Assessment of the safety of supplementation with different amounts of vitamin E in healthy older adults[1–4]

Simin Nikbin Meydani, Mohsen Meydani, Jeffrey B Blumberg, Lynette S Leka, Marcos Pedrosa, Richard Diamond, and Ernst J Schaefer

ABSTRACT We showed previously that supplementation for 30 d with 800 IU (727 mg) vitamin E/d did not adversely affect healthy elderly persons. We have now assessed the effects of 4 mo of supplementation with 60, 200, or 800 IU (55, 182, or 727 mg) *all-rac*-α-tocopherol/d on general health, nutrient status, liver enzyme function, thyroid hormone concentrations, creatinine concentrations, serum autoantibodies, killing of *Candida albicans* by neutrophils, and bleeding time in 88 healthy subjects aged >65 y participating in a double-blind, placebo-controlled trial. No side effects were reported by the subjects. Vitamin E supplementation had no effect on body weight, plasma total proteins, albumin, glucose, plasma lipids or the lipoprotein profile, total bilirubin, alkaline phosphatase, serum aspartate aminotransferase, serum alanine aminotransferase, lactate dehydrogenase, serum urea nitrogen, total red blood cells, white blood cells or white blood cell differential counts, platelet number, bleeding time, hemoglobin, hematocrit, thyroid hormones, or urinary or serum creatinine concentrations. Values from all supplemented groups were within normal ranges for older adults and were not significantly different from values in the placebo group. Vitamin E supplementation had no significant effects on plasma concentrations of other antioxidant vitamins and minerals, glutathione peroxidase, superoxide dismutase, or total homocysteine. There was no significant effect of vitamin E on serum nonspecific immunoglobulin concentrations or anti-DNA and anti-thyroglobulin antibodies. The cytotoxic ability of neutrophils against *Candida albicans* was not compromised. Thus, 4 mo of supplementation with 60–800 IU vitamin E/d had no adverse effects. These results are relevant for determining risk-to-benefit ratios for vitamin E supplementation. *Am J Clin Nutr* 1998; 68:311–8.

KEY WORDS Vitamin E, safety, elderly, supplementation, *all-rac*-α-tocopherol, toxicity, bleeding time, neutrophil cytotoxicity

INTRODUCTION

Evidence from clinical trials and epidemiologic studies suggests that vitamin E supplementation is associated with reduced risk for some forms of cancer, diabetes, cataract, and heart disease (1). Furthermore, several studies suggest that vitamin E may be valuable as an adjunctive therapeutic agent in the treatment of some of these conditions, eg, in coronary artery disease (2), diabetes (3), colorectal adenomas (4), and relative anergy in older adults (5, 6). However, the application of these observations to the general public or an individual patient requires that the potential benefits be weighed against the potential risks, including those of acute and chronic toxicity (7).

The literature concerning the safety of vitamin E has been reviewed in detail (8–11). Subacute and subchronic toxicity studies in laboratory animals have consistently found no evidence of carcinogenic, hematologic, mutagenic, or adverse reproductive effects with high doses of vitamin E (12). Nonetheless, most of the clinical trials testing the efficacy and safety of vitamin E supplementation were of short duration and examined only a single dose. We previously characterized the clinical toxicology of short-term (30 d), high-dose (800 IU/d, or 727 mg) vitamin E supplementation in elderly volunteers (13). We present here toxicologic data from a long-term, dose-response study of vitamin E supplementation in healthy older adults, the segment of the US population with perhaps the highest consumption of vitamin E supplements (14). Furthermore, in addition to the indexes tested in our short-term study (13), the present study also focused on the effect of vitamin E on plasma lipoprotein concentrations, bleeding time, serum autoantibody concentrations, and neutrophil cytotoxicity.

[1] From the Jean Mayer US Department of Agriculture Human Nutrition Research Center on Aging at Tufts University, Boston; the Department of Gastroenterology, Veterans' Affairs Medical Center, Boston; and the Infectious Disease Program, University Hospital, Boston University.

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[4] Address reprint requests to SN Meydani, Nutritional Immunology Laboratory, Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University, 711 Washington Street, Boston, MA 02111. E-mail: S_Meydani_im@hnrc.tufts.edu.

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TABLE 1
Methods and reference ranges

Analyte	Method reference	Reference range[1]
Vitamin A (μmol/L)	15	1.05–3.15
Vitamin B-6 (nmol/L)	16–19	14.6–72.8
Vitamin B-12 (pmol/L)	Unpublished[2]	111–886
Folate (nmol/L)	Unpublished[2]	6.8–68.0
Total carotenoids (μmol/L)	20	0.74–4.46
Vitamin C (μmol/L)	21, 22	22.7–124.9
Vitamin E (μmol/L)	15	11.6–41.8
Glutathione peroxidase (U/L)	23	Unknown
Superoxide dismutase (U/L)	Unpublished[3]	20.0–80.0
Copper (μmol/L)	24	8.6–27.5
Iron (μmol/L)	25	9.0–30.4
Zinc (μmol/L)	26	10.7–19.9
Total homocysteine (μmol/L)	27	5.5–15.0
White blood cell count ($\times 10^9$/L)	28	(3.9–10.6), (3.5–10.0)
Lymphocyte	28	0.13–0.52
Monocyte	28	0.01–0.08
Basophil	28	0–0.02
Neutrophil	28	0.37–0.80
Erythrocyte count ($\times 10^{12}$/L)	29–32[4]	[(4.5–6.0), (4.0–5.5)]
Hemoglobin (g/L)	29–32	[(130–180), (115–160)]
Hematocrit	29–32	[(0.40–0.53), (0.36–0.49)]
Platelet count ($\times 10^9$/L)	29–32	[140–400]
Albumin (g/L)	33	[(37–54), (36–53)]
Alanine aminotransferase (μkat/L)	34	[0.07–0.62]
Alkaline phosphatase (μkat/L)	35	[0.50–1.71]
Aspartate aminotransferase (μkat/L)	34	[0.15–0.57]
Bilirubin, total (μmol/L)	36	[0–25.6]
Lactate dehydrogenase (μkat/L)	37	[1.7–3.48]
Total protein (g/L)	38	[60–80]
Urine creatinine (mmol/L)	39	(2.30–17.68), (1.77–14.14)
Creatinine excretion (mmol/d)	39	(7.1–19.4), (5.3–15.9)
Serum creatinine (μmol/L)	39	[44.2–114.9]
Blood urea nitrogen (mmol/L)	40	[2.5–8.2]
Blood urea nitrogen:creatinine	—	[22–186]
Blood glucose (mmol/L)	41	[3.6–6.2]
Plasma triacylglycerols		
(mmol/L)	42–44	(0.56–2.39), (0.46–2.30)
(mg/dL)	42–44	(50–212), (41–204)
Plasma total cholesterol		
(mmol/L)	45	(3.4–6.5), (3.4–7.2)
(mg/dL)	45	(130–252), (130–278)
Plasma HDL		
(mmol/L)	45, 46	(0.8–1.8), (1.0–2.1)
(mg/dL)	45, 46	(32–70), (37–80)
Plasma LDL		
(mmol/L)	47	(1.9–4.7), (1.6–4.9)
(mg/dL)	47	(73–182), (62–189)
Plasma VLDL		
(mmol/L)	47	(0.1–0.8), (0.08–0.88)
(mg/dL)	47	(5–31), (3–34)
LDL:HDL	—	(1.1–5.9), (0.8–4.9)
Thyroxine, total (nmol/L)	Unpublished[5]	57–161
Free thyroxine index	—	[1.6–3.2]
Triiodothyronine uptake	Unpublished[6]	0.25–0.35
Triiodothyronine uptake ratio[7]	—	[0.82–1.13]

[1] Values in parentheses are for males and females, respectively. Values in square brackets are intralaboratory ranges.

[2] Quantaphase II B12/Folate Radioassay; BioRad Laboratories, Hercules, CA.

[3] Superoxide dismutase assay kit; Calbiochem-Novabiochem Corporation, San Diego.

[4] Manual DS-014, System 9000 Automated Cell Counter, 1988; Baker Instrument Corporation, Allentown, PA.

[5] Magic T_4 (iodine) radioimmunoassay, revision D, document 103007001, 1988; Ciba Corning Magnetic Immunochemistries, Medfield, MA.

[6] Magic T_3 uptake (iodine) radioimmunoassay, document 103043001, 1988; Ciba Corning Magnetic Immunochemistries.

[7] (Bound:nonbound thyroxine) × standardization factor.

SUBJECTS AND METHODS

Study subjects and experimental design

Characteristics of the study subjects, the exclusion criteria, and the experimental design were reported previously (6). Briefly, 88 free-living, healthy, nonsmoking men and women aged ≤65 y, with no known medical illnesses and taking neither prescription medication nor dietary supplements, participated in a double-blind, placebo-controlled trial of the effect of supplementation with different doses of vitamin E on the immune response. Not all subjects finished the study. In addition, samples for biochemical analysis of some indexes were not available from all subjects at all time points. For details of subject recruitment, the dropout rate, and other specifics, *see* our previous report (6). Subjects were randomly assigned to groups given a placebo or 60, 200, or 800 IU (55, 182, or 727 mg) vitamin E/d. Each group received 2 capsules daily to be ingested with dinner. Vitamin E capsules contained 30, 100, or 400 IU (27, 91, or 364 mg) *all-rac*-α-tocopherol in soybean oil (Roche Vitamins, Inc, Parsippany, NJ); the placebo capsules contained soybean oil only and were identical in taste and appearance to the vitamin E capsules. Plasma vitamins, trace elements, antioxidant status, hematologic status, hepatic and renal function, intermediary metabolism, bleeding time, serum autoantibodies, and the ability of neutrophils to kill *Candida albicans* were assessed before and after 4 mo of supplementation with vitamin E. The experimental protocol was approved by the Human Investigation Review Committee of the New England Medical Center and Tufts University. All of the participating subjects signed an approved consent form.

Procedures

With the exception of lipid, neutrophil killing, and autoantibody measurements, all analyses were performed by the Nutritional Evaluation Laboratory of the Jean Mayer US Department of Agriculture Human Nutrition Research Center on Aging at Tufts University by using standard methods as described previ-

TABLE 2
Effect of vitamin E supplementation on serum or plasma vitamin status in healthy elderly[1]

	Placebo		60 IU (55 mg)		200 IU (182 mg)		800 IU (727 mg)	
Nutrient	Before	After	Before	After	Before	After	Before	After
Vitamin A (μmol/L)	1.9 ± 0.3	1.9 ± 0.4	2.0 ± 0.3	1.8 ± 0.4	1.9 ± 0.3	1.9 ± 0.4	2.0 ± 0.4	1.9 ± 0.5
Total carotenoids (μmol/L)	2.32 ± 0.78	2.27 ± 0.76	2.56 ± 0.85	2.48 ± 1.00	2.76 ± 1.44	2.74 ± 0.99	2.13 ± 0.81	2.26 ± 0.82
Vitamin C (μmol/L)	84 ± 35	75 ± 33	97 ± 20	91 ± 26	90 ± 32	79 ± 35	95 ± 41	81 ± 31
Vitamin E (μmol/L)	24.5 ± 4.7	23.3 ± 2.2	27.2 ± 6.1	38.4 ± 5.3[2]	25.6 ± 5.7	51.0 ± 13.6[3]	25.8 ± 6.3	71.5 ± 26.5[2]
Vitamin B-6 (nmol/L)	33.0 ± 16.5	32.9 ± 15.8	50.4 ± 28.1	40.1 ± 20.8[3]	45.0 ± 25.0	45.1 ± 27.8	36.7 ± 19.0	28.8 ± 9.7
Vitamin B-12 (pmol/L)	249 ± 63	233 ± 66	299 ± 164	292 ± 133	264 ± 73	262 ± 79	241 ± 103	231 ± 116
Folate (nmol/L)	15.1 ± 7.1	14.8 ± 7.6	19.7 ± 13.2	21.1 ± 14.6	20.5 ± 11.9	19.0 ± 11.1	18.6 ± 9.1	17.3 ± 10.9
Total homocysteine (μmol/L)	10.5 ± 2.2	11.2 ± 2.5	9.7 ± 2.3	9.7 ± 2.2	10.0 ± 1.8	9.7 ± 2.0	10.1 ± 2.6	11.1 ± 3.8

[1] $\bar{x}$ ± SD; n = 16–20. All measurements were made in plasma except for vitamin E, which was measured in serum. Repeated-measures ANOVA indicated a significant time and group interaction for vitamins E and B-6, $P < 0.05$.

[2,3] Significantly different from before supplementation (paired Student's t test followed by Bonferroni correction for multiple comparisons): [2] $P < 0.001$, [3] $P < 0.03$.

ously (13). For assays in which commercial kits were used, the reference ranges were established by the manufacturers. For assays developed by the Nutritional Evaluation Laboratory, reference ranges were established by using values from a normally distributed healthy adult population (**Table 1**) (48). Methods for assessing nutrient status, hematologic status, hepatic and renal function, and intermediary metabolism are referenced in Table 1. Methods for serum autoantibody and neutrophil cytotoxicity were published previously (6). Bleeding time was assessed by using Simplate II (Organon Teknika, Durham, NC) as described by Kumar et al (49).

Statistical analysis

Data were analyzed by repeated-measures analysis of variance (ANOVA) for the effect of time. Differences in dose response were tested as group-by-time interactions in ANOVA. Data within each group were analyzed by Student's t test (for variables having normal distributions) or the paired Wilcoxon signed-rank test (for variables not having normal distributions). Adjustments for multiple comparisons were made by placing Bonferroni bounds on P values. Data are reported as means ± SDs; significance was determined at $P < 0.05$.

RESULTS

As reported earlier, there was no significant effect of vitamin E supplementation on body weight or the general health of subjects except for a significant improvement in in vivo indexes of T cell–mediated function (6). In addition, vitamin E–supplemented subjects tended to show a nonsignificant ($P = 0.09$) 30% reduction in incidence of self-reported infections (6). Plasma or serum vitamin concentrations and trace element concentrations in subjects receiving placebo or different doses of vitamin E are shown in **Table 2** and **Table 3**. Plasma vitamin and trace mineral concentrations before supplementation were not significantly different in the placebo and vitamin E–supplemented groups. As reported previously (6) and as shown in Table 2, serum vitamin E concentrations increased significantly in all 3 vitamin E–supplemented groups whereas no significant change was observed in the placebo group. With the exception of a decrease in vitamin B-6 concentrations in the group receiving 60 IU/d, there was no significant change in concentrations of other fat- or water-soluble vitamins, trace elements (Table 3), or other minerals (data not shown) in any of the groups. The reason for the decrease in vitamin B-6 in the 60-IU/d group and not in either of the groups supplemented with higher amounts of vitamin E is not clear. However, it should be noted that even in the 60-IU/d group the postsupplementation values remained within the normal range. Furthermore, no effects of vitamin E supplementation on glutathione peroxidase activity, superoxide dismutase activity, or total homocysteine concentrations were observed. In a previous study (13), we reported a small but significant increase in plasma zinc concentrations after 1 mo of supplementation with 800 IU vitamin E/d. In this study, no such effect was observed. It is possible that our previous finding reflected a transient change in plasma zinc concentrations that is not sustained after long-term vitamin E supplementation.

No significant differences were observed in hematologic status (including white blood cell differentials as well as blood cell and platelet counts) between the 4 groups before or after supplementation. Furthermore, none of the groups showed a significant change in these indexes with time with the exception of a small

TABLE 3
Effect of vitamin E supplementation on trace element status in healthy elderly[1]

	Placebo		60 IU (55 mg)		200 IU (182 mg)		800 IU (727 mg)	
Nutrient	Before	After	Before	After	Before	After	Before	After
Copper (μmol/L)	17 ± 3	17 ± 3	19 ± 3	19 ± 2	18 ± 3	18 ± 3	19 ± 3	19 ± 3
Iron (μmol/L)	20 ± 7	19 ± 8	18 ± 5	18 ± 4	17 ± 6	18 ± 7	19 ± 7	19 ± 6
Zinc (μmol/L)	14 ± 2	14 ± 2	14 ± 1	13 ± 1	13 ± 1	13 ± 1	14 ± 2	13 ± 2
Glutathione peroxidase (U/L)	203 ± 24	207 ± 27	215 ± 29	218 ± 27	210 ± 34	220 ± 33	205 ± 30	217 ± 33
Superoxide dismutase (10^3 U/L)	48 ± 6	50 ± 4	47 ± 5	48 ± 4	47 ± 5	47 ± 5	47 ± 5	46 ± 4

[1] $\bar{x}$ ± SD; n = 15–20. No statistically significant differences were observed.

TABLE 4
Effect of vitamin E supplementation on hematologic status of healthy elderly[1]

	Placebo		60 IU (55 mg)		200 IU (182 mg)		800 IU (727 mg)	
Index	Before	After	Before	After	Before	After	Before	After
Total WBC count ($\times 10^9$/L)	6.1 ± 1.0	5.9 ± 1.0	5.6 ± 1.1	5.6 ± 1.3	6.0 ± 1.5	6.1 ± 1.4	5.6 ± 1.1	5.4 ± 1.1
Lymphocyte	0.31 ± 0.06	0.30 ± 0.06	0.31 ± 0.06	0.34 ± 0.07[2]	0.30 ± 0.06	0.30 ± 0.07	0.30 ± 0.06	0.30 ± 0.06
Monocyte	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.02	0.06 ± 0.01	0.05 ± 0.02	0.05 ± 0.02	0.06 ± 0.01	0.05 ± 0.01
Basophil	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.01 ± 0	0.01 ± 0	0 ± 0.01	0 ± 0
Neutrophil	0.60 ± 0.07	0.61 ± 0.06	0.61 ± 0.07	0.58 ± 0.08	0.61 ± 0.06	0.61 ± 0.07	0.62 ± 0.07	0.62 ± 0.07
Erythrocyte count ($\times 10^{12}$/L)	4.6 ± 0.3	4.6 ± 0.3	4.5 ± 0.4	4.6 ± 0.4	4.6 ± 0.3	4.7 ± 0.4	4.6 ± 0.4	4.6 ± 0.4
Hemoglobin (g/L)	138 ± 9	138 ± 10	135 ± 8	136 ± 11	139 ± 10	139 ± 12	139 ± 12	138 ± 13
Hematocrit	0.41 ± 0.03	0.41 ± 0.03	0.40 ± 0.03	0.41 ± 0.03	0.41 ± 0.03	0.42 ± 0.04	0.41 ± 0.03	0.41 ± 0.03
Platelet count ($\times 10^9$/L)	247 ± 59	251 ± 74	247 ± 39	245 ± 37	249 ± 59	256 ± 62	259 ± 49	253 ± 54

[1] $\bar{x}$ ± SD; n = 17–20. Repeated-measures ANOVA indicated a significant time and group interaction, $P = 0.055$. WBC, white blood cell.
[2] Significantly different from before supplementation, $P < 0.05$ (paired Student's t test followed by Bonferroni correction for multiple comparisons).

but significant increase in percentage lymphocytes in the 60-IU/d group. However, the change in the 60-IU/d group was not significantly different from that in the placebo group. All values in the placebo and vitamin E–supplemented groups remained within normal ranges throughout the course of the study (**Table 4**).

Hepatic (**Table 5**) and renal (**Table 6**) function indexes were within normal ranges both before and after supplementation in all groups and were not significantly different. As shown in Table 5, there was a small (4.5%) but significant decrease in plasma albumin concentrations in subjects supplemented with 60 IU vitamin E/d but not in subjects supplemented with higher doses. The reason for this decrease in the subjects consuming the lowest dose of vitamin E is not clear; however, it is doubtful that this change would be of clinical significance to the elderly because postsupplementation values remained within the normal range. There was also a significant decrease in alanine aminotransferase in the subjects consuming 800 IU vitamin E/d. This, again, would not be of clinical significance because postsupplementation values were well within the normal range. If anything, the decrease should be considered a beneficial rather than an adverse effect.

Blood glucose and thyroid hormone concentrations were not different in the placebo and vitamin E–supplemented groups before supplementation and did not change significantly in any group during the study period (**Table 7**). Plasma lipid and lipoprotein values are shown in **Table 8**. There were no significant differences in plasma lipid or lipoprotein concentrations before supplementation among the different treatment groups. There was no significant change in any of the plasma lipid indexes in subjects consuming placebo, 60 IU vitamin E/d, or 800 IU vitamin E/d. There was no significant change in total cholesterol, HDL, LDL, or the ratio of LDL to HDL cholesterol in subjects consuming 200 IU vitamin E/d. There was, however, a significant increase in

TABLE 5
Effect of vitamin E supplementation on hepatic function in healthy elderly[1]

	Placebo		60 IU (55 mg)		200 IU (182 mg)		800 IU (727 mg)	
Index	Before	After	Before	After	Before	After	Before	After
Total protein (g/L)	68 ± 4	68 ± 3	70 ± 5	69 ± 5	68 ± 4	69 ± 4	69 ± 3	68 ± 4
Albumin (g/L)	42 ± 2	42 ± 3	44 ± 4	42 ± 3[2]	42 ± 2	42 ± 3	43 ± 4	42 ± 2
Bilirubin, total (μmol/L)	9 ± 2	10 ± 4	9 ± 4	10 ± 3	10 ± 4	11 ± 4	10 ± 4	10 ± 4
Alanine aminotransferase (μkat/L)	0.30 ± 0.09	0.28 ± 0.07	0.25 ± 0.07	0.24 ± 0.04	0.32 ± 0.08	0.32 ± 0.11	0.29 ± 0.11	0.25 ± 0.08[2]
Alkaline phosphatase (μkat/L)	1.1 ± 0.3	1.1 ± 0.4	1.1 ± 0.3	1.1 ± 0.3	1.1 ± 0.3	1.1 ± 0.3	1.1 ± 0.3	1.2 ± 0.3
Aspartate aminotransferase (μkat/L)	0.34 ± 0.07	0.33 ± 0.07	0.34 ± 0.07	0.33 ± 0.04	0.36 ± 0.04	0.34 ± 0.06	0.35 ± 0.09	0.32 ± 0.08
Lactate dehydrogenase (μkat/L)	2.5 ± 0.4	2.7 ± 0.5	2.6 ± 0.3	2.6 ± 0.3	2.8 ± 0.4	2.8 ± 0.5	2.4 ± 0.5	2.4 ± 0.4

[1] $\bar{x}$ ± SD; n = 16–20. Repeated-measures ANOVA indicated a significant time and group interaction for albumin and alanine aminotransferase, $P < 0.05$.
[2] Significantly different from before supplementation, $P < 0.05$ (paired Student's t test followed by Bonferroni correction for multiple comparisons).

TABLE 6
Effect of vitamin E supplementation on renal function in healthy elderly[1]

	Placebo		60 IU (55 mg)		200 IU (182 mg)		800 IU (727 mg)	
Index	Before	After	Before	After	Before	After	Before	After
Urinary creatinine (mmol/L)	7 ± 4	6 ± 3	4 ± 3	5 ± 5	4 ± 2	4 ± 2	5 ± 3	5 ± 3
Creatinine excretion (mmol/d)	10 ± 3	10 ± 3	8 ± 3	8 ± 3	10 ± 3	10 ± 3	9 ± 3	10 ± 3
Serum creatinine (μmol/L)	87 ± 17	86 ± 16	72 ± 15	72 ± 15	77 ± 16	76 ± 18	76 ± 15	73 ± 16
Blood urea nitrogen (mmol/L)	6 ± 2	6 ± 2	5 ± 1	5 ± 2	6 ± 1	6 ± 1	5 ± 1	5 ± 1
Blood urea nitrogen:creatinine	69 ± 15	66 ± 17	71 ± 16	68 ± 15	76 ± 20	79 ± 18	67 ± 20	68 ± 18

[1] $\bar{x}$ ± SD; n = 16–20. No statistically significant differences were observed.

TABLE 7
Effect of vitamin E supplementation on intermediary metabolites in healthy elderly[1]

	Placebo		60 IU (55 mg)		200 IU (182 mg)		800 IU (727 mg)	
Index	Before	After	Before	After	Before	After	Before	After
Blood glucose (mmol/L)	5.2 ± 0.8	5.3 ± 1.0	5.0 ± 0.4	5.0 ± 0.4	5.1 ± 0.4	4.9 ± 0.4	5.0 ± 0.4	5.1 ± 0.5
Thyroxine, total (nmol/L)	106 ± 17	104 ± 16	103 ± 19	102 ± 18	100 ± 19	101 ± 20	100 ± 12	102 ± 12
Free thyroxine index	2.2 ± 0.2	2.2 ± 0.3	2.2 ± 0.3	2.2 ± 0.4	2.2 ± 0.4	2.2 ± 0.4	2.2 ± 0.4	2.2 ± 0.3
Triiodothyronine uptake	0.28 ± 0.03	0.28 ± 0.03	0.28 ± 0.02	0.28 ± 0.02	0.28 ± 0.02	0.28 ± 0.03	0.28 ± 0.03	0.28 ± 0.03
Triiodothyronine uptake ratio	0.91 ± 0.07	0.91 ± 0.05	0.92 ± 0.08	0.92 ± 0.06	0.94 ± 0.07	0.93 ± 0.08	0.95 ± 0.10	0.93 ± 0.09

[1] $\bar{x}$ ± SD; n = 15–20. No statistically significant differences were observed.

TABLE 8
Effect of vitamin E supplementation on plasma lipid concentrations in healthy elderly[1]

	Placebo		60 IU (55 mg)		200 IU (182 mg)		800 IU (727 mg)	
Index	Before	After	Before	After	Before	After	Before	After
Plasma triacylglycerol (mmol/L)	1.18 ± 0.50	1.20 ± 0.44	1.06 ± 0.36	1.09 ± 0.35	1.04 ± 0.35	1.21 ± 0.44[2]	1.19 ± 0.41	1.23 ± 0.43
Plasma total cholesterol (mmol/L)	5.11 ± 0.71	5.11 ± 0.65	5.20 ± 0.72	5.19 ± 0.64	5.31 ± 0.83	5.46 ± 0.96	5.49 ± 0.79	5.69 ± 0.92
HDL (mmol/L)	1.55 ± 0.46	1.57 ± 0.55	1.59 ± 0.38	1.59 ± 0.44	1.62 ± 0.32	1.57 ± 0.29	1.45 ± 0.40	1.50 ± 0.37
LDL (mmol/L)	3.03 ± 0.75	3.00 ± 0.73	3.13 ± 0.80	3.11 ± 0.70	3.22 ± 0.73	3.35 ± 0.82	3.44 ± 0.81	3.58 ± 0.95
VLDL (mmol/L)	0.53 ± 0.23	0.54 ± 0.20	0.48 ± 0.17	0.49 ± 0.16	0.47 ± 0.16	0.54 ± 0.20[2]	0.54 ± 0.19	0.56 ± 0.20
LDL:HDL	2.14 ± 0.82	2.18 ± 0.93	2.14 ± 0.91	2.16 ± 0.92	2.06 ± 0.60	2.19 ± 0.62	2.54 ± 0.92	2.54 ± 0.96

[1] $\bar{x}$ ± SD; n = 17–20. Repeated-measures ANOVA indicated an overall significant time effect, $P < 0.05$.
[2] Significantly different from before supplementation, $P < 0.05$ (paired Student's t test followed by Bonferroni correction for multiple comparisons).

plasma triacylglycerol (1.04 ± 0.35 mmol/L before compared with 1.21 ± 0.44 mmol/L after supplementation) and VLDL (0.47 ± 0.16 mmol/L before and 0.54 ± 0.20 mmol/L after supplementation) concentrations in subjects consuming 200 IU vitamin E/d. However, the changes in plasma triacylglycerol and VLDL in this group were not significantly different from those in the placebo group. Furthermore, postsupplementation values for both plasma triacylglycerol and VLDL were well below the normal range (<2.30 mmol/L for triacylglycerol and <0.8 mmol/L for VLDL). The reason for this change in the 200-IU/d group and not in the other vitamin E–supplemented groups is not clear; however, it is unlikely that it is of biological significance.

Because vitamin E has been shown to decrease concentrations of eicosanoids involved in platelet aggregation (50–52), which, in turn, might adversely affect blood coagulation, we measured bleeding time before and after supplementation. As shown in **Table 9**, bleeding time in all groups before supplementation was within the range reported by others using Simplate II (49). There was no significant change in bleeding time in either the placebo group or any of the vitamin E–supplemented groups.

DISCUSSION

Because consideration is being given to determining new dietary allowances for vitamin E, it is important to consider the safety margin of a range of dietary and supplemental intakes. We observed no significant adverse effects after 4 mo of consumption of 60–800 IU (55–727 mg) vitamin E/d on a variety of outcome measures, including general health, nutrient status, liver enzyme function, thyroid hormones, creatinine concentrations, serum autoantibodies, killing of *C. albicans* by neutrophils, and bleeding time.

Concern about the safety of vitamin E supplementation has been raised because of vitamin E's potential to affect platelet aggregation and blood coagulation. Similar to the results of Stampfer et al (53), we observed no effect of vitamin E supplementation on platelet aggregation or bleeding time. It should be noted, however, that subjects in our study were healthy elderly with normal vitamin K statuses and who were not regularly taking any other anticoagulants, including nonsteroidal antiinflammatory drugs such as aspirin. The effect of vitamin E supplementation on the bleeding time of subjects consuming anticoagulants needs to be determined. Adverse effects on prothrombin time in human studies have been reported but only in subjects who have vitamin K deficiency or use anticoagulant medications (10). Although a higher mortality from hemorrhagic stroke was noted in the vitamin E treatment group in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (study group 1994), the 50-IU/d dose of *all-rac*-α-tocopheryl acetate used in the trial was substantially lower than those doses found to affect platelet function (54). Antioxidant supplements containing 30 IU vitamin E/d were not associated with any hematologic morbidity or mortality in the Linxian Chemoprevention Trial (55).

It has also been suggested (56) that the immunostimulatory effect of vitamin E on T cell–mediated function in the elderly (6, 13) might be associated with increased autoantibody formation, resulting in increased incidence of autoimmune diseases. As reported previously (6), we did not observe a significant change

TABLE 9
Effect of vitamin E supplementation on bleeding time in healthy elderly[1]

Group	Before	After
	s	
Placebo	302 ± 97	263 ± 77
60 IU (55 mg)	310 ± 78	345 ± 94
200 IU (182 mg)	331 ± 68	293 ± 64
800 IU (727 mg)	310 ± 89	288 ± 72

[1] $\bar{x}$ ± SD; n = 15–19. No statistically significant differences were observed.

TABLE 10
Effect of long-term vitamin E supplementation on the ability of polymorphonuclear cells from healthy elderly to kill *Candida albicans*[1]

	Unopsonized		Opsonized	
Group	30 min	60 min	30 min	60 min
	% killed			
Placebo	6 ± 1	23 ± 2	23 ± 4	48 ± 3
60 IU (55 mg)	4 ± 7	28 ± 2	18 ± 5	38 ± 4
200 IU (182 mg)	2 ± 4	23 ± 2	22 ± 4	50 ± 3
800 IU (727 mg)	4 ± 3	27 ± 3	22 ± 5	54 ± 4

[1] $\bar{x}$ ± SEM; n = 5–7. No statistically significant differences were observed. These data were reported previously (6).

in serum concentrations of anti-DNA or anti-thyroglobulin antibodies after vitamin E supplementation in this study. Another concern regarding vitamin E supplementation is the possible adverse effect on phagocytic cell cytotoxicity, a process that is dependent on free radical formation. As reported previously (6) and as shown in **Table 10**, supplementation had no effect on the ability of neutrophils to kill *C. albicans*.

Controlled, double-blind clinical trials of vitamin E in which doses of 100–3200 IU/d were used confirm the absence of vitamin E toxicity in healthy subjects (13, 57–60), in smokers (61), and in patients with angina pectoris (62, 63), diabetes (3, 64–66), epilepsy (67), Parkinson disease (68), tardive dyskinesia (69), and vascular disease (70). Although some questions have been raised about the prudence of recommending vitamin E supplementation given the absence of established safety tests over several decades (71), long-term prospective epidemiologic surveys such as the Nurses' Health Study (72) indicate no increase in relative risk for major coronary disease, ischemic stroke, or cardiovascular or overall mortality associated with vitamin E supplementation; indeed, the data suggest modest beneficial although nonsignificant effects on these outcomes.

We conclude that 4 mo of supplementation with ≤800 IU *all-rac*-α-tocopherol/d does not have an adverse effect on hepatic or renal function, intermediary metabolism, hematologic status, plasma lipid and lipoprotein concentrations, bleeding time, plasma vitamins, serum trace elements, serum autoantibody concentrations, or the ability of neutrophils to kill *C. albicans* in healthy older subjects. These results confirm our previously reported finding with short-term vitamin E supplementation (13). The data presented here will be useful in determining the risk-to-benefit ratio of vitamin E supplementation in the elderly.

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