starch 4.6669; choline dihydrogen citrate, 3.497; vitamin A palmitate (500,000 units/g), 0.3964; DL-α-tocopheryl acetate (1000 IU/g) 0.1212; dry vitamin D_3 (500,000 IU/g), 0.0044; *p*-aminobenzoic acid, 0.1101; ascorbic acid, coated (97.5%), 1.0166; vitamin B_{12} (0.1% trituration in mannitol), 0.0297; calcium pantothenate, 0.0661; folic acid, 0.002; inositol, 0.1101; menadione, 0.0496; niacin, 0.0991; pyridoxine HCl, 0.022; riboflavin, 0.022; thiamin HCl, 0.022.

[c] Biotin enriched (0.04/kg diet).

[d] Ethoxyquin (0.02 g/kg diet).

[e] Mineral mix (g/kg): $CaHPO_4$, 19.767; KCl, 3.051; NaCl, 3.05; $MgSO_4$, 2.4752; $MnSO_4 \cdot H_2O$, 0.1662; $FeSO_4 \cdot 7H_2O$, 0.2; KIO_3, 0.0004; $CuSO_4 \cdot 5H_2O$, 0.0151; $CrK(SO_4)_2 \cdot 12H_2O$, 0.0193; Na_2SeO_3 (0.0445% in sucrose), 0.5.

[f] Mineral Mix (g/kg): $CaHPO_4$, 20.5; NaCl, 9.15; KCl, 7.63; $MgSO_4$, 2.4752; Na_2SeO_3 (0.0445% in sucrose), 0.5; $FeSO_4 \cdot 7H_2O$, 0.2; $MnSO_4 \cdot H_2O$, 0.1662; $CrK(SO_4)_2 \cdot 12H_2O$, 0.0193; $CuSO_4 \cdot 5H_2O$, 0.0151; KIO_3, 0.0004.

diets. The diets, obtained from Teklad, Inc., Madison, WI, were assayed for Zn content by atomic absorption spectrophotometry and Zn was added to the basal diet in the form of Zn carbonate. The diets were then reanalyzed for Zn to ensure proper formulation and mixing.

Experiment 1. In the first experiment, rats were fed an egg white solid (EWS diet, Table I) Zn-deficient (<0.9 μg Zn/g), or a Zn-adequate (50.9 μg Zn/g) diet for 21 days. Since the level of dietary protein influences both food consumption and carcass weight gain during Zn depletion (21), three levels of dietary protein were examined. Thus, diets utilized in the first experiment contained either 10, 20, or 30% egg white solids as examples of low, adequate, and excess dietary protein.

At all three levels of dietary protein, groups of five rats each were fed diets that were (i) Zn-deficient *ad libitum*; (ii) Zn-adequate pair-fed to the Zn-deficient group; and (iii) Zn-adequate *ad libitum*. Food consumption was recorded daily and body weight recorded weekly for 21 days. At the end of this period, a blood sample was obtained from each rat via cardiac puncture using metofane anesthesia, and each rat was killed by cervical dislocation. Plasma was prepared from heparinized blood by centrifugation at 2500*g* for 3 min and immediately frozen on dry ice. Both femurs from each rat were then dissected and frozen at −20°C for later analysis of tissue Zn content.

Plasma α-tocopherol and retinol concentrations were determined by reverse phase high pressure liquid chromatography according to the method of Driskel *et al.* (22). Plasma Zn concentrations were measured by flame atomic absorption spectrophotometry after diluting specimens 10-fold with distilled deionized water. Absorption of samples was compared with Zn nitrate samples prepared in 2.5% glycerol. The Zn content of liver and bone was determined by atomic absorption spectrophotometry after wet ashing in 50% H_2O_2 and HNO_3 (26). Plasma cholesterol concentrations were measured by a specific total enzymatic procedure (23). Plasma albumin and total plasma protein concentrations were determined by colorimetric procedures (24, 25).

Experiment 2. In the second experiment, Zn-deficient and Zn-supplemented diets containing 0, 25, 125, 500, and 1500 units of all-rac-α-tocopherol acetate were fed to groups of five female Sprague-Dawley rats for 28 days. All diets contained 20% protein (EWS diet, Table I). The Zn-deficient diets were fed *ad libitum* and the Zn-supplemented diets containing the corresponding level of vitamin E were pair-fed to the level of intake of the Zn-deficient group. Food consumption was recorded daily and body weight weekly. In addition, a Zn-adequate *ad libitum*-fed control group of five rats fed the EWS diet and two treatment groups consisting of five rats each were fed an alternative source of dietary protein. The alternative source of dietary protein was a diet formulated with crystalline amino acids (CAA diet, Table I) instead of spray-dried egg white solids. At the end of this period plasma α-tocopherol and plasma Zn concentrations were determined in all animals as outlined in Experiment 1.

Data were evaluated statistically utilizing analysis of variance employed at the Memorial Sloan-Kettering Department of Medical Physics Computer Facility (27, 28).

Results

Experiment 1. At all levels of dietary protein, Zn deficiency resulted in significant reductions in carcass weight gain and significant elevations in efficiency of feed utilization compared with Zn-adequate *ad libitum*-fed controls (Table II). The carcass weight gain during the 21-day experiment for Zn-adequate pair-fed rats was not significantly different from that of the Zn-deficient *ad libitum*-fed experimental group. Plasma and femur Zn concentrations were significantly reduced in Zn-deficient rats compared with both Zn-adequate pair-fed and Zn-adequate *ad libitum*-fed controls.

Dietary Zn deficiency results in a significant reduction in plasma α-tocopherol concentrations compared with that in both Zn-adequate *ad libitum*-fed and pair-fed controls (Fig. 1). Significant reductions in plasma retinol and plasma triglyceride concentrations were also observed (Table III). Plasma cholesterol concentration was significantly ($P \leq 0.05$) reduced only in rats fed 30% protein. It is important to note that the decreases in plasma α-tocopherol were out of proportion to that of plasma cholesterol and plasma triglycerides. Thus, Zn deficiency resulted in significant reductions in the ratio of plasma α-tocopherol:plasma cholesterol (Fig. 2). Plasma albumin and total plasma protein concentrations were not affected by dietary Zn or dietary protein level in this experiment (Table III).

Experiment 2. In the second experiment, Zn-deficient and Zn-supplemented diets containing 0, 25, 125, 500, and 1500 units of all-rac-α-tocopherol acetate per kg were fed to female Sprague-Dawley rats for 28 days. The Zn-deficient diets were fed *ad libitum*, and Zn-supplemented diets containing equivalent levels of α-tocopherol were pair-fed to the level of intake of the Zn-depleted animals.

The concentration of α-tocopherol in plasma of Zn-deficient rats was observed to be lower than that observed in the Zn-supplemented pair-fed rats at all levels of dietary vitamin E supplementation (Fig. 3). An elevation in plasma α-tocopherol concentration was observed in the Zn-deficient rats with increasing vitamin E supplementation. However, feeding diets containing 12 times that level of vitamin E to the deficient group of rats did not bring the plasma level of α-tocopherol up to that observed in the +Zn group fed 125 mg of E. In addition, the Zn-supplemented *ad libitum*-fed rats displayed plasma α-tocopherol concentrations that were not significantly different ($P > 0.05$) from that observed in the Zn-adequate pair-fed rats. Mean carcass weight gains for all groups of Zn-adequate pair-fed animals were not significantly different from corresponding Zn-deficient *ad libitum*-fed experimental groups (data not shown). Rats fed the Zn-supplemented diet *ad libitum* gained 130.3 ± 4.0 g of carcass

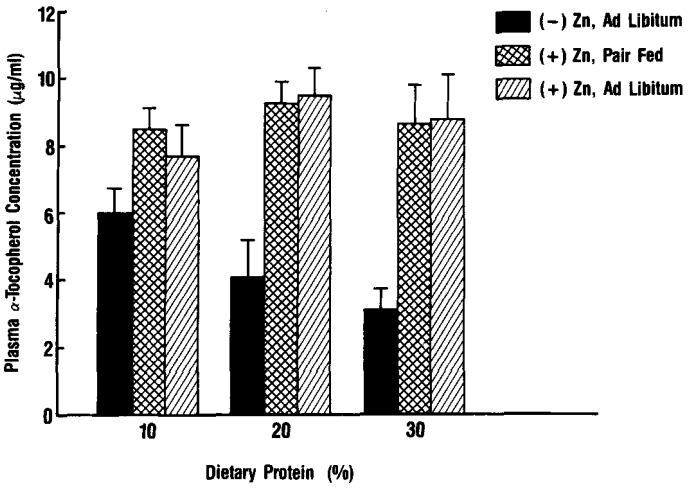

Figure 1. Effect of dietary zinc and protein on plasma α-tocopherol concentration in female Sprague-Dawley rats. Values represent mean ± SE. By analysis of variance the α-tocopherol data showed a Zn effect, $F = 29.95$, $P \leq .0001$; a protein effect, $F = 0.5$, $P \leq 0.610$; and a Zn × protein effect, $F = 1.48$, $P \leq 0.23$, Experiment 1.

Table II. Response of Female Sprague-Dawley Rats to Dietary Zinc and Dietary Protein (Experiment 1)

Dietary treatment			Cumulative carcass weight gain[b] (g)	Feed/gain[b,c] (g/g)	Plasma Zn[b] (μg/dl)	Femur Zn[b] (μg/g)
Zn[a] (μg/g)	Protein (%)	Level				
0.9	10	*Ad libitum*	15.24 ± 0.97	9.68 ± 0.84	62.0 ± 11.2	103.5 ± 8.1
50.9	10	Pair-fed	22.82 ± 3.17	5.71 ± 0.70	115.0 ± 6.9	314.6 ± 4.7
50.9	10	*Ad libitum*	77.52 ± 4.23	3.72 ± 0.13	133.0 ± 7.2	333.4 ± 6.3
0.9	20	*Ad libitum*	15.98 ± 0.76	7.51 ± 0.49	54.0 ± 1.9	98.2 ± 7.1
50.9	20	Pair-fed	15.88 ± 2.00	6.99 ± 0.87	122.5 ± 8.8	405.4 ± 13.7
50.9	20	*Ad libitum*	100.36 ± 6.63	2.76 ± 0.19	132.0 ± 4.1	373.5 ± 7.2
0.9	30	*Ad libitum*	13.08 ± 1.04	8.73 ± 0.65	62.5 ± 4.3	106.1 ± 6.8
50.9	30	Pair-fed	22.30 ± 2.13	4.76 ± 0.44	143.0 ± 4.4	434.3 ± 14.5
50.9	30	*Ad libitum*	109.64 ± 5.04	2.38 ± 0.65	139.0 ± 6.8	451.8 ± 8.9
Statistical analysis						
Protein[d]			6.45 (.0041)	3.17 (.0544)	5.75 (.0070)	62.86 (.0001)
Zinc[e]			496.07 (.0001)	81.23 (.0001)	110.65 (.0001)	1028.63 (.0001)
Protein × zinc[f]			8.79 (.0001)	3.17 (.0254)	1.23 (.3175)	17.63 (.0001)

[a] Zinc added as $ZnCO_3$ to basal diet shown in Table I containing ≤0.9 g of Zn diet as described in Materials and Methods.
[b] Mean ± SEM for five female rats per treatment group.
[c] Mean ± SEM for grams of feed consumed per gram of carcass weight gain during 21-day experiment.
[d] By analysis of variance, protein effect with 2 df; *F* (*P* value).
[e] By analysis of variance, zinc effect with 2 df; *F* (*P* value).
[f] By analysis of variance, protein × zinc interaction with 4 df; *F* (*P* value).

Table III. Plasma Levels of Lipid and Protein Constituents under Different Dietary Treatments in Female Rats (Experiment I)

Dietary treatment			Retinol[a]	Triglycerides[a]	Cholesterol[a]	Albumin[b]	Protein[b]
Zn[a] (μg/g)	Protein (%)	Level	(μg/dl)	(μg/dl)	(mg/dl)	(g/dl)	(g/dl)
0.9	10	*Ad libitum*	17.97 ± 2.80	20.40 ± 3.95	70.60 ± 7.44	3.28 ± 0.15	4.78 ± 0.20
50.9	10	Pair-fed	29.62 ± 1.05	56.80 ± 12.96	74.60 ± 3.36	2.84 ± 0.17	4.52 ± 0.22
50.9	10	*Ad libitum*	41.32 ± 4.42	88.40 ± 10.32	60.40 ± 5.35	2.96 ± 0.51	4.90 ± 0.07
0.9	20	*Ad libitum*	17.23 ± 1.90	18.20 ± 3.28	52.80 ± 12.41	3.28 ± 0.13	4.80 ± 0.22
50.9	20	Pair-fed	24.48 ± 2.29	59.75 ± 6.01	73.00 ± 5.46	3.00 ± 0.13	4.53 ± 0.15
50.9	20	*Ad libitum*	30.69 ± 2.46	79.40 ± 7.62	69.00 ± 3.36	3.36 ± 0.07	5.36 ± 0.10
0.9	30	*Ad libitum*	7.35 ± 1.21	14.25 ± 3.07	43.50 ± 4.48	3.23 ± 0.19	4.67 ± 0.22
50.9	30	Pair-fed	22.97 ± 1.02	58.80 ± 14.17	79.00 ± 10.47	3.18 ± 0.04	5.00 ± 0.06
50.9	30	*Ad libitum*	33.14 ± 3.44	48.80 ± 4.07	63.80 ± 9.18	3.18 ± 0.02	5.14 ± 0.06
Statistical analysis							
Protein[c]			6.55 (.0040)	2.64 (.0860)	0.38 (.6870)	2.25 (.0952)	1.57 (.2220)
Zinc[d]			47.17 (.0001)	34.46 (.0001)	4.63 (.0166)	3.84 (.0314)	6.03 (.0057)
Protein × zinc[e]			1.54 (.2123)	1.44 (.2431)	1.55 (.2089)	1.55 (.2228)	1.70 (.1731)

[a] See footnotes *a* and *b*, Table II.
[b] Differences not significant ($P > 0.05$).
[c] See footnote *c*, Table II.
[d] See footnote *e*, Table II.
[e] See footnote *f*, Table II.

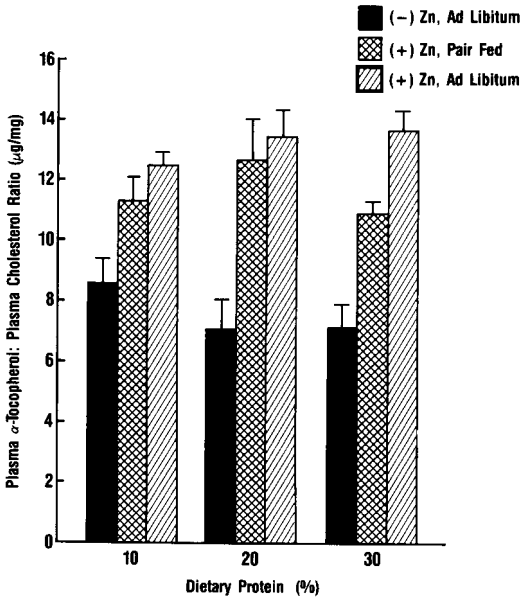

Figure 2. Effect of dietary zinc and protein on plasma ratio of α-tocopherol:cholesterol in female Sprague-Dawley rats. Values represent mean ± SE. By analysis of variance, the plasma E:plasma cholesterol data showed a Zn effect, $F = 37.49$, $P \leq .0001$; a protein effect, $F = 0.440$, $P \leq .8705$; and a Zn × protein effect, $F = 1.37$, $P \leq .2667$, Experiment 1.

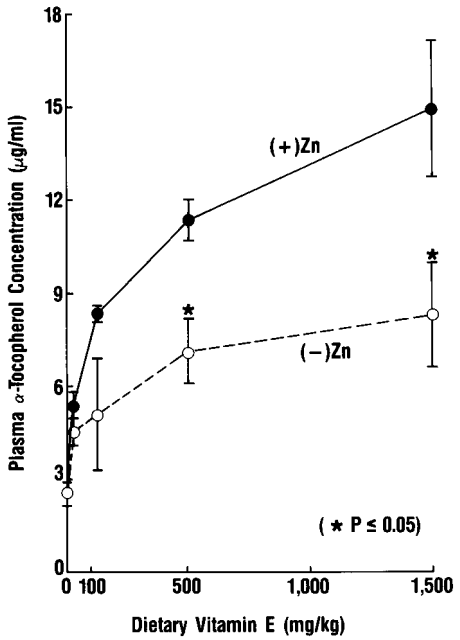

Figure 3. Effect of dietary vitamin E supplementation upon the zinc-plasma vitamin E interaction in female Sprague-Dawley rats. Vertical bars represent mean ± SE, Experiment 2.

weight, which was comparable to that observed in the first experiment.

The source of dietary amino acids appeared to have no effect upon the interaction between Zn and plasma α-tocopherol concentration as Zn-deficient rats fed diets containing crystalline amino acids also displayed significantly reduced plasma α-tocopherol concentrations compared with Zn-supplemented rats fed the same amino acid (5.9 ± 1.3 μg/ml vs 11.3 ± 1.1 μg/ml; $P \leq 0.05$).

Discussion

The present experiments were conducted to examine the relationship between dietary Zn and E metabolism. The results demonstrate that dietary Zn deficiency significantly reduces both the absolute concentration of E in plasma as well as the plasma E: plasma cholesterol ratio, which is considered a more accurate

indicator of E nutritional status (29). In addition, even under conditions of excess dietary E supplementation, the plasma increments of E were lower in Zn-depleted than in Zn-adequate pair-fed animals.

Deficiencies of Zn and E result in profound alterations in reproductive capacity, immune function, membrane stability, and experimental carcinogenesis. Both Zn and E are reduced in plasma of subjects with sickle cell anemia and administration of either nutrient reduces the number of irreversibly sickled cells (30, 31). Recent findings also suggest that epithelial pathologies attributed to Zn deficiency in animal model systems may be alleviated by E supplementation (18). Therefore, the present study may have important implications for several human disease states and the significance of the interaction between dietary Zn deficiency and plasma E relating to the nutritional and physiologic conditions mentioned above remains to be clarified.

Zn-depleted humans with acrodermatitis enteropathica (32) and protein-calorie malnutrition (33) display similar symptoms which may be reversed by Zn supplementation. No studies, however, have considered the possibility that immune and/or reproductive disorders associated with these human conditions may be due in part to E deficiency, which is secondary to Zn depletion. Patients with chronic cholestatic liver disease, moreover, display low plasma E levels and are known to be Zn depleted (34). To our knowledge no studies have indicated that Zn supplementation may improve E status in these patients.

Although this study has only considered plasma concentrations of α-tocopherol and ratios of plasma α-tocopherol:plasma cholesterol in Zn-depleted rats, these results provide a strong foundation for looking further at the mechanism by which plasma α-tocopherol levels are lowered by dietary Zn deficiency and more closely at the nutritional significance of this interaction.

Possible mechanisms for the reduction in plasma E concentrations observed during Zn depletion include effects upon absorption or transport of tocopherol by the intestinal mucosa and the blood transport system, or a combination of the above. Specific mechanisms to explain this interaction include the consideration that (i) since both bile and pancreatic secretions are necessary for hydrolysis of tocopheryl-acetate (the form of vitamin E in our diets) and absorption of free tocopherol, Zn depletion may impact upon plasma tocopherol concentrations by adversely affecting pancreatic and/or hepatic function; (ii) the transfer of tocopherol across the epithelial cell is thought to require several stages of processing and Zn depletion may affect any number of these stages; and/or (iii) tocopherols circulate in the lymph and blood bound to the lipoproteins and are generally distributed according to the lipid composition of each fraction. Since the concentrations of both plasma triglycerides and plasma α-tocopherol were significantly reduced, these data strongly suggest an effect of Zn deficiency upon transport of lipid constituents in plasma.

At present there is no evidence of a specific transport protein for α-tocopherol in blood; therefore, possible effects of Zn depletion upon pancreatic function, lipid absorption, and/or lipoprotein metabolism warrant further investigation. Additional studies are presently under way to elucidate the precise effects of Zn deficiency on vitamin E absorption and transport.

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