The Effect of Vitamin E on Endothelial Function of Micro- and Macrocirculation and Left Ventricular Function in Type 1 and Type 2 Diabetic Patients

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We examined the effects of high-dosage vitamin E treatment over a 12-month period on the vascular reactivity of micro- and macrocirculation and left ventricular function in diabetic patients. Subjects (n = 89) were randomized to vitamin E (1,800 IU daily) or placebo and were followed for 12 months. High-resolution ultrasound images were used to measure the flow-mediated dilation (FMD; endothelium dependent) and nitroglycerin-induced dilation (NID; endothelium independent) of the brachial artery. Laser Doppler perfusion imaging was used to measure vascular reactivity in the forearm skin. Left ventricular function was evaluated using transthoracic echocardiogram. At the end of the 6-month period, a worsening in endothelium-dependent skin vasodilation (P = 0.02) and rise in endothelin levels (P = 0.01) were found in the vitamin E compared with the placebo group. At the end of the 12-month period, a worsening was observed in NID (P = 0.02) and a marginal worsening was seen in systolic blood pressure (P = 0.04) and FMD (P = 0.04) in the vitamin E compared with the placebo group. In addition C-reactive protein levels decreased marginally in the vitamin E compared with the placebo group (P = 0.05). No changes were observed in left ventricular function. We concluded that long-term treatment with 1,800 IU of vitamin E has no beneficial effects on endothelial or left ventricular function in diabetic patients. Because vitamin E–treated patients had a worsening in some vascular reactivity measurements when compared with control subjects, the use of high dosages of vitamin E cannot be recommended. *Diabetes* 54:204–211, 2005

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Ach, acetylcholine chloride; CAM, cellular adhesion molecule; CRP, C-reactive protein; FMD, flow-mediated dilation; LV, left ventricular; NID, nitroglycerin-induced dilation; PAI-1, plasminogen activator inhibitor-1; PKC, protein kinase C; TNF-α, tumor necrosis factor-α; tPA, tissue plasminogen activator.

Diabetes is associated with endothelial dysfunction and a proinflammatory response; both conditions play an important role in the development of cardiovascular disease. Endothelial dysfunction is present in both macro- and microcirculation and leads to reduced vasodilation and increased expression of several markers of endothelial activation molecules, including cellular adhesion molecules (CAMs), vasoconstrictors (e.g., endothelin-1), and plasminogen activator inhibitor-1 (PAI-1) (1). Reduced nitric oxide (NO) production and/or bioavailability due to increased oxidative stress has been proposed to be a major mechanism responsible for this endothelial dysfunction.

Cardiomyopathy in the absence of coronary disease or any other possible cause, such as alcoholism or valvular disease, is also more common in diabetes and results in a higher incidence of congestive heart failure (2,3). Although the etiology is multifactorial, activation of the protein kinase C (PKC) β isoform has been proposed as a major contributing factor (4–6).

Vitamin E is one of the most readily available dietary antioxidants. Treatment with vitamin E inactivates the circulating free radicals that quench NO before it reaches vascular smooth muscle and improves the endothelial-dependent vasodilation under experimental conditions (7). In addition, results from animals studies have suggested that vitamin E ameliorates endothelial dysfunction, possibly by inhibiting the activation of PKC (8,9). Thus, vitamin E can act through multiple pathways that are related not only to endothelial function, but also to the development of long-term diabetes complications. It therefore seems reasonable to suggest that long-term treatment with this agent could be helpful in preventing these complications.

In the present study, we examined the effects of high-dosage vitamin E (1,800 IU daily) treatment over a 12-month period in diabetic patients. The main end points were the vascular reactivity of micro- and macrocirculation and left ventricular (LV) function.

RESEARCH DESIGN AND METHODS

For this study, 32 type 1 diabetic and 57 type 2 diabetic subjects, ages 21–80 years, were recruited. Diabetes was defined according to the recommendations of the American Diabetes Association's Expert Committee on the Classification and Diagnosis of Diabetes (10).

TABLE 1
Clinical characteristics of participants

	All	Type 1 diabetes	Type 2 diabetes	*P*
n	89	32	57	—
Age (years)	53 ± 14	44 ± 15	59 ± 9	<0.0001
Men	48 (54)	14 (44)	34 (60)	NS
Diabetes duration (years)	15 ± 15	25 ± 16	9 ± 9	<0.0001
BMI (kg/m^2)	29.2 ± 6.5	25.3 ± 3.8	31.4 ± 6.7	<0.0001
Systolic blood pressure (mmHg)	128 ± 19	122 ± 16	132 ± 20	0.023
Diastolic blood pressure (mmHg)	77 ± 9	73 ± 8	79 ± 9	0.002
Fasting glucose (mg/dl)	177 ± 79	195 ± 86	164 ± 70	NS
HbA_{1c} (%)	7.5 ± 1.4	7.8 ± 1.0	7.3 ± 1.5	NS
Total cholesterol (mg/dl)	186 ± 32	187 ± 28	185 ± 34	NS
LDL cholesterol (mg/dl)	101 ± 28	104 ± 21	100 ± 31	NS
HDL cholesterol (mg/dl)	59 ± 16	69 ± 18	54 ± 11	<0.0001
Triglycerides (mg/dl)	132 ± 105	71 ± 55	166 ± 111	<0.0001
Urine albumin/creatinine ratio (mg/g)	24 ± 50	12 ± 15	32 ± 61	<0.01
Antihypertensive treatment				
No treatment	46 (53)	23 (72)	23 (40)	—
β-Blockers	3 (3)	0	3 (5)	—
ACE inhibitors	23 (26)	7 (22)	16 (28)	—
Diuretics	2 (2)	0	2 (4)	—
Calcium antagonists	3 (3)	0	3 (5)	—
Combination	10 (12)	2 (6)	8 (15)	—
Diabetes treatment				
Diet	11 (13)	—	11 (19)	—
Oral agents	38 (43)	—	38 (67)	—
Insulin	40 (45)	32 (100)	8 (14)	—
Statin treatment	23 (26)	4 (13)	19 (33)	—
Aspirin	17 (19)	2 (6)	15 (26)	—

Data are means ± SD or *n* (%). *P* values indicate differences between type 1 and type 2 diabetes.

The following exclusion criteria were applied: serious long-term diabetes complications, unstable coronary artery disease, cardiac arrhythmia, congestive heart failure, uncontrolled hypertension, recent stroke, lower extremities bypass surgery for peripheral vascular disease, liver disease, renal disease (including macroalbuminuria), severe dyslipidemia (triglycerides >600 mg/dl or cholesterol >300 mg/dl), history of smoking during the previous 6 months, serious chronic disease requiring active treatment, ingestion of vitamin E during the previous 2 months, and current treatment with lipid-lowering agents, glucocorticoids, antineoplastic agents, psychoactive agents, or bronchodilators. The protocol was approved by the Institutional Review Board at the Joslin Diabetes Center and the Beth Israel Deaconess Medical Center, and all participants gave written informed consent. Volunteers for the study were recruited through local advertisement.

This was a prospective, randomized, double-blind, placebo-controlled study of a total duration of 12 months. The baseline visit included physical examination, blood tests, vascular reactivity evaluation of micro- and macrocirculation, retinal photography, and LV function measurements. Participants were seen for a middle visit 6 months after recruitment and for an exit visit at 12 months. The 6-month visit included a physical examination, blood tests, and vascular reactivity measurements. The exit visit included all the above plus retinal photography and LV function measurements. Subjects fasted overnight before all visits.

Vascular reactivity tests. Laser Doppler perfusion imaging measurements were used before and after the iontophoresis of acetylcholine chloride (Ach; endothelium-dependent vasodilation) and sodium nitroprusside (endothelium-independent vasodilation), as previously described (11). Flow-mediated dilation (FMD) was measured using a high-resolution ultrasound with a 10.0-MHz linear array transducer and the HDI Ultramark 9 system (Advanced Technology Laboratories, Bothel, WA), according to standard guidelines (12). The results were analyzed before the codes were broken in a blinded fashion. Information about the coefficient of variation of these techniques, which is <15%, has been provided elsewhere (1).

LV function measurements. LV functions were evaluated using transthoracic echocardiogram according to the American Society of Echocardiography guidelines (13,14). The following measurements were obtained: LV end-diastolic and end-systolic diameters, LV fractional shortening, the ejection fraction, and LV mass.

Evaluation of retinopathy. Evaluation of retinopathy was performed at the Eye Unit of the Joslin Diabetes Center. The photographs were scored according to the following scale: 0 = no signs of retinopathy; 1 = very mild nonproliferative; 2 = mild to moderate nonproliferative; 3 = mild and other; 4 = evidence of laser treatment; 5 = evidence of laser treatment plus additional abnormalities; 6 = evidence of overall retinal vascular tortuosity; 7 = severe nonproliferative; 8 = mild proliferative; 9 = mild proliferative plus additional abnormalities; 10 = moderate proliferative; 11 = moderate to severe proliferative; and 12 = severe plus additional abnormalities.

Treatment with vitamin E. Participants were randomized to 1,800 IU of vitamin E (in the form of 600-IU softgel capsules, all-racemic form) or corresponding placebo (485 mg of soybean oil). All capsules were taken as one dose in the morning before food. Compliance was evaluated by counting the returned capsules. The α-tocopherol levels were measured in six randomly selected vitamin E–treated and six placebo-treated patients. Baseline α-tocopherol levels were 24.9 ± 4.9 vs. 32.9 ± 4.5 μmol/l in the placebo and vitamin E groups, and posttreatment levels were 23.7 ± 6.9 and 56.2 ± 30.7 μmol/l, respectively.

Data analysis. The Minitab statistical package (Minitab, State College, PA) and the Statistical Analysis System (SAS, Cary, NC) were used for statistical analysis. The primary hypothesis of this study was to determine if there were micro- or macrocirculation changes through the use of vitamin E. Therefore, the primary analysis examined the difference in mean change between placebo- and vitamin E groups of these measures. This analysis was also done in each subset of diabetes type. The distribution of the change variables for baseline versus 6 month and baseline versus exit were tested for normality using Kolmogorov-Smirnov tests. Variables that did not meet the criteria for normality under these tests were analyzed with nonparametric methods (Kruskal-Wallis two-sample test) and are presented as medians with the 25th−75th percentile.

Because initial studies examining antioxidative agents have suggested a positive effect of vitamin E on circulation, preliminary power calculations were done estimating a 20% improvement in circulation measures in the vitamin E group. These were done using a power of 80% and a 0.05 level of significance to test the a priori hypothesis that vitamin E should increase circulation-related parameters by ≥20% in comparison with the placebo group. In the final analysis of the data, the authors felt that a significance threshold of $P < 0.025$ for significance would be a better criterion considering the number of measures being examined, but still appreciating the initial estimates of the power calculations.

The change between baseline and exit visit or baseline and 6-month visit

TABLE 2
Baseline measurements of vascular reactivity and biochemical markers of endothelial function

	All	Type 1 diabetes	Type 2 diabetes	*P*
Resting brachial artery diameter (mm)	3.62 ± 0.75	3.23 ± 0.58	3.85 ± 0.75	<0.0001
FMD	6.1 ± 3.3	6.3 ± 3.3	6.0 ± 3.2	NS
NID	12.6 ± 7.1	12.6 ± 6.8	12.6 ± 7.3	NS
Baseline blood flow before the iontophoresis of acetylcholine (volts)	0.94 ± 0.22	0.92 ± 16	0.95 ± 0.25	NS
Ach-induced skin vasodilation	58 ± 27	59 ± 29	57 ± 25	NS
Sodium nitroprusside−induced skin vasodilation	36 ± 18	40 ± 17	33 ± 17	0.03
Intracellular adhesion molecule (ng/ml)	260 ± 53	262 ± 35	259 ± 62	NS
Vascular cellular adhesion molecule (ng/ml)	729 ± 141	726 ± 117	730 ± 156	NS
Endothelin-1 (pg/ml)	0.91 ± 0.41	0.75 ± 0.22	1.01 ± 0.46	0.01
TNF-α (pg/ml)	3.36 ± 2.32	2.57 ± 1.60	3.82 ± 2.56	0.04
High-sensitivity CRP (mg/l)	3.9 ± 5.5	2.98 ± 3.63	4.52 ± 6.38	NS
von Willebrand factor (%)	128 ± 41	127 ± 45	129 ± 40	NS
PAI-1 antigen (ng/ml)	26 ± 22	16 ± 14	31 ± 24	0.01
tPA antigen (ng/ml)	6.1 ± 2.9	4.4 ± 1.6	7.1 ± 3.0	<0.0001

Data are means ± SD. Data for FMD, NID, Ach-induced skin vasodilation, and sodium nitroprusside–induced skin vasodilation represent percent of increase over baseline. *P* values indicate differences between type 1 and type 2 diabetes.

in each group was evaluated using the paired *t* test for parametrically distributed data and Wilcoxon's matched pair signed-rank test for nonparametrically distributed data. The *t* test was used to compare baseline characteristics between active and placebo groups. Correlation between variables was tested using both univariate and multivariate analyses (Pearson's correlation and Spearman's correlation analysis were used for parametrically and nonparametrically distributed data and analysis and multiple stepwise regression analysis). The results are presented as means ± SD or medians with the 25th−75th percentile.

RESULTS

The baseline demographics of the whole group of enrolled subjects and type 1 and type 2 diabetic patients separately are shown in Table 1. Type 1 diabetic patients were younger than type 2 diabetic patients ($P < 0.0001$), had a longer duration of diabetes ($P < 0.0001$), had lower BMI ($P < 0.0001$), had lower systolic ($P < 0.05$) and diastolic

TABLE 3
Comparison of changes between vitamin E– and placebo-treated patients

	Vitamin E	Placebo	*P*
Systolic blood pressure (mmHg)			
6 months	1.27 ± 17.6	−2.76 ± 12.0	0.04
12 months	12.1 ± 18.4	2.1 ± 18.8	0.05
HbA_{1c} (%)			
6 months	0.1 (−0.3 to 0.9)	−0.1 (−0.5 to 0.2)	0.04*
12 months	0.0 (−0.3 to 0.7)	0.0 (−0.5 to 0.4)	0.53*
FMD			
6 months	0.0 (−0.65 to 1.24)	0.132 (−0.58 to 1.42)	0.91*
12 months	−0.48 (−2.77 to 1.49)	0.71 (−0.19 to 2.48)	0.04*
NID			
6 months	−0.28 (−1.67 to 1.56)	−0.44 (−0.86 to 0.31)	0.84*
12 months	−1.3 (−5.65 to 0.04)	0.00 (−0.66 to 0.48)	0.02*
Cholesterol (mg/dl)			
6 months	0.0 (−9.6 to 16.8)	−6.5 (−28.5 to 10.5)	0.04*
12 months	0.5 (−14.0 to 21.5)	−14 (−26.0 to 5.0)	0.03*
LDL cholesterol (mg/dl)			
6 months	8.76 ± 22.4	−3.0 ± 28.8	0.08
12 months	5.9 ± 22.4	−1.65 ± 25.8	0.22
Ach			
6 months	−6 (−15 to 13)	11.3 (0–33)	0.02*
12 months	−2 (−21 to 23)	−6 (−23 to 15)	0.71*
Endothelin (pg/ml)			
6 months	0.1 (−0.1 to 0.2)	−0.1 (−0.2 to 0.0)	0.01*
12 months	0 (−0.1 to 0.2)	−0.1 (−0.2 to 0.1)	0.23*
tPA (μg/ml)			
6 months	0.25 (−0.58 to 1.72)	−0.40 (−2.3 to 0.5)	0.04*
12 months	0.4 (−1.0 to 2.3)	−0.3 (−1.2 to 0.8)	0.17*
CRP at 12 months (μg/ml)	−0.045 (−0.17 to 0.01)	0.02 (−0.02 to 0.11)	0.05*

Data are means ± SD or median (25th−75th percentile). Data for FMD, NID, and Ach represent percent of increase over baseline. Because of multiple comparisons, $P < 0.025$ was used for significance level. *Kruskal-Wallis test for significant difference was used for these nonnormally distributed data.

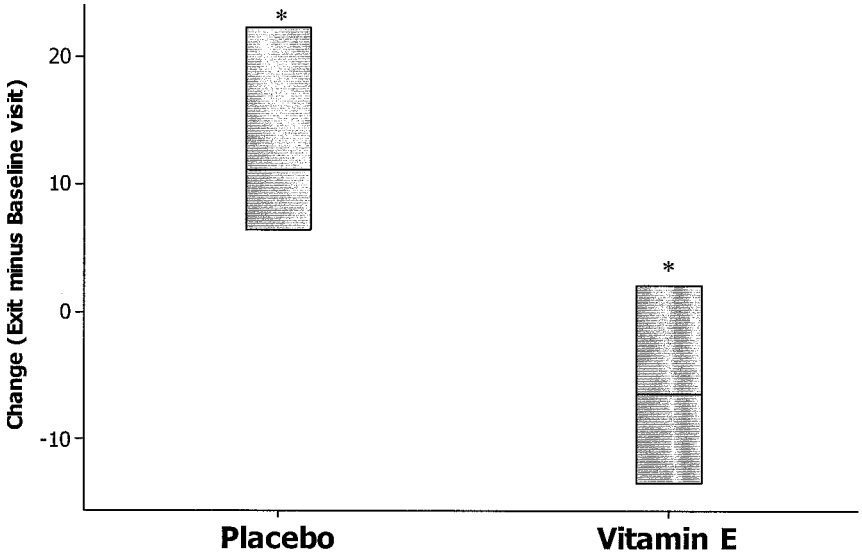

FIG. 1. Changes in the vasodilatory response to acetylcholine iontophoresis (endothelium dependent) at the forearm skin after 6 months of treatment with placebo or vitamin E (1,800 IU daily). Compared with the placebo group, the vitamin E group had a deterioration in its response. Data are shown as medians and interquartile range box. *$P < 0.01$.

($P < 0.05$) blood pressure, had higher HDL ($P < 0.0001$), and had lower triglycerides ($P < 0.0001$). The results of vascular reactivity and biochemical markers of endothelial function are shown in Table 2. Compared with type 1 diabetic patients, type 2 diabetic patients had higher levels of endothelin ($P < 0.05$), tumor necrosis factor-α (TNF; $P < 0.05$), PAI-1 ($P = 0.01$), and tissue plasminogen activator (tPA; $P < 0.0001$). No differences existed between active and placebo groups regarding treatment of diabetes, hypertension, or dyslipidemia, and glycemic control. There were no significant changes in the medications the participants were receiving during the study.

Of those initially enrolled, 26 subjects (9 type 1 and 17 type 2) did not complete the study; 5 patients did not finish the study because of nonserious adverse events not related to the study medication, and 21 subjects withdrew from the study for personal reasons or were lost to follow-up. Of these 26 subjects, 23 also did not return for the 6-month visit, whereas the remaining 3 returned for the 6-month visit but did not return for the exit visit. The results presented regarding the 6-month visit include data from the three subjects who were seen during this visit, but subsequently dropped out of the study. The subjects who did not complete the study were not different in terms of their average age, BMI, HbA_{1c}, FMD, nitroglycerin-induced dilation (NID), or Ach dilation from those who completed the study. In addition, the distribution of sex and use of a multivitamin was not different among those who did not complete the study compared with those who did.

The measurements that showed a significant difference in the changes between vitamin E and placebo groups are shown in Table 3. Because of multiple comparisons, significance was assumed at a level of $P < 0.025$. During the first 6 months of the study, improvement in endothelium-dependent skin vasodilation ($P = 0.02$) (Fig. 1) and a reduction in the endothelin ($P = 0.01$) were observed in the placebo compared with the vitamin E group. In addition, compared with the vitamin E group, the placebo group had a marginal improvement in systolic blood pressure ($P = 0.04$) and cholesterol ($P = 0.04$) and a marginal reduction in tPA ($P = 0.04$). During the entire 12-month period of the study, when compared with the control group, the vitamin E group showed a marginal increase in systolic blood pressure ($P = 0.05$). The FMD (endothelium dependent) was marginally different between the groups, mainly due to an improvement in the

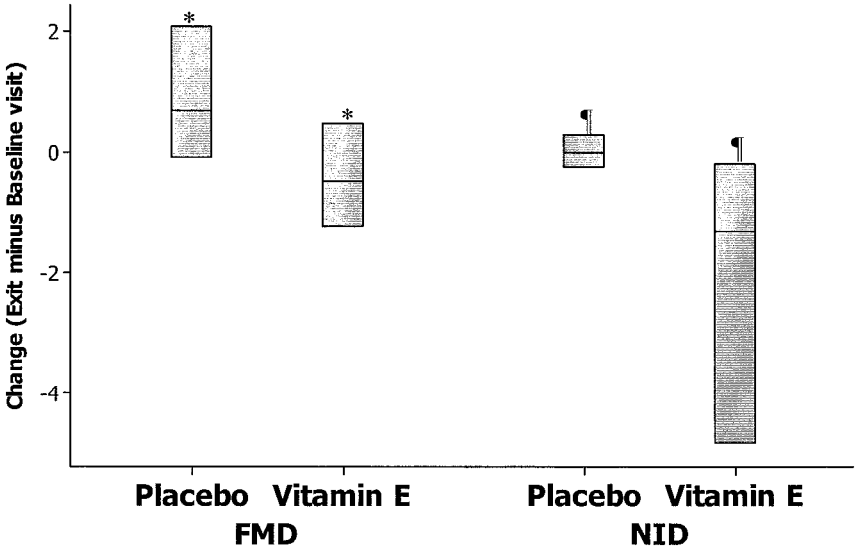

FIG. 2. Changes in brachial artery FMD (endothelium dependent) and NID (endothelium independent) after 12 months of treatment with placebo or vitamin E (1,800 IU daily). Compared with the placebo group, the vitamin E group had a deterioration in both the FMD and NID. Data are shown as medians and interquartile range box. *$P < 0.05$, ¶$P < 0.05$.

TABLE 4
Changes in endothelial function between baseline and middle (6-month) visit

	All		Type 1 diabetes		Type 2 diabetes	
	Vitamin E	Placebo	Vitamin E	Placebo	Vitamin E	Placebo
n	34	32	9	12	25	20
Brachial artery diameter (mm)						
Baseline	3.78 ± 0.78	3.43 ± 0.59	3.32 ± 0.71	3.16 ± 0.55	3.93 ± 0.76	3.60 ± 0.57
6 months	3.89 ± 0.86	3.53 ± 0.77	3.29 ± 0.68	3.31 ± 0.86	4.09 ± 0.82	3.67 ± 0.69
P	NS	NS	NS	NS	NS	NS
FMD						
Baseline	5.7 ± 2.5	5.7 ± 2.8	5.7 ± 1.8	6.6 ± 2.1	5.6 ± 2.8	5.2 ± 3.0
6 months	6.4 ± 3.4	5.9 ± 2.9	6.8 ± 1.5	5.8 ± 3.6	6.3 ± 3.8	5.9 ± 2.4
P	NS	NS	NS	NS	NS	NS
NID						
Baseline	13.2 ± 4.2	15.5 ± 5.7	15.2 ± 4.7	16.4 ± 6.2	12.4 ± 3.9	14.9 ± 5.4
6 months	12.8 ± 4.9	15.7 ± 6.5	15.8 ± 5.1	17.6 ± 9.1	11.6 ± 4.4	14.6 ± 4.0
P	NS	NS	NS	NS	NS	NS
Ach						
Baseline	58 ± 27	62 ± 26	74 ± 35	57 ± 25	53 ± 22	65 ± 27
6 months	55 ± 30	76 ± 35	57 ± 36	78 ± 35	54 ± 28	75 ± 36
P	NS	0.01	NS	0.03	NS	NS
Sodium nitroprusside						
Baseline	32 ± 17	38 ± 19	41 ± 18	40 ± 21	29 ± 15	37 ± 18
6 months	37 ± 17	46 ± 27	42 ± 19	52 ± 28	36 ± 17	43 ± 27
P	NS	NS	NS	NS	NS	NS
Endothelin (pg/ml)						
Baseline	0.90 ± 0.47	0.94 ± 0.37	0.66 ± 0.23	0.79 ± 0.23	1.01 ± 0.55	1.03 ± 0.42
6 months	0.98 ± 0.65	0.84 ± 0.40	0.60 ± 0.15	0.63 ± 0.25	1.13 ± 0.71	0.98 ± 0.43
P	NS	0.02	NS	0.002	NS	NS
HbA_{1c} (%)						
Baseline	7.2 ± 1.3	7.5 ± 1.2	7.6 ± 1.3	7.9 ± 1.1	7.1 ± 1.3	7.2 ± 1.2
6 months	7.4 ± 1.5	7.2 ± 1.1	7.8 ± 1.5	7.8 ± 0.9	7.2 ± 1.5	7.0 ± 1.2
P	NS	0.05	NS	NS	NS	NS
Total cholesterol (mg/dl)						
Baseline	182 ± 35	194 ± 31	173 ± 25	205 ± 16	185 ± 36	188 ± 37
6 months	186 ± 36	186 ± 31	182 ± 36	184 ± 25	188 ± 37	187 ± 35
P	NS	NS	NS	0.01	NS	NS
HDL cholesterol (mg/dl)						
Baseline	56 ± 14	64 ± 18	68 ± 14	76 ± 21	52 ± 12	56 ± 12
6 months	55 ± 13	60 ± 15	67 ± 11	67 ± 16	50 ± 11	56 ± 13
P	NS	0.06	NS	0.02	NS	NS

Data are means ± SD. Data for FMD, NID, and Ach represent percent of increase over baseline. *P* indicates differences between baseline and mid-visit values.

control group ($P = 0.04$). A difference was also observed in the NID (endothelium independent), mainly due to a reduction in the vitamin E group ($P = 0.02$) (Fig. 2). In addition, a marginal reduction was also observed in the C-reactive protein (CRP) levels in the vitamin E group ($P = 0.05$).

Changes in the endothelial function measurements between baseline and the 6-month visit are shown in Table 4. Compared with baseline, the placebo-treated patients showed an improvement in response to the iontophoresis of Ach (endothelium-dependent vasodilation) and a reduction in endothelin levels. A reduction in HbA_{1c}, total cholesterol, and HDL was also observed in the placebo group. The changes between baseline and exit visit in the active and placebo groups regarding demographic characteristics, endothelial function, and biochemical markers of endothelial function are shown in Table 5. No changes were observed in weight or BMI between the placebo and vitamin E groups (data not shown). The responses to vitamin E and placebo treatment were similar in type 1 and type 2 diabetic patients

The results of the eye examination and LV function are shown in Table 6. There was no difference in the changes during the study between the vitamin E and placebo groups. However, both groups showed a similar deterioration during the 12-month period of the study. No differences were observed in the changes in LV function from baseline to the 12-month visit between the active and control groups.

No correlations were observed between the changes in vascular reactivity of micro- and macrocirculation and any changes in glycemic control, lipids, or other biochemical markers of endothelial function.

DISCUSSION

The main findings of this study were that vitamin E does not have any beneficial effect on the vascular reactivity and ventricular function in patients with long-duration type 1 or type 2 diabetes when given at rather high dosages for a relative prolonged time period. In addition, the actively treated group showed a worsening in certain

TABLE 5
Changes in endothelial function between baseline and exit (12-month) visit

	All		Type 1 diabetes		Type 2 diabetes	
	Vitamin E	Placebo	Vitamin E	Placebo	Vitamin E	Placebo
n	32	31	10	13	22	18
Brachial artery diameter (mm)						
Baseline	3.78 ± 0.74	3.41 ± 0.60	3.46 ± 0.59	3.21 ± 0.52	3.93 ± 0.76	3.55 ± 0.63
Exit	3.97 ± 0.72	3.55 ± 0.68	3.59 ± 0.62	3.40 ± 0.70	4.15 ± 0.71	3.66 ± 0.67
P	0.03	NS	NS	NS	NS	NS
FMD						
Baseline	5.8 ± 2.3	5.8 ± 2.8	6.0 ± 3.3	6.3 ± 2.0	5.6 ± 2.7	5.5 ± 3.2
Exit	5.5 ± 3.8	6.8 ± 2.5	5.6 ± 3.7	7.0 ± 3.0	5.4 ± 3.9	6.7 ± 2.1
P	NS	0.05	NS	NS	NS	NS
NID						
Baseline	13.4 ± 4.2	15.7 ± 5.4	14.4 ± 4.9	15.5 ± 5.7	12.9 ± 3.8	15.8 ± 5.4
Exit	10.2 ± 6.9	14.9 ± 6.3	10.3 ± 7.2	14.6 ± 6.0	10.1 ± 6.9	15.1 ± 6.7
P	0.02	NS	NS	NS	0.05	NS
Ach						
Baseline	59 ± 30	64 ± 27	63 ± 41	60 ± 26	58 ± 23	67 ± 28
Exit	61 ± 39	64 ± 38	59 ± 40	52 ± 33	61 ± 40	72 ± 40
P	NS	NS	NS	NS	NS	NS
Sodium nitroprusside						
Baseline	34 ± 18	39 ± 18	40 ± 17	41 ± 21	31 ± 18	38 ± 17
Exit	38 ± 17	39 ± 17	38 ± 12	34 ± 14	38 ± 19	43 ± 18
P	NS	NS	NS	NS	NS	NS

Data are means ± SD. Data for FMD, NID, and Ach represent percent of increase over baseline. *P* indicates differences between baseline and exit values.

measurements of vascular reactivity compared with the placebo group. This is a novel finding that is contrary to our original hypothesis and one that has never been described in previous studies.

A considerable number of large clinical trials have been conducted over the last decade regarding the effect of vitamin E on preventing cardiovascular disease in at-risk populations, such as in subjects with hypercholesterolemia or a history of myocardial infarction and postmenopausal women, but the results are inconclusive (15–17). Thus, despite initial positive results in the CHAOS (Cambridge Heart Antioxidant Study), most subsequent studies have shown negative results (18). Furthermore, recent studies have also suggested that administration of vitamin E may prevent the beneficial effects of statins on HDL and the progression of coronary artery stenosis or increase the morbidity and mortality in postmenopausal women with coronary artery disease (19,20).

With regard to microvascular outcomes, no beneficial effects were detected in the diabetic patients who partic-

TABLE 6
Results of left ventricular function and eye examinations between baseline and 12-month visit

	All		Type 1 diabetes		Type 2 diabetes	
	Vitamin E	Placebo	Vitamin E	Placebo	Vitamin E	Placebo
Septal wall thickness (mm)						
Baseline	9.9 ± 1.5	10.7 ± 2.5	9.2 ± 1.2	10.4 ± 2.1	10.4 ± 1.6	11.0 ± 3.1
Exit	10.0 ± 1.7	10.7 ± 3.0	9.1 ± 1.5	10.5 ± 2.3	10.6 ± 1.7	10.9 ± 3.8
P	NS	NS	NS	NS	NS	NS
Posterior wall thickness (mm)						
Baseline	9.8 ± 1.3	9.8 ± 3.0	9.4 ± 1.1	9.3 ± 2.5	10.1 ± 1.5	10.4 ± 3.7
Exit	10.3 ± 1.9	10.0 ± 2.3	9.4 ± 1.5	9.8 ± 2.0	11.0 ± 2.0	10.3 ± 3.0
P	NS	NS	NS	NS	NS	NS
Mitral valve E wave (m/s)						
Baseline	0.84 ± 0.16	0.77 ± 0.25	0.82 ± 0.12	0.81 ± 0.25	0.85 ± 0.19	0.73 ± 0.26
Exit	0.83 ± 0.16	0.74 ± 0.26	0.75 ± 0.13	0.70 ± 0.29	0.88 ± 0.16	0.76 ± 0.25
P	NS	NS	NS	NS	NS	NS
Mitral valve E/A ratio*						
Baseline	1.19 ± 0.35	1.22 ± 0.54	1.29 ± 0.52	1.43 ± 0.78	1.13 ± 0.19	1.08 ± 0.26
Exit	1.08 ± 0.32	0.98 ± 0.32	1.16 ± 0.40	1.04 ± 0.45	1.03 ± 0.27	0.94 ± 0.22
P	NS	0.055	NS	NS	0.05	0.05
Average of both eyes						
Baseline	3.1 ± 3.4	4.0 ± 3.7	3.5 ± 3.3	4.8 ± 3.4	2.9 ± 3.5	3.5 ± 4.0
Exit	4.0 ± 3.7	5.6 ± 4.5	4.4 ± 3.8	7.2 ± 4.0	3.8 ± 3.8	4.5 ± 4.6
P	0.05	0.05	NS	NS	NS	NS

Data are means ± SD unless otherwise indicated. *Mitral valve peak early (E) and late (A) transmitral filling velocities ratio.

ipated in the HOPE (Heart Outcomes Prevention Evaluation) trial and were treated with 400 IU of vitamin E for an average of 4.5 years (21). In contrast, a previous study that used the same dosage as this study showed that vitamin E increased retinal blood flow to normal levels in type 1 diabetic patients who were considerably younger and had a shorter duration of diabetes compared with the patients in the present study (22). The differences in age and diabetes duration may have been the main reason for the observed discrepancies between the two studies. Furthermore, in previous, short-duration studies, vitamin E decreased PAI-1 and CRP levels and the albumin excretion rate and improved nerve electrophysiological parameters (23–26). Regarding macrocirculation, most short-term studies have also reported beneficial effects of vitamin E on endothelial function, but these results have not been confirmed in studies of longer duration (27–32). In diabetic patients, 3- to 6-month studies have shown negative results in type 2 diabetes and positive results in type 1 diabetes with vitamin E (33–35).

In the present study, we followed patients for 12 months, the longest-duration study of vitamin E treatment in diabetes. Our results indicate that by the end of the study, the vitamin E group showed no changes in FMD, whereas a small improvement was noticed in the placebo group, resulting in a marginal difference in the change between the two groups (Table 3). Vitamin E–treated patients also showed a deterioration in NID, whereas the control subjects remained stable. Furthermore, both groups showed a minor increase in systolic blood pressure, but this increase was more pronounced in the vitamin E group, resulting in a marginal difference between the two groups. It is also of interest that, in contrast to previous studies, we observed the same trend in both type 1 and type 2 diabetic patients, namely, the lack of any beneficial effect of vitamin E

We have also investigated changes in skin microcirculation, an end point that has not been previously examined. A difference in endothelium-dependent vasodilation at the 6-month visit was found between the active and placebo groups, but this difference did not persist to the end of the study. These changes were accompanied by a similar change, although relatively small to be characterized as clinically significant, in the endothelin levels. These results suggest that these changes in endothelin may be related to the changes in endothelium-dependent vasodilation. The finding of no change in endothelium-dependent vasodilatation and endothelin levels at the end of the 12-month study period indicates that these abnormalities were transient, but further studies will be required before any solid conclusions can be made.

Another interesting finding in this study was the slight improvement in HbA_{1c}, a minor lowering of total and LDL cholesterol, an improvement in the Ach response, and a reduction of endothelin levels in the control group. Finally, when comparisons were made between the placebo and active groups, a marginal improvement in HbA_{1c} and total cholesterol levels was noticed during the first 6 months in the control group, whereas tPA was higher in the active group (Table 3). The reasons for these findings are not clear, but were not related to any additional care in the placebo group. In addition, as the placebo pills contained only a very small amount of soybean oil, it seems that the observed results are related mostly to a placebo nonspecific effect rather than a specific effect of the soybean oil.

Vitamin E had no influence in LV function measurements. To our knowledge, this is the first study to examine this end point. As no major changes were observed between the baseline and end of the study in the active and placebo groups, these results indicate a slow progression of ventricular changes in diabetes. This is in contrast to the retinal evaluation, which showed a deterioration of diabetic retinopathy in both groups, with no effect of the vitamin treatment.

Acute administration of vitamin E can prevent the postprandial increase of PAI-1 and inflammatory cytokines such as TNF-α, intracellular adhesion molecule, vascular CAM, and CRP that is related to the ingestion of a high-fat meal (36). In this study, we observed a small reduction in CRP levels after 12 months of treatment. The possible anti-inflammatory effects of vitamin E should not be overlooked, given the mounting evidence that CRP may be an important factor in the pathogenesis of atherosclerosis (37).

In conclusion, the results of this study indicate that long-term treatment with 1,800 IU of vitamin E daily has no beneficial effects on endothelial or LV function in diabetic patients. Because vitamin E–treated patients had a worsening in some vascular reactivity measurements when compared with control subjects, the use of high dosages of vitamin E cannot be recommended.

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REFERENCES

1. Caballero AE, Arora S, Saouaf R, Lim SC, Smakowski P, Park JY, King GL, LoGerfo FW, Horton ES, Veves A: Microvascular and macrovascular reactivity is reduced in subjects at risk for type 2 diabetes. *Diabetes* 48:1856–1862, 1999
2. Rubler S, Dlugash J, Yuceoglu YZ, Kumral T, Branwood AW, Grishman A: New type of cardiomyopathy associated with diabetic glomerulosclerosis. *Am J Cardiol* 30:595–602, 1972
3. Kannel WB, Hjortland M, Castelli WP: Role of diabetes in congestive heart failure: the Framingham Study. *Am J Cardiol* 34:29–34, 1974
4. Zarich SW, Nesto RW: Diabetic cardiomyopathy. *Am Heart J* 118:1000–1012, 1989
5. Wakasaki H, Koya D, Schoent FJ, Jirousek MR, Ways DK, Hoit BD, Walsh RA, King GL: Targeted overexpression of protein kinase C β2 isoform in myocardium causes cardiomyopathy. *Proc Natl Acad Sci U S A* 94:9320–9325, 1997
6. Koya D, King GL: Protein kinase C activation and the development of diabetic complications. *Diabetes* 47:859–866, 1998
7. Block ER: Hydrogen peroxide alters the physical state and function of the plasma membrane of pulmonary artery endothelial cells. *J Cell Physiol* 146:362–369, 1991
8. Keaney JF Jr, Guo Y, Cunningham D, Shwaery GT, Xu A, Vita JA: Vascular incorporation of alpha-tocopherol prevents endothelial dysfunction due to oxidized LDL by inhibiting protein kinase C stimulation. *J Clin Invest* 98:386–394, 1996
9. Kunisaki M, Fumio U, Nawata H, King GL: Vitamin E normalizes diacylglycerol-protein kinase C activation induced by hyperglycemia in rat vascular tissues. *Diabetes* 45 (Suppl.):S117–S119, 1996

10. American Diabetes Association: Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 20:1183–1197, 1997
11. Veves A, Akbari CM, Primavera J, Donaghue VM, Zacharoulis D, Chrzan JS, DeGirolami U, LoGerfo FW, Freeman R: Endothelial dysfunction and the expression of endothelial nitric oxide synthetase in diabetic neuropathy, vascular disease and foot ulceration. *Diabetes* 47:457–463, 1998
12. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, Deanfield J, Drexler H, Gerhard-Herman M, Herrington D, Vallance P, Vita J, Vogel R: Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J Am Coll Cardiol* 39:257–265, 2002
13. Sahn DJ, DeMaria A, Kisslo J, Weyman A: Recommendations regarding quantitation in M-mode echocardiography: results of a survey of echocardiographic measurements. *Circulation* 58:1072–1083, 1978
14. Nishimura RA, Tajik AJ: Quantitative hemodynamics by Doppler echocardiography: a noninvasive alternative to cardiac catheterization. *Prog Cardiovasc Dis* 36:309–342, 1994
15. Stephens NG, Parsons A, Schofield PM, Kelly F, Cheeseman K, Mitchinson MJ: Randomised controlled trial of vitamin E in patients with coronary disease: Cambridge Heart Antioxidant Study (CHAOS). *Lancet* 347:781–786, 1996
16. Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico: Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. *Lancet* 354:447–455, 1999
17. Heart Protection Study Collaborative Group: MRC/BHF Heart Protection Study of antioxidant vitamin supplementation in 20 536 high-risk individuals: a randomised placebo-controlled trial. *Lancet*. 360:7–22, 2002
18. Jialal I, Devaraj S: Antioxidants and atherosclerosis: don't throw out the baby with the bath water. *Circulation* 107:926–928, 2003
19. Brown BG, Zhao XQ, Chait A, Fisher LD, Cheung MC, Morse JS, Dowdy AA, Marino EK, Bolson EL, Alaupovic P, Frohlich J, Albers JJ: Simvastatin and niacin, antioxidant vitamins, or the combination for the prevention of coronary disease. *N Engl J Med* 345:1583–1592, 2001
20. Waters DD, Alderman EL, Hsia J, Howard BV, Cobb FR, Rogers WJ, Ouyang P, Thompson P, Tardif JC, Higginson L, Bittner V, Steffes M, Gordon DJ, Proschan M, Younes N, Verter JI: Effects of hormone replacement therapy and antioxidant vitamin supplements on coronary atherosclerosis in postmenopausal women: a randomized controlled trial. *JAMA* 288:2432–2440, 2002
21. Lonn E, Yusuf S, Hoogwerf B, Pogue J, Yi Q, Zinman B, Bosch J, Dagenais G, Mann JF, Gerstein HC, HOPE Study, MICRO-HOPE Study: Effects of vitamin E on cardiovascular and microvascular outcomes in high-risk patients with diabetes: results of the HOPE study and MICRO-HOPE substudy. *Diabetes Care* 25:1919–1927, 2002
22. Bursell SE, Clermont AC, Aiello LP, Aiello LM, Schlossman DK, Feener EP, Laffel L, King GL: High-dose vitamin E supplementation normalizes retinal blood flow and creatinine clearance in patients with type 1 diabetes. *Diabetes Care* 22:1245–1251, 1999
23. Devaraj S, Chan AV Jr, Jialal I: α-Tocopherol supplementation decreases plasminogen activator inhibitor-1 and p-selectin levels in type 2 diabetic patients. *Diabetes Care* 25:524–529, 2002
24. Upritchard JE, Sutherland WH, Mann JI: Effect of supplementation with tomato juice, vitamin E, and vitamin C on LDL oxidation and products of inflammatory activity in type 2 diabetes. *Diabetes Care* 23:733–738, 2000
25. Gaede P, Poulsen HE, Parving HH, Pedersen O: Double-blind, randomised study of the effect of combined treatment with vitamin C and E on albuminuria in type 2 diabetic patients. *Diabet Med* 18:756–760, 2001
26. Tutuncu NB, Bayraktar M, Varli K: Reversal of defective nerve conduction with vitamin E supplementation in type 2 diabetes: a preliminary study. *Diabetes Care* 21:1915–1918, 1998
27. Title LM, Cummings PM, Giddens K, Nassar BA: Oral glucose loading acutely attenuates endothelium-dependent vasodilation in healthy adults without diabetes: an effect prevented by vitamins C and E. *J Am Coll Cardiol* 36:2185–2191, 2000
28. Plotnick GD, Corretti MC, Vogel RA: Effect of antioxidant vitamins on the transient impairment of endothelium-dependent brachial artery vasoactivity following a single high-fat meal. *JAMA* 278:1682–1686, 1997
29. Nappo F, Esposito K, Cioffi M, Giugliano G, Molinari AM, Paolisso G, Marfella R, Giugliano D: Postprandial endothelial activation in healthy subjects and in type 2 diabetic patients: role of fat and carbohydrate meals. *J Am Coll Cardiol* 39:1145–1150, 2002
30. Koh KK, Blum A, Hathaway L, Mincemoyer R, Csako G, Waclawiw MA, Panza JA, Cannon RO: Vascular effects of estrogen and vitamin E therapies in postmenopausal women. *Circulation* 100:1851–1857, 1999
31. Engler MM, Engler MB, Malloy MJ, Chiu EY, Schloetter MC, Paul SM, Stuehlinger M, Lin KY, Cooke JP, Morrow JD, Ridker PM, Rifai N, Miller E, Witztum JL, Mietus-Snyder M: Antioxidant vitamins C and E improve endothelial function in children with hyperlipidemia: Endothelial Assessment of Risk From Lipids in Youth (EARLY) trial. *Circulation* 108:1059–1063, 2003
32. Kinlay S, Behrendt D, Fang JC, Delagrange D, Morrow J, Witztum JL, Rifai N, Selwyn AP, Creager MA, Ganz P: Long-term effect of combined vitamins E and C on coronary and peripheral endothelial function. *J Am Coll Cardiol* 43:629–634, 2004
33. Skyrme-Jones RA, O'Brien RC, Berry KL, Meredith IT: Vitamin E supplementation improves endothelial function in type I diabetes mellitus: a randomized, placebo-controlled study. *J Am Coll Cardiol* 36:94–102, 2000
34. Beckman JA, Goldfine AB, Gordon MB, Garrett LA, Keaney JF Jr, Creager MA: Oral antioxidant therapy improves endothelial function in type 1 but not type 2 diabetes mellitus. *Am J Physiol* 285:H2392–H2398, 2003
35. Gazis A, White DJ, Page SR, Cockcroft JR: Effect of oral vitamin E (alpha-tocopherol) supplementation on vascular endothelial function in type 2 diabetes mellitus. *Diabet Med* 16:304–311, 1999
36. Carroll MF, Schade DS: Timing of antioxidant vitamin ingestion alters postprandial proatherogenic serum markers. *Circulation* 108:24–31, 2003
37. Devaraj S, Xu DY, Jialal I: C-reactive protein increases plasminogen activator inhibitor-1 expression and activity in human aortic endothelial cells: implications for the metabolic syndrome and atherothrombosis. *Circulation* 107:398–404, 2003