Effects of long-term supplementation with moderate pharmacologic doses of vitamin E are saturable and reversible in patients with type 1 diabetes[1–3]

Wendy Engelen, Begoña Manuel y Keenoy, Jan Vertommen, and Ivo De Leeuw

ABSTRACT

Background: Vitamin E supplementation has been proposed as adjunctive therapy to counteract the increased LDL oxidation in diabetes and thus prevent or delay cardiovascular complications.

Objective: The objective of this study was to investigate the effect of a moderate pharmacologic dose of vitamin E for ≤1 y in patients with type 1 diabetes.

Design: The study was double blind and the subjects were randomly assigned to 2 groups: the supplemented group (group S; n = 22) received 250 IU (168 mg) *RRR*-α-tocopherol 3 times/d for 1 y and the placebo group (group P; n = 22) received a placebo for 6 mo followed by 250 IU (168 mg) *RRR*-α-tocopherol 3 times/d for an additional 6 mo.

Results: Serum vitamin E doubled after 3 mo of supplementation, from a mean (±SD) of 36.9 ± 10.9 to 66.4 ± 18.3 μmol/L ($P < 0.0005$). Although lipid profiles, glycated hemoglobin, and blood biochemistry values did not change significantly, copper-induced in vitro peroxidizability of LDL and VLDL decreased after 3 mo of supplementation: the production of thiobarbituric acid–reactive substances decreased by 30–60% ($P < 0.005$) and the lag time for the appearance of fluorescent products increased from 107 ± 25 to 123 ± 30 min in group S ($P = 0.002$ compared with group P). Vitamin E supplementation for an additional 3–9 mo resulted in no further changes in serum vitamin E and lipoprotein peroxidizability. Values returned to baseline after supplementation ended.

Conclusions: Because the improvement in lipoprotein peroxidizability is saturable and reversible, life-long supplementation with vitamin E should be considered in patients with type 1 diabetes. *Am J Clin Nutr* 2000;72:1142–9.

KEY WORDS Type 1 diabetes, vitamin E, LDL and VLDL peroxidation, thiobarbituric acid–reactive substances, cardiovascular disease risk factors

INTRODUCTION

In diabetic patients, the risk of atherosclerosis is 3- to 4-fold higher than in nondiabetic persons and cardiovascular disease is the major cause of premature death (1). This is partly explained by a clustering of risk factors such as hypertension and dyslipidemia in this patient group and by the direct consequences of hyperglycemia and glycation (2–4), which favor the oxidation and modification of LDL particles and thus accelerate the development of atherosclerosis (5). The process of peroxidation of polyunsaturated fatty acids in lipoproteins can be inhibited by vitamin E when given either in vitro or in vivo (6–8). Indeed, evidence from clinical trials and epidemiologic studies suggests that vitamin E supplementation is associated with a reduced risk of cardiovascular disease (9–11).

Although plasma or tissue vitamin E concentrations are not always low in diabetic patients (12–15), vitamin E supplements might help counteract the heightened oxidative conditions observed in diabetes and thus serve as an adjunctive therapeutic agent for the prevention and even treatment of the cardiovascular complications associated with diabetes (16). In patients with type 2 diabetes, vitamin E supplementation led to a decrease in the susceptibility of LDL to in vitro peroxidation (17, 18) and to a decrease of in vivo peroxidation products in young (mean age: 12 y) patients with type 1 diabetes (19).

However, several aspects of vitamin E supplementation in diabetic patients should be assessed critically. First, vitamin E supplementation in the different human studies reviewed below ranged from 10 to 100 times the recommended dietary allowance (20) of 10 mg α-tocopherol equivalents (15 IU) and thus do not distinguish between nutritional repletion and pharmacologic effects. Second, careful evaluation is needed of the nonantioxidative effects of vitamin E supplementation, such as a lowering of blood triacylglycerols (19, 21, 22), a decrease in glycation (21–23), an improvement in insulin action (24), an inhibition of platelet adhesion (25) and activation (15, 26–28), and, in diabetic rats, an improvement in vascular reactivity (29). Third, in most of these studies, supplements were given for <3 mo; therefore, long-term effects have been less well studied. Fourth, the patient characteristics varied widely in the different studies, especially regarding metabolic control. A consensus on

[1] From the Laboratory of Endocrinology, the University of Antwerp, Antwerp, Belgium.

[2] The vitamin E (E-MED FORTE) and placebo capsules were supplied by OMEGA PHARMA NV (Leuven, Belgium).

[3] Address reprint requests to B Manuel y Keenoy, University of Antwerp, Laboratory of Endocrinology, Universiteitsplein 1, B-2610 Wilrijk-Antwerp, Belgium. E-mail: begona@uia.ua.ac.be.

Received July 19, 1999.

Accepted for publication March 30, 2000.

TABLE 1
Patient characteristics at baseline[1]

Characteristic	Group S (n = 22)	Group P (n = 22)
Age (y)	42 (26–54)[2]	40 (23–65)
Sex (male/female)	13/9	15/7
Duration of diabetes (y)	16 ± 10	16 ± 7
Insulin dose (U/d)	45 ± 13	47 ± 15
Smoker (yes/no)	6/16	5/17
BMI (kg/m^2)	24.0 ± 3.3	24.3 ± 3.2
Blood pressure (mm Hg)		
Systolic	130 (104–160)	131 (104–159)
Diastolic	78 (65–95)	80 (66–100)
Glycated hemoglobin	0.08 ± 0.012	0.079 ± 0.01
Fasting glycemia (mmol/L)	10.2 ± 4.9	7.6 ± 4.3
Serum vitamin E (μmol/L)	36.9 ± 10.9	33.9 ± 10.9
Serum triacylglycerol (mmol/L)	0.99 ± 0.46	0.92 ± 0.36
Serum total cholesterol (mmol/L)	5.33 ± 1.06	5.30 ± 1.01
Serum HDL cholesterol (mmol/L)	1.63 ± 0.54	1.60 ± 0.49
Serum LDL cholesterol (mmol/L)	3.26 ± 0.98	3.28 ± 0.93
Lipoprotein(a) (mg/L)	1.22 (1.06–10.6)	1.21 (0.83–16.2)

[1] Group S received vitamin E for 12 mo; group P received placebo for 6 mo followed by vitamin E for an additional 6 mo. There were no significant differences between the 2 groups, indicating satisfactory randomization.
[2] Median; range in parentheses for nonnormally distributed data.

the correct selection of high-risk patients who could eventually derive more benefit from such supplements is difficult to obtain from such data. Last, the possible risks of vitamin E supplementation should be considered, especially long-term supplementation in patients with a chronic disease.

Although vitamin E is not mutagenic, carcinogenic, or teratogenic, and human studies showed that oral supplementation—even at doses as high as 3200 IU (3 g) *all-rac*-α-tocopherol/d—resulted in no adverse effects (30–32), these studies were conducted in healthy humans or animals. The effects of long-term vitamin E supplementation in patients with impaired platelet, coagulation, lipid, and antioxidant statuses or taking medication regularly, as diabetic patients often do, need further investigation (33). Because of this lack of consensus on the risk-benefit balance of vitamin E supplementation, we investigated the effect on cardiovascular risk markers of pharmacologic but moderate dosages [750 IU (503 mg) *RRR*-α-tocopherol/d] for ≤1 y to well-controlled, patients with type 1 diabetes.

SUBJECTS AND METHODS

Patient population and study design

Forty-four middle-aged, metabolically well-controlled patients with type 1 diabetes with initial cardiovascular risk factors such as lipidemia and blood pressure within accepted limits were randomly assigned to 2 groups in a double-blind manner. Patient characteristics at baseline are shown in **Table 1**. Exclusion criteria were intake of drugs that interfere with the oxidant-antioxidant status and signs on a standard electromyogram of axonal involvement or demyelinization. Group S received 250 IU (168 mg) *RRR*-α-tocopherol 3 times/d with meals for 1 y. Group P received a placebo (280 mg soybean oil containing 0.25 mg tocopherol per capsule and identical in taste and appearance to the vitamin E capsules) for the first 6 mo and then 250 IU (168 mg) *RRR*-α-tocopherol 3 times/d for an additional 6 mo. Patients were monitored at baseline (visit 1, n = 22 in each group); after 3 mo (visit 2, n = 22 in each group), 6 mo (visit 3, n = 21 in each group), 9 (visit 4, n = 20 in each group), and 12 mo (visit 5, n = 19 in group S and 20 in group P) mo of supplementation; and 3 mo after vitamin E supplementation ended (visit 6, n = 18 in group S and 14 in group P).

All patients were consuming a standard diet for diabetic patients that provided 7.5–8.5 MJ/d (50% of energy as carbohydrates, 20% as protein, and 30% as fat). This diet ensures a daily intake of ≥3 mg vitamin E, 3000 mg vitamin A, 150 mg vitamin C, and 26 mg flavonoids. The experimental protocol was in accord with the Helsinki Declaration and was approved by the ethical committee of Antwerp University Hospital, Belgium. Participating subjects signed an approved consent form.

Analytic methods

Routine blood tests (blood count, urea nitrogen, creatinine, uric acid, glucose, liver enzymes, protein, albumin, total and HDL cholesterol, triacylglycerols, sodium, potassium, calcium, parathyroid hormone, iron, total-iron-binding capacity, bilirubin, C-reactive protein, and alkaline phosphatase) were analyzed in the routine laboratory of Antwerp University Hospital. Total analytic variability, expressed as the CV, was 2%, 1.9%, and 0.9% for total cholesterol, HDL cholesterol, and triacylglycerol, respectively. LDL cholesterol was calculated according to the Friedewald equation (34). Glycated hemoglobin (Hb A_{1c}) was measured by using an HPLC cation exchange column (Modular Diabetic Monitoring System; Bio-Rad, Richmond, CA); the CV was 1.5%. Oxidant-antioxidant balance was evaluated by measuring the concentrations of individual antioxidants and the susceptibility of lipoproteins to oxidative attack in vitro (ie, peroxidizability).

Vitamins E and A in serum were measured by HPLC (Shimadzu, Kyoto, Japan) with a reversed-phase C_{18} column (Bio-Rad Laboratories, Hercules, CA) with 100% methanol mobile phase and detection at 292 and 325 nm, respectively, with CVs of 10% and 13%, respectively (35). Glutathione in whole blood was measured by using a colorimetric method with a CV of 7% (36). Glutathione peroxidase activity in the hemolysate was measured with a commercial kit (Randox Laboratories Ltd, Crumlin, United Kingdom) with a CV of 6%. The susceptibility of LDL and VLDL to copper-catalyzed oxidation was measured by isolating these 2 groups of lipoproteins by dextran sulfate–magnesium chloride precipitation and incubation of a suspension containing 200 mg cholesterol/L with 46 μmol $CuSO_4$/L for ≤180 min at 37°C, during which samples were taken every 30 min for the measurement of thiobarbituric acid–reactive substances (TBARS). Fluorescence at 360-nm excitation and 430-nm emission wavelengths was monitored continuously. We distinguished 3 phases in this process: lag time, during which the antioxidants within the lipoprotein prevent the peroxidation from proceeding as a chain reaction (0–60 min); early initiation (60–90 min) and propagation, during which the reaction causes a linear increase in TBARS and fluorescence production (120–150 min); and saturation (180 min), during which fluorescence reaches a plateau and gives an estimate of the total amount of lipid oxidized (37). The CVs of these variables ranged from 3% to 11%.

Statistical methods

Data were analyzed by using SPSS software (SPSS Inc, Chicago). Although there were no significant differences between groups in sex or smoking habits by chi-square test, in all variables tested by analysis of variance, and in age, duration of diabetes, and body mass index (BMI; in kg/m^2) by the Mann-Whitney *U* test, the results of comparisons of the effects of vitamin E with those of placebo were adjusted for the covariates age, duration of diabetes, and BMI and for the factors sex and smoking habit. The effect of vitamin E supplementation for 3–6 mo on the various variables was studied by repeated-measures analysis of variance in 2 ways: *1*) a between-group comparison between groups S and P in the first 6 mo of the study, and *2*) a within-group comparison of the effect of placebo (first 6 mo) and that of vitamin E (from 6 to 12 mo) in group P. Within-group comparisons of the effect of vitamin E supplementation for 9–12 mo in group S and of the effect of discontinuation of vitamin E supplementation for 3 mo in groups S and P were also conducted.

The *P* values given below refer to the significance of either the change over time (analyzed by repeated contrasts) within one group since the previous visit or to the between-group effect of vitamin E supplementation compared with that of placebo on changes over a given period of time. *P* values <0.05 were considered significant. Results are presented as means ± SDs unless noted otherwise.

RESULTS

Randomization at baseline resulted in 2 groups of patients with clinical characteristics that were not significantly different from each other (Table 1). Initial serum vitamin E concentrations were not related to lipid concentrations (correlation with total cholesterol: $r = 0.26$, $P = 0.10$; $n = 42$), BMI, insulin dose, glycemic control (fasting glycemia, daily insulin dose, and Hb A_{1c}) or to the other biochemical and clinical variables investigated.

In group S, serum vitamin E concentrations increased from 36.9 ± 10.9 μmol/L at baseline to 66.4 ± 18.3 μmol/L after 3 mo ($P < 0.0005$ for the within-group and between-group comparisons) (**Figure 1**A). After 6, 9, and 12 mo of continuous vitamin E supplementation, serum vitamin E concentrations did not increase further (60.8 ± 19.0, 62.0 ± 25.8, and 66.2 ± 22.3 μmol/L, respectively). This suggests that the transport capacity of vitamin E in serum was already saturated after 3 mo of supplementation. In group P, serum vitamin E remained stable during the first 6 mo of the study; however, 3 mo after vitamin E supplementation began, serum vitamin E concentrations doubled ($P < 0.0005$ compared with the first 6 mo of the study) and then remained stable during the remaining period of supplementation (months 9–12). Serum vitamin E concentrations returned to baseline values in both groups 3 mo after vitamin E supplementation ended (Figure 1A).

During the study there were no significant changes in routine blood biochemistry values, red blood cell antioxidants (glutathione and glutathione peroxidase), or lipid profiles—total, HDL, and LDL cholesterol; lipoprotein(a) [Lp(a)]; and triacylglycerols (Figure 1, B and C)—in either of the 2 groups. Likewise, there were no significant time trends for Hb A_{1c} (Figure 1D), BMI, insulin dose, or blood pressure and no significant differences between groups.

In both groups, in vitro peroxidizability of LDL and VLDL decreased significantly during vitamin E supplementation but not during placebo administration. This improvement in peroxidizability was indicated by a 20% prolongation in the lag time for the appearance of fluorescent products of peroxidation in group S (from 107 ± 25 min at baseline to 123 ± 30 min after 3 mo; Figure 1E) and by a decrease in the production of TBARS of 30–60% from baseline during the initiation and early propagation phases (90–150 min after incubation with copper) (Figure 1F and **Figure 2**). For example, 120 min after incubation with copper, TBARS from group S decreased from 58.1 ± 21.4 nmol MDA/mg non-HDL cholesterol at baseline to 43.1 ± 22.4 and 40.3 ± 23.9 nmol MDA/mg non-HDL cholesterol after 3 and 6 mo, respectively. In group P, these values were 52.2 ± 20.6, 58.6 ± 16.7, and 56.0 ± 18.7 nmol MDA/mg non-HDL cholesterol at baseline and after 3 and 6 mo, respectively ($P = 0.001$ compared with the change over time in group S). In group P, TBARS concentrations after 120 min of incubation with copper decreased to 35.7 ± 16.8 and 36.8 ± 16.5 nmol MDA/mg non-HDL cholesterol after supplementation for 3 and 6 mo, respectively ($P < 0.0005$ for the comparison of the change in group P between the first and last 6 mo of the study). This decrease in peroxidizability was already significant after 3 mo of vitamin E supplementation (in the within-group comparison: $P < 0.0005$ for both groups S and P). Supplementation for an additional 3 mo in both groups or for a total of 12 mo in group S resulted in no further significant changes. Thus, it seems that peroxidizability was maintained at this lower level during long-term supplementation. As seen for serum vitamin E, lipid peroxidizability variables returned to baseline values after supplementation ended (Figures 1 and 2).

The factors affecting peroxidizability at baseline were analyzed by stepwise multiple regression. Only serum cholesterol affected peroxidizability significantly ($r = 0.43$, $F = 7.7$, $P = 0.009$), accounting for 18% of the variability in TBARS production after 120 min of incubation with copper. This variable did not correlate with serum vitamin E concentrations ($r = 0.14$, $P = 0.37$; $n = 44$). After 3 mo of supplementation, the decrease in TBARS production after 120 min of incubation with copper correlated with the increase in serum vitamin E ($r = –0.43$, $P = 0.005$; $n = 41$) but this relation disappeared after 6–12 mo of supplementation ($r = –0.22$, $P = 0.196$; $n = 38$ after 6 mo).

Likewise, there was no correlation between fluorescence lag time at baseline and serum vitamin E, even when standardized for LDL cholesterol ($r = –0.19$, $P = 0.23$; $n = 40$), or between the extent of prolongation of the lag time of fluorescence and the increase in serum vitamin E after supplementation. These relations were not affected significantly by the smoking habits of the patient.

In 10 of the 42 patients (4 in group P and 6 in group S; NS), the production of TBARS during in vitro lipoprotein oxidation did not decrease after 3 mo of vitamin E supplementation. These nonresponders were characterized by having significantly lower TBARS concentrations ($P < 0.01$ for TBARS at 30–120 min) and a tendency for lower Lp(a) concentrations at baseline (median: 1.21 mg/L; minimum: 0.83 mg/L; maximum: 632 mg/L) than were the responders (1.81, 1.06, and 16.20 mg/L; $P = 0.08$ by Mann-Whitney *U* test) as well as lower cholesterol (NS) and higher triacylglycerol (NS) concentrations. There were no significant differences in Hb A_{1c}, BMI, or insulin dose between the 2 groups. The proportion of smokers was not significantly different between the 2 groups.

Although there was no significant difference in initial serum vitamin E concentrations between groups, even when standardized

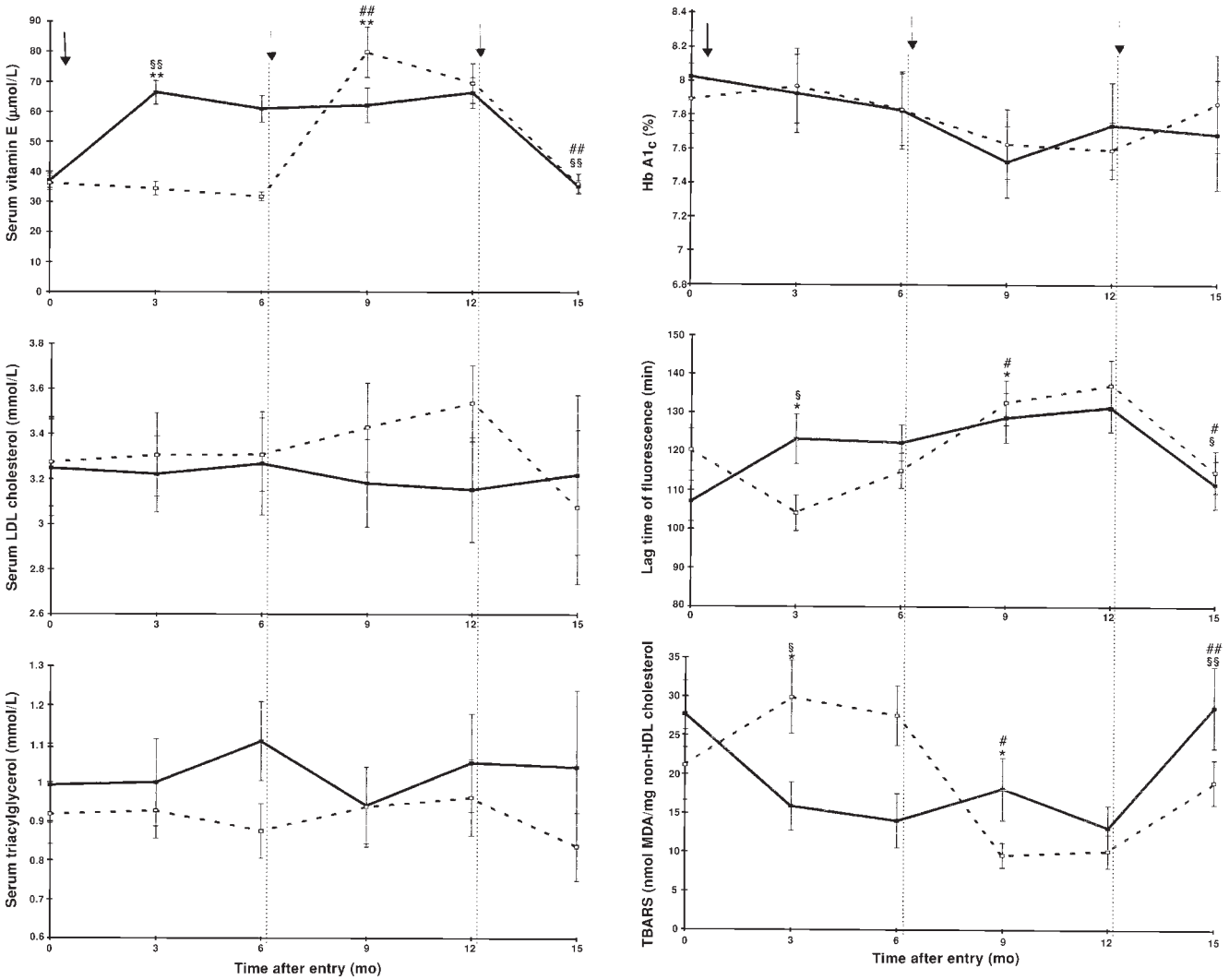

FIGURE 1. Mean (±SEM) changes in serum vitamin E, serum LDL cholesterol, serum triacylglycerols, serum glycated hemoglobin (Hb A_{1c}), lag time of fluorescence, and production of thiobarbituric acid–reactive substances (TBARS) 90 min after incubation with copper in the group receiving vitamin E [750 IU (503 mg) *RRR*-α-tocopherol/d] for 1 y (group S; solid line) and in the group receiving placebo for the first 6 mo and then vitamin E for the following 6 mo (group P; dashed line). After 12 mo, both groups stopped taking vitamin E for 3 mo and variables were then monitored for the last time (15 mo after the start of the study). *,**Significantly different from other group: $^{*}P < 0.01$, $^{**}P < 0.0005$. §,§§Significant change since the previous visit in group S: $^{§}P < 0.01$, $^{§§}P < 0.0005$. $^{\#,\#\#}$Significant change since the previous visit in group P: $^{\#}P < 0.01$, $P < 0.0005$.

for LDL cholesterol, the increase in serum vitamin E was lower in the nonresponders than in the responders (25.3 ± 15.2 compared with 42.6 ± 29.4 μmol/L; $P = 0.04$), suggesting either worse compliance in this group or less transport capacity because of their lower cholesterol concentrations. However, only 7% of the women compared with 33% of the men were nonresponders ($\chi^2 = 3.78$, $P = 0.052$); therefore, when the effects of sex and serum cholesterol were corrected for, these differences in the 2 response groups were no longer significant. Even when the multivariate analysis accounted for all of the lipid variables together [cholesterol, ln Lp(a), triacylglycerols, and LDL cholesterol], there were still no significant differences between the 2 response groups (Hotelling's $F = 1.62$, $P = 0.19$). Thus, in this particular group of patients, no given variable or combination of variables, except for higher initial lipoprotein peroxidizability, could predict the subsequent response to vitamin E.

DISCUSSION

Oral supplementation with moderate amounts of vitamin E (750 IU/d for ≤1 y) resulted in an increase in serum α-tocopherol concentrations, which reached a steady state of only 2–4 times the presupplementation concentrations. This same steady state was reached in other studies in which higher doses, even 100 times the recommended daily allowance (38), were given. This may have been due to the limited capacity of the hepatic binding protein responsible for the preferential secretion of *RRR*-α-tocopherol into the nascent VLDL (39). Moreover, the turnover and

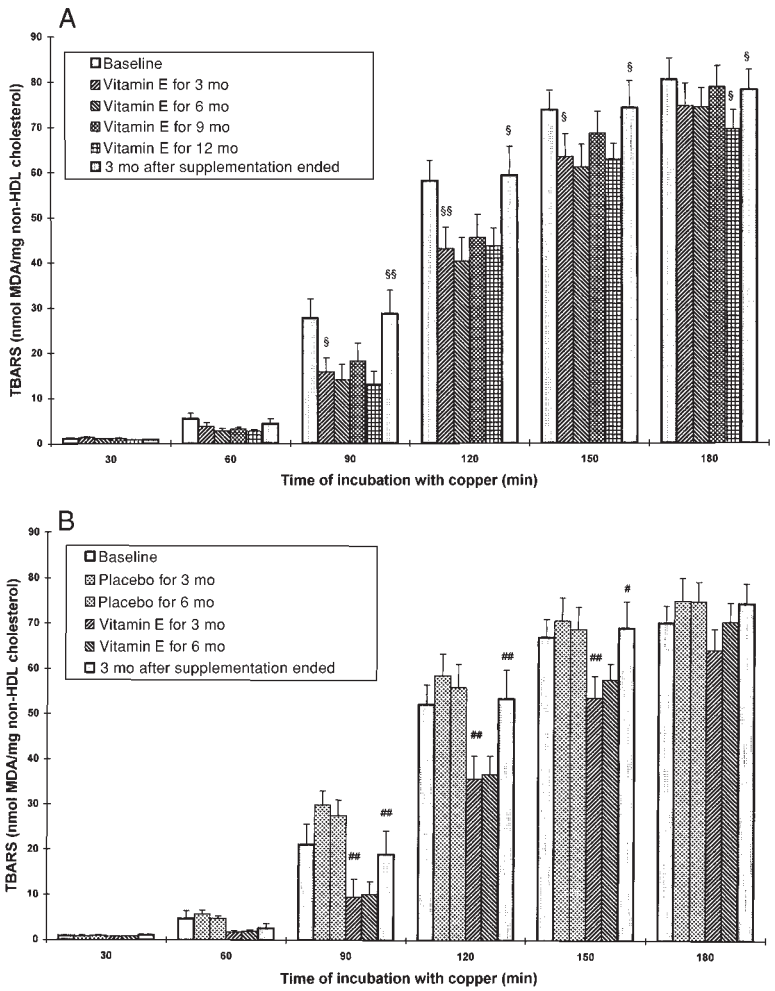

FIGURE 2. Time course of production of thiobarbituric acid–reactive substances (TBARS) during in vitro incubation of LDL and VLDL lipoproteins with copper in the group receiving vitamin E [750 IU (503 mg) *RRR*-α-tocopherol/d] for 1 y (group S; panel A) and in the group receiving placebo for the first 6 mo and then vitamin E for an additional 6 mo (group P; panel B). §,§§Significant change since the previous visit in group S: $^{\S}P < 0.01$, $^{\S\S}P < 0.0005$. #,##Significant change since the previous visit in group P: $^{\#}P < 0.01$, $^{\#\#}P < 0.0005$.

exchange between plasma and liver and between the various lipoproteins is very quick: 6–9 h after an oral dose of vitamin E, the concentration of α-tocopherol is the same in all lipoproteins (38). Concentrations in plasma are also highly dependent on transport capacity, ie, on cholesterol and triacylglycerol concentrations (40). In our group of patients, none of whom had extreme dyslipidemia or abnormally low serum vitamin E concentrations, there was no correlation between serum vitamin E and lipids. Despite a doubling of serum vitamin E during the study, lipid concentrations remained stable, as was also observed by Fuller et al (22). In healthy adults, lipids also remained unchanged or even increased slightly (30, 32). These observations contrast with the findings of one study in which lower dosages (100 IU/d, or ≈67 mg α-tocopherol equivalents) of vitamin E were given to children with type 1 diabetes (19). In that study, a doubling of serum vitamin E was accompanied by a significant decrease in serum triacylglycerol (19). In contrast with the results of a pediatric study (21) and one by Ceriello et al (23), and in concordance with the results of Fuller et al (22), Hb A_{1c} was not affected significantly by vitamin E supplementation in our group of relatively well-controlled diabetic patients (initial Hb A_{1c}: ≈0.08). These differences in response may have been due to the different ages of the study populations (≈40 y in our study and 12 y in the pediatric study) and to the pathologically high initial triacylglycerol concentrations (2.9 mmol/L) and Hb A_{1c} values (0.13) in the pediatric patients. These conflicting outcomes on the effects of vitamin E on metabolic control stress the importance of initial status on the subsequent response; those with greater lipidemia and glycation (21, 23) are more likely to improve after an intervention.

Although the concentration of vitamin E in plasma does not reflect the concentration in tissues, passage into tissues is ensured by several different compensatory mechanisms (41); therefore, we assume that the concentrations reached in tissues during supplementation were normal to high, depending on the type of tissue. Nevertheless, plasma concentrations measured 3 mo after supplementation ended returned to baseline values. This reversal had already occurred 8–12 d after supplementation ended in 2 other studies (42, 43), confirming the rapid turnover of plasma tocopherol and of its compensatory mobilization from the tissues to the plasma in humans. In dogs fed a vitamin E–deficient diet, the time necessary for half of the adipose tissue

tocopherol to be depleted was 100 d (38). In type 1 diabetes particularly, exogenous insulin might, by stimulating lipoprotein lipase, promote passage of vitamin E from the lipoproteins to the membranes. This can also lead to an increase in turnover and thus tend to decrease concentrations in plasma, as seen during intravenous infusion of insulin (44). All of these observations suggest that to maintain high-normal vitamin E concentrations in both plasma and tissues, uninterrupted supplementation is preferable to short-duration "cures" with high-dose supplementation or intravenous loading.

The analysis of the factors affecting lipoprotein peroxidizability at baseline confirms other reports of the lack of correlation between lipoprotein peroxidizability and vitamin E concentrations (45, 46). In fact, lipoprotein peroxidizability is strongly modulated by other factors, such as particle density (47), glycation (18, 48), autoxidative glycation (49), content in polyunsaturated fatty acids (50–52), lipid hydroperoxides (53), and concentrations of other antioxidants, such as ubiquinol-10 (52, 54, 55). In vivo, factors in the microenvironment around the LDL particle—notably vitamin C in plasma (56) and bilirubin (57)—smoking (58), and ketosis (59) also influence the peroxidation process. In view of the strong role played by these other factors, some authors have even argued against the involvement of vitamin E as an antioxidant in LDL challenged with copper (60) or emphasized the importance of the oxidative conditions on its prooxidant or antioxidant activity (53, 61).

Nevertheless, in the present study, the time course of changes during the vitamin E supplementation or placebo period showed unequivocally that vitamin E supplementation resulted in a decrease in lipoprotein peroxidizability (a decrease of ≈50% in TBARS production and a prolongation of ≈20% for the lag time of fluorescent products) and that, after 3 mo, the decrease in TBARS production was related to the increase in serum vitamin E. Similar decreases were seen after 1600 mg vitamin E/d in patients with type 2 diabetes (7) and after 500, 1000, or 1500 IU/d in healthy volunteers (62). The minimum dosage needed to reduce susceptibility of LDL to oxidation was 400 IU/d in healthy subjects (63). Epidemiologic studies have pinpointed a minimum daily intake of 100 IU for protection against coronary disease in healthy women (11). This suggests that the reduction in lipoprotein peroxidizability observed in our study after a daily intake of 750 IU (503 mg) is clinically relevant. It must be stressed, however, that the capacity of an antioxidant to inhibit lipid peroxidation induced in vitro and measured by techniques (TBARS and fluorescence measurements in this study) that reflect only a given stage in the peroxidation cascade is no absolute measure of its in vivo antiatherogenic potency.

The antiperoxidative effect of vitamin E supplementation was not related to the initial serum concentrations of vitamin E or lipids. Thus, the benefit was not derived from a replenishment of possibly deficient stores and was pharmacologic rather than nutritional. Moreover, the benefit was not proportional to the dose accumulated during months 3–12 of the study; the decrease in peroxidizability reached a steady state after just 3 mo of supplementation, as was also observed for serum vitamin E concentrations. This indicates the saturability of its effect, the strong modulation by other factors, or both. Furthermore, the beneficial effects of vitamin E on lipoprotein peroxidizability were not permanent because they disappeared after supplementation ended. These observations invite a discussion on the need for maintenance supplementation and on the minimum effective dose. Studies in healthy subjects indicate that, despite substantial interindividual variability, chronic administration of vitamin E at different dosages (≈400–1500 mg/d) causes the same increase in plasma tocopherol concentrations (≈80%) (43) and, in parallel, the same reduction in LDL peroxidizability (62, 63). In diabetic patients, the dose needed for the same end result might be higher. This observation is supported by the following considerations. First, it is recommended that diabetic patients consume low-fat diets, which are known to diminish intestinal absorption, liver secretion of vitamin E in VLDL, or both (43). Second, insulin-induced transport of vitamin E from plasma to tissue membranes (44) tends to decrease concentrations of vitamin E in lipoproteins. Last, high doses of vitamin E might be required to counteract the heightened oxidative conditions due to hyperglycemia and the derangement of other factors (mentioned above), which also play a role in lipoprotein peroxidation.

Another aspect concerns the selection of patients most likely to benefit from such lifelong and expensive supplements. Our results corroborate the belief that the effect of vitamin E supplementation is pharmacologic and was not a correction of an initial impairment or nutritional deficiency. No given variable or combination of variables could predict the subsequent response to vitamin E, except for higher initial lipoprotein oxidizability.

In summary, vitamin E supplementation decreased lipoprotein susceptibility to in vitro oxidation by copper but had no effect on other cardiovascular risk factors, such as lipid profiles, glycation, and glycemic control. This decrease in peroxidizability was limited in extent and to the period of supplementation, reversed after supplementation ended, and could not be predicted by initial patient characteristics. Therefore, continuous supplementation at a minimum effective dose and cosupplementation with modulatory factors such as vitamin C and ubiquinol-10 should be considered carefully.

We are grateful to the patients who participated in the study; to I Fiers, S Schrans, P Aerts, and M Vinckx for their skillful technical assistance; and to L Nonnemans and the nursing staff of the Antwerp University Hospital.

REFERENCES

1. Ruderman NB, Haudenschild C. Diabetes as an atherogenic factor. Prog Cardiovasc Dis 1984;26:373–412.
2. Kannel WB, McGee DL. Diabetes and glucose tolerance as risk factors for cardiovascular disease: the Framingham Study. Diabetes Care 1979;2:120–6.
3. Lyons TJ. Oxidized low density lipoproteins: a role in the pathogenesis of atherosclerosis in diabetes? Diabet Med 1991;8:411–9.
4. Witzum JL. Role of modified lipoproteins in diabetic macroangiopathy. Diabetes 1997;46:S112–4.
5. Berliner JA, Heinecke JW. The role of oxidized lipoproteins in atherogenesis. Free Radic Biol Med 1996;20:707–27.
6. Esterbauer H, Rotheneder M, Striegl G, Waeg G, Sattler W, Jürgens G. Vitamin E and other lipophilic antioxidants protect LDL against oxidation. Fat Sci Technol 1989;91:316–24.
7. Reaven PD, Khouw A, Beltz W, Parthasarathy S, Witzum JL. Effect of dietary antioxidant combinations in humans: protection of LDL by vitamin E, but not by β-carotene. Arterioscler Thromb 1993;13:590–600.
8. Vinson J, Hsu C, Possanza C, et al. Lipid peroxidation and diabetic complications: effect of antioxidant vitamins C and E. Adv Exp Med Biol 1994;366:430–2.
9. Gey KF, Puska P, Jordan P, Moser UK. Inverse correlation between plasma vitamin E and mortality from ischemic heart disease in cross-cultural epidemiology. Am J Clin Nutr 1991;53(suppl):326S–34S.

10. Rimm EB, Stampfer MJ, Ascherio A, Giovannucci E, Colditz GA, Willett WC. Vitamin E consumption and the risk of coronary heart disease in men. N Engl J Med 1993;328:1450–6.
11. Stampfer MJ, Hennekens CH, Manson JE, Colditz GA, Willett WC. Vitamin E consumption and the risk of coronary heart disease in women. N Engl J Med 1993;328:1444–9.
12. Vatassery GT, Morley JE, Kuskowski MA. Vitamin E in plasma and platelets of human diabetic patients and control subjects. Am J Clin Nutr 1983;37:641–4.
13. Karpen CW, Cataland S, O'Dorisio TM, Panganamala RV. Production of 12-hydroxyeicosatetraenoic acid and vitamin E status in platelets from type 1 human diabetic subjects. Diabetes 1985;34:526–31.
14. Martinoli L, Di-Felice M, Seghieri G, et al. Plasma retinol and alpha-tocopherol concentrations in insulin-dependent diabetes mellitus: their relationship to microvascular complications. Int J Vitam Nutr Res 1993;63:87–92.
15. Jain SK, Krueger KS, McVie R, Jaramillo JJ, Palmer M, Smith T. Relationship of blood thromboxane-B2 (TxB2) with lipid peroxides and effect of vitamin E and placebo supplementation on TxB2 and lipid peroxide concentrations in type 1 diabetic patients. Diabetes Care 1998;21:1511–6.
16. Gazis A, Page S. Vitamin E and cardiovascular protection in diabetes. BMJ 1997;314:1845–6.
17. Reaven P. Dietary and pharmacologic regimens to reduce lipid peroxidation in non-insulin-dependent diabetes mellitus. Am J Clin Nutr 1995;62(suppl):1483S–9S.
18. Li D, Devaraj S, Fuller C, Bucala R, Jialal I. Effect of α-tocopherol on LDL oxidation and glycation: in vitro and in vivo studies. J Lipid Res 1996;37:1978–86.
19. Jain SK, McVie R, Jaramillo JJ, et al. The effect of modest vitamin E supplementation on lipid peroxidation products and other cardiovascular risk factors in diabetic patients. Lipids 1996;31:S87–90.
20. National Research Council. Recommended dietary allowances. 9th ed. Washington, DC: National Academy Press, 1980.
21. Jain SK, McVie R, Jaramillo JJ, Palmer M, Smith T. The effect of modest vitamin E supplementation on blood glycated hemoglobin and triacylglycerol levels and red blood cell indices in type 1 diabetic patients. J Am Coll Nutr 1996;15:458–61.
22. Fuller CJ, Chandalia M, Garg A, Grundy SM, Jialal I. *RRR*-α-tocopheryl acetate supplementation at pharmacologic doses decreases low-density-lipoprotein oxidative susceptibility but not protein glycation in patients with diabetes mellitus. Am J Clin Nutr 1996;63:753–9.
23. Ceriello AD, Giugliano A, Quatraro C, Donzella C, Dipalo G, Lefebvre PJ. Vitamin E reduction of protein glycosylation in diabetes. New prospects for prevention of diabetic complications. Diabetes Care 1991;14:68–72.
24. Paolisso G, D'Amore A, Giugliano D, Ceriello A, Varricchio M, D'Onofrio F. Pharmacological doses of vitamin E improve insulin action in healthy subjects and non-insulin-dependent diabetic patients. Am J Clin Nutr 1993;57:650–9.
25. Jandak J, Steiner M, Richardson PD. α-Tocopherol, an affective inhibitor of platelet adhesion. Blood 1989;73:141–9.
26. Colette C, Pares-Herbute N, Monnier LH, Cartry E. Platelet function in type I diabetes: effects of supplementation with large doses of vitamin E. Am J Clin Nutr 1988;47:256–61.
27. Gisinger C, Jeremy J, Speiser P, Mikhailidis D, Dandona P, Schernthaner G. Effect of vitamin E supplementation on platelet thromboxane A2 production in type 1 diabetic patients. Double blind crossover trial. Diabetes 1988;37:1260–4.
28. Gerster H. Prevention of platelet dysfunction by vitamin E in diabetic atherosclerosis. Z Ernährungswiss 1993;32:243–61.
29. Karasu C, Ozansuy G, Bozkurt O, Erdogan D, Omeroglu S, for the ADIC Study Group. Antioxidant and triacylglycerol-lowering effects of vitamin E associated with the prevention of abnormalities on the reactivity and morphology of aorta from streptozotocin-diabetic rats. Metabolism 1997;46:872–9.
30. Stocker R. Lipoprotein oxidation: mechanistic aspects, methodological approaches and clinical relevance. Curr Opin Lipidol 1994;5:422–33.
31. Bendich A, Machlin LJ. Safety of oral intake of vitamin E. Am J Clin Nutr 1988;48:612–9.
32. Meydani SN, Meydani M, Rall LC, Morrow F, Blumberg JB. Assessment of the safety of high-dose, short-term supplementation with vitamin E in healthy older adults. Am J Clin Nutr 1994;60:704–9.
33. Meydani SN, Meydani M, Blumberg JB, et al. Assessment of the safety of supplementation with different amounts of vitamin E in healthy older adults. Am J Clin Nutr 1998;68:311–8.
34. Friedewald WT, Levy RI, Frederickson DS. Estimation of the concentration of low density lipoprotein cholesterol in plasma without the use of the preparative ultracentrifuge. Clin Chem 1972;18:499–502.
35. Caye-Vaugien C, Krempf M, Lamarche P, Charbonnel B, Pieri J. Determination of α-tocopherol in plasma, platelets and erythrocytes of type I and II diabetic patients by HPLC. Int J Vitam Nutr Res 1990;60:324–30.
36. Beutler E. Red cell metabolism; a manual of biochemical methods. New York: Grune and Stratton, 1975.
37. Zhang A, Vertommen J, Van Gaal L, De Leeuw I. A rapid and simple method for measuring the susceptibility of low-density-lipoprotein and very-low-density-lipoprotein to copper-catalysed oxidation. Clin Chim Acta 1994;227:159–73.
38. Kayden HJ, Traber MG. Absorption, lipoprotein transport, and regulation of plasma concentrations of vitamin E in humans. J Lipid Res 1993;34:343–58.
39. Traber MG, Burton GW, Ingold KU, Kayden HJ. *RRR*- and *SRR*-α-tocopherols are secreted without discrimination in human chylomicrons, but *RRR*-α-tocopherol is preferentially secreted in very low density lipoproteins. J Lipid Res 1990;31:675–85.
40. Horvitt MK, Harvey CC, Dahm DH, Searcy MT. Relationship between tocopherol and serum lipid levels for determination of nutritional adequacy. Ann N Y Acad Sci 1972;203:223–36.
41. Kayden HJ, Hatam LJ, Traber MG. The measurement of nanograms of tocopherol from needle aspiration biopsies of adipose tissue: normal and abetalipoproteinemic subjects. J Lipid Res 1983;24:652–6.
42. Machlin LJ, Gabriel E. Kinetics of tissue α-tocopherol uptake and depletion following administration of high levels of vitamin E. Ann N Y Acad Sci 1982;393:48–60.
43. Dimitrov NV, Meyer C, Gilliland D, Ruppenthal M, Chenoweth W, Malone W. Plasma tocopherol concentrations in response to supplemental vitamin E. Am J Clin Nutr 1991;53:723–9.
44. Galvan AQ, Muscelli E, Catalano C, et al. Insulin decreases circulating vitamin E levels in humans. Metabolism 1996;45:998–1003.
45. Smith D, O'Leary VJ, Darley-Usmar VM. The role of α-tocopherol as a peroxyl radical scavenger in human low density lipoprotein. Biochem Pharmacol 1993;45:2195–201.
46. Babiy AV, Gebicki JM, Sullivan DR. Vitamin E content and low density lipoprotein oxidizability induced by free radicals. Atherosclerosis 1990;81:175–82.
47. Sevanian A, Hwang J, Cazzolato G, Avogaro P, Bittol-Bon G. Contributions of an in vivo oxidized LDL to LDL oxidation and its association with dense LDL subpopulations. Arterioscler Thromb Vasc Biol 1996;16:784–93.
48. Bucala R. Lipid and lipoprotein modification by advanced glycosylation end-products: role in atherosclerosis. Exp Physiol 1997;82:327–37.
49. Hunt JV, Smith CCT, Wolff SP. Autoxidative glycosylation and possible involvement of peroxides and free radicals in LDL modification by glucose. Diabetes 1990;39:1420–4.
50. Bonanome A, Pagnan A, Biffanti S, et al. Effect of dietary monounsaturated and polyunsaturated fatty acids on the susceptibility of plasma low density lipoproteins to oxidative modification. Arterioscler Thromb 1992;12:529–33.
51. Kleinveld HA, Naber AHJ, Stalenhoef AFH, Demacker PNM. Oxidation resistance, oxidation rate, and extent of oxidation of human

low-density lipoprotein depend on the ratio of oleic acid content to linoleic acid content: studies in vitamin E deficient subjects. Free Radic Biol Med 1993;15:273–80.
52. Kontush A, Hübner C, Finckh B, Kohlschütter A, Beisiegel U. Low density lipoprotein oxidizability by copper correlates to its initial ubiquinol-10 and polyunsaturated fatty acid content. FEBS Lett 1994;341:69–73.
53. Frei B, Gaziano JM. Content of antioxidants, preformed lipid hydroperoxides, and cholesterol as predictors of the susceptibility of human LDL to metal ion-dependent and -independent oxidation. J Lipid Res 1993;34:2135–45.
54. Stocker R, Bowry VW, Frei B. Ubiquinol-10 protects human low density lipoprotein more efficiently against lipid peroxidation than does a-tocopherol. Proc Natl Acad Sci U S A 1991;88:1646–50.
55. Tribble DL, Van den Berg JM, Motchnik PA, et al. Oxidative susceptibility of low density lipoprotein subfractions is related to their ubiquinol-10 and α-tocopherol content. Proc Natl Acad Sci U S A 1994;91:1183–7.
56. Frei B, Stocker R, Ames BN. Antioxidant defenses and lipid peroxidation in human plasma. Proc Natl Acad Sci U S A 1988;85:9748–52.
57. Neuzil J, Stocker R. Free and albumin-bound bilirubin are efficient co-antioxidants for α-tocopherol, inhibiting plasma and low density lipoprotein lipid peroxidation. J Biol Chem 1994;269:16712–9.
58. Zoppini G, Targher G, Monauni T, et al. Increase in circulating products of lipid peroxidation in smokers with IDDM. Diabetes Care 1996;19:1233–6.
59. Jain SK, McVie R, Jaramillo JJ, Chen Y. Hyperketonemia (acetoacetate) increases the oxidizability of LDL + VLDL in type-I diabetic patients. Free Radic Biol Med 1998;24:175–81.
60. Maiorino M, Zamburlini A, Roveri A, Ursini F. Copper-induced lipid peroxidation in liposomes, micelles, and LDL: which is the role of vitamin E? Free Radic Biol Med 1995;18:67–74.
61. Neuzil J, Thomas SR, Stocker R. Requirement for, promotion, or inhibition by alpha-tocopherol of radical-induced initiation of plasma lipoprotein lipid peroxidation. Free Radic Biol Med 1997;22:57–71.
62. Simons LA, Von Konigsmark M, Balasubramaniam S. What dose of vitamin E is required to reduce susceptibility of LDL to oxidation? Aust N Z J Med 1996;26:496–503.
63. Jialal I, Fuller CJ, Huet BA. The effect of α-tocopherol supplementation on LDL oxidation. A dose-response study. Arterioscler Thromb Vasc Biol 1995;15:190–8.

The American Journal of Clinical Nutrition