High-Dose Vitamin E Supplementation Normalizes Retinal Blood Flow and Creatinine Clearance in Patients With Type 1 Diabetes

SVEN-ERIK BURSELL, PHD
ALLEN C. CLERMONT, MS
LLOYD PAUL AIELLO, MD, PHD
LLOYD M. AIELLO, MD
DEBORAH K. SCHLOSSMAN, MD
EDWARD P. FEENER, PHD
LORI LAFFEL, MD, MPH
GEORGE L. KING, MD

OBJECTIVE — To determine the effectiveness of vitamin E treatment in normalizing retinal blood flow and renal function in patients with <10 years of type 1 diabetes.

RESEARCH DESIGN AND METHODS — An 8-month randomized double-masked placebo-controlled crossover trial evaluated 36 type 1 diabetic and 9 nondiabetic subjects. Subjects were randomly assigned to either 1,800 IU vitamin E/day or placebo for 4 months and followed, after treatment crossover, for a further 4 months. Retinal blood flow was measured using video fluorescein angiography, and renal function was assessed using normalized creatinine clearance from timed urine collections.

RESULTS — After vitamin E treatment, serum levels of vitamin E were significantly elevated ($P < 0.01$) in both type 1 diabetic and control patients. Hemoglobin A_{1c} was not affected by vitamin E treatment. Diabetic patient baseline retinal blood flow (29.1 ± 7.5 pixel²/s) was significantly ($P = 0.030$) decreased compared with that of nondiabetic subjects (35.2 ± 7.2 pixel²/s). After vitamin E treatment, diabetic patient retinal blood flow (34.5 ± 7.8 pixel²/s) was significantly increased ($P < 0.001$) and was comparable with that of nondiabetic subjects. Additionally, vitamin E treatment significantly ($P = 0.039$) normalized elevated baseline creatinine clearance in diabetic patients.

CONCLUSIONS — Oral vitamin E treatment appears to be effective in normalizing retinal hemodynamic abnormalities and improving renal function in type 1 diabetic patients of short disease duration without inducing a significant change in glycemic control. This suggests that vitamin E supplementation may provide an additional benefit in reducing the risks for developing diabetic retinopathy or nephropathy.

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From the Beetham Eye Institute (S.-E.B., A.C.C., L.P.A., L.M.A., D.K.S.), Joslin Diabetes Center Eye Research Section; the Departments of Ophthalmology (S.-E.B., L.P.A., L.M.A., D.K.S.) and Medicine (E.P.F., L.L., G.L.K.), Harvard Medical School; and the Research Division (L.P.A., E.P.F., L.L., G.L.K.), Joslin Diabetes Center, Boston, Massachusetts.

Address correspondence and reprint requests to George L. King, MD, Research Division, Joslin Diabetes Center, 1 Joslin Place, Boston, MA 02215. E-mail: kingg@joslab.harvard.edu.

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Abbreviations: AER, albumin excretion ratio; ANOVA, analysis of variance; DAG, diacylglycerol; DCCT, Diabetes Control and Complications Trial; ETDRS, Early Treatment Diabetic Retinopathy Study; MCT, mean circulation time; PAI-1, plasminogen activator inhibitor-1; PKC, protein kinase C; PT, prothrombin time; PTT, partial thromboplastin time; VFA, video fluorescein angiogram.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Hyperglycemia is a major causative factor in the development of endothelial dysfunction in diabetes and the subsequent development of vascular complications. In diabetes, the vascular endothelium demonstrates an impaired synthesis or action of vasodilators (1,2), and increased vasoconstrictor release (3) resulting in an imbalance of vascular homeostasis. Hyperglycemia can mediate endothelial cell dysfunction through a number of potential pathways (4), including increased oxidative stress (5,6), impaired endothelium-derived relaxing factor/nitric oxide (7,8), and activation of the diacylglycerol (DAG)/protein kinase C (PKC) pathway (9–12). Clinical studies have shown that both hyperglycemia and diabetes impair nitric oxide–related endothelium-dependent vasodilation (1,8) and that antioxidant treatment may ameliorate endothelial cell dysfunction in diabetic patients (13). Thus, antioxidants such as vitamin E have received considerable attention with respect to their potential ability to ameliorate diabetic complications.

Retinal blood flow decreases in early diabetes are causally related to elevated retinal DAG levels and PKC-β isoform activation (9,10,14,15). Vitamin E treatment in diabetic rats reduced retinal and renal DAG levels, normalized PKC-β activation, normalized retinal blood flow (16) and glomerular filtration rates (14), and restored aortic nitric oxide–mediated endothelium-dependent relaxation (17), despite still chronically elevated blood glucose.

In diabetic patients with no or minimal diabetic retinopathy, retinal blood flow is also reduced, and this reduction is associated with glycemic control (18,19). Similarly, renal hyperfiltration is more commonly found in diabetic patients with poor glycemic control (20). Clinical studies have shown that short-term antioxidant treatment can improve endothelium-dependent function in type 1 diabetic patients (13). These clinical results, together with prior animal studies, provided support for performing a clinical study to evaluate the effectiveness of vitamin E treatment in normalizing retinal

blood flow and renal hyperfiltration in type 1 diabetic patients. Retinal blood flow normalization was considered an appropriate surrogate clinical end point for this study. The alternative use of development or progression of retinopathy would require the study of large numbers of patients over several years.

RESEARCH DESIGN AND METHODS

Study design

A single-center double-masked randomized placebo-controlled crossover clinical trial was performed with a total duration of 8 months and a treatment group crossover at 4 months. A total of 46 type 1 diabetic patients with no (Early Treatment Diabetic Retinopathy Study [ETDRS] retinopathy severity level 10) or minimal (ETDRS level 20) diabetic retinopathy (21) and 13 nondiabetic subjects were recruited. Ten diabetic and four nondiabetic subjects withdrew from the study after the initial study visit. Patient recruitment was based on variances of prior retinal blood flow measurements and the desire to detect a 20% change in retinal blood flow at a significance of $P = 0.05$ and a power of 0.8 (18). Patients were randomly assigned to either 1,800 IU vitamin E/day or placebo and were crossed over to the other treatment after 4 months. The vitamin E was encapsulated in #10 oval soft elastic gelatin (Henkel, LaGrange, IL). Each capsule (600 IU) contained 450 mg D-α-tocopherol acetate dissolved in 50 mg of edible vegetable oil. Placebo capsules contained 500 mg of soybean oil dissolved in the same 50 mg of vehicle. Placebo capsules were the same size, shape, and color as the vitamin E capsules. The vitamin E dose was chosen based on results from our prior animal study data (16).

Eligibility criteria

Type 1 diabetic patients between the ages of 18 and 45 years with diabetes for <10 years and no or minimal diabetic retinopathy (ETDRS retinopathy level 10 or 20) were eligible. Patients with microalbuminuria, those taking cardiac medications, anticoagulants, hypertension medications, or antihistamines within 1 month before study onset, or those with a history of fluorescein dye allergies, migraines, or ocular abnormalities other than diabetic retinopathy were excluded. Women of child-bearing potential were required to use a medically accepted form of contraception for the study duration and were excluded from the study if pregnant or planning pregnancy. Eligible patients were asked to discontinue vitamin E supplementation for 2 months before starting the study. Nondiabetic subjects of similar age range served as control subjects. The study received Joslin Diabetes Center Committee on Human Studies approval, and all patients signed a written informed consent.

Protocol

Patients underwent a screening evaluation of ETDRS 7-standard field stereo fundus photography, ETDRS visual acuity, D15 Color Hue test, Amsler Grid test, intraocular pressure measurement and a urinalysis. All patient study visits occurred between 9:00 and 11:00 A.M. Potentially eligible female subjects underwent a screening urine pregnancy test. Eligible patients returned for the initial visit with their timed overnight urine collection sample for timed albumin excretion rate and creatinine clearance. At this visit, seated blood pressures, heart rates, and retinal blood flow measurements were performed. Blood samples were acquired for clinical laboratory analysis of blood glucose, glycohemoglobin (HbA_{1c}), blood chemistries (hematology, electrolytes and lipids), thyroid function (T3, T4), liver function (aspartate aminotransferase, lactate dehydrogenase, prothrombin time [PT] and partial thromboplastin time [PTT]), urinalysis (total protein, microalbumin/creatinine and creatinine clearance) and serum vitamin E levels. Patients were then randomized to either 1,800 IU vitamin E p.o./day or placebo. Four weeks later, patients returned for limited blood testing (T3, T4, PT, PTT, complete blood count and platelets, lipids and serum vitamin E levels). At this visit, and at all subsequent visits, the patients answered a questionnaire reporting symptoms (headaches, muscle weakness, dizziness, diarrhea, intestinal cramps, nausea, breast pain or breast masses, emotional changes, and fatigue) potentially associated with high-dose vitamin E treatment. At the 4-month crossover visit, patients underwent all protocols as described under the initial visit. Patients were then crossed over so that patients initially receiving vitamin E were given placebo and patients previously receiving placebo received 1,800 IU/day vitamin E. Patients returned 4 weeks later for limited blood testing and serum vitamin E determinations. At 8 months, patients underwent the same protocols as for the initial visit. Compliance was assessed by capsule counts at each visit and by serum vitamin E levels.

Blood and urine assays were performed by the Joslin Diabetes Center clinical laboratory. Creatinine clearance was determined from timed overnight urine collections and normalized to 1.73 m^2 body surface area. SmithKline Beecham Clinical Laboratories (Waltham, MA) performed the liver function tests. Eisai (Tsukaba, Japan) performed plasma and erythrocyte membrane vitamin E assays using high-pressure liquid chromatography (22). Serum and erythrocyte samples for vitamin E assays were stored at −20°C before shipment on dry ice. The samples for vitamin E assays were not stored in antioxidant; thus, degradation may have occurred, resulting in lower measured levels than were actually present at collection. Plasminogen activator inhibitor-1 (PAI-1) assays were performed on a subset of 23 diabetic patients for whom serum samples were available both at baseline and after vitamin E treatment. Blood samples were collected in EDTA anticoagulant and stored at −80°C. PAI-1 antigen was determined using IMUBIND plasma PAI-1 enzyme-linked immunosorbent assay (American Diagnostica, Greenwich, CT).

Retinal blood-flow measurements

Retinal blood flows were determined from video recordings of fluorescein dye passage through the retinal circulation using a scanning laser ophthalmoscope (Rhodenstock/Canon, Lake Success, NY) as previously described (18). For video fluorescein angiograms (VFAs), a 0.75 ml bolus of 10% sodium fluorescein dye (Akorn, Abita Springs, LA) was rapidly injected, to ensure a sharp dye front in the retinal vasculature, into the antecubital vein catheter used for study blood sample withdrawals. VFAs were recorded for both eyes of each patient. Frame-by-frame analysis of VFA images was performed on the four major retinal artery/vein pairs exiting from the optic disc perfusing the four retinal quadrants. Measurements of retinal vessel diameters and retinal vessel fluorescence intensities were made at a 1,500-μm radius from the optic disc center. Parameters determined from analysis of the vessel fluorescence intensity curves provided mean circulation times (MCTs) for each quadrant. The calculated quadrant retinal blood flow was proportional to the sum of the squares of the artery and vein diameters divided by the MCT for that artery/vein pair (18). Final values for retinal vessel diameters, MCT, and blood

Table 1—*Baseline characteristics for diabetic groups*

	Nondiabetic group	Diabetic group
n	9	36
Age (years)	31.2 ± 6.8	31.6 ± 7.1
Duration of diabetes (years)	N/A	4.3 ± 2.7
Sex (M/F)	7/6	26/20
Hemoglobin A_{1c} (%)	5.3 ± 0.4	7.9 ± 1.5*
Blood glucose (mmol/l)	4.92 ± 0.62	11.97 ± 5.53*
Cholesterol (mmol/l)	4.73 ± 0.97	4.84 ± 0.86
LDL (calculated) (mmol/l)	3.15 ± 0.95	3.01 ± 0.78
Heart rate (beats/min)	64.3 ± 20.6	72.4 ± 12.8
Systolic blood pressure (mmHg)	119.2 ± 13.4	114.7 ± 11.1
Diastolic blood pressure (mmHg)	75.0 ± 13.3	73.3 ± 9.8
Mean intra-ocular pressure (mmHg)	14.7 ± 2.4	14.2 ± 2.4
Vitamin E		
Plasma (μg/ml)	8.0 ± 2.1	8.8 ± 1.8
Red blood cell (μg/ml)	0.29 ± 0.09	0.35 ± 0.13
Retinopathy grade (ETDRS 10/20)	9/0	30/6

Data are means ± SD unless otherwise indicated. *$P < 0.001$ compared with nondiabetic groups.

flow represent the averages of all four individual quadrants.

Statistical analysis

All values are quoted as mean ± 1 SD. Statistical analyses were performed using the SigmaStat (Jandel Scientific, San Rafael, CA) statistical analysis package. Group comparisons were performed using one-way analysis of variance (ANOVA). Populations were tested for normality using the Kolmogorov-Smirnov test and variance equality using the Levene Median test. Distributions failing normality or variance equality tests were analyzed using the Mann-Whitney rank-sum test for two-group comparisons and the Kruskal-Wallis ANOVA on ranks for multiple group comparisons. Vitamin E treatment effects in the same individuals were analyzed using either paired *t* test or one-way repeated measures ANOVA.

RESULTS — A total of 36 diabetic and 9 nondiabetic patients completed the study. Baseline characteristics are summarized in Table 1. Age was not significantly different between diabetic and nondiabetic groups, and subjects were evenly distributed with respect to sex. Average duration of diabetes was 4.3 ± 2.7 years. Average hemoglobin A_{1c} and blood glucose were significantly ($P < 0.001$) greater in the diabetic group. Baseline diabetic and nondiabetic serum and red blood cell vitamin E levels were not significantly different. Five diabetic patients had ETDRS retinopathy level 20 (microaneurisms only) in one eye only and one had retinopathy level 20 in both eyes. All others had no retinopathy. There were no statistically significant differences between the 10 diabetic and 4 nondiabetic subjects who did not complete the study and those who did (diabetic subjects not completing the study had an average MCT = 6.6 ± 2.6 s, blood flow = 27.2 ± 8.3 pixel²/s and creatinine clearance = 140.1 ± 15.2 ml · min^{-1} · 1.73 m^{-2}).

For diabetic patients, there was no significant change in average HbA_{1c} (baseline: 7.9 ± 1.5, placebo: 8.1 ± 2.1, vitamin E: 8.1 ± 1.7, withdrawal: 8.1 ± 1.6%) or blood glucose level (baseline: 11.97 ± 5.53, placebo: 10.21 ± 5.41, vitamin E: 11.01 ± 5.86, withdrawal: 10.2 ± 5.98 mmol/l) over the study duration, as was true for the nondiabetic groups. Serum cholesterol (vitamin E: 4.99 ± 0.68, withdrawal: 5.18 ± 0.78 mmol/l) and LDL levels (vitamin E: 3.19 ± 0.63, withdrawal: 3.26 ± 0.80 mmol/l) showed a nonsignificant trend toward increasing after vitamin E treatment in diabetic patients.

After vitamin E treatment, diabetic patient serum (8.8 ± 1.8 to 15.2 ± 5.3 μg/ml, $P < 0.001$) and red blood cell (0.35 ± 0.16 to 0.81 ± 0.57 μg/ml, $P < 0.001$) vitamin E levels significantly increased. Comparable vitamin E increases were measured in nondiabetic subjects (serum: 8.0 ± 2.1 to 17.9 ± 9.5 μg/ml, $P = 0.004$; red blood cell: 0.29 ± 0.09 to 0.98 ± 1.04 μg/ml, $P = 0.014$). During withdrawal, serum (10.3 ± 1.9 μg/ml) and red blood cell (0.43 ± 0.17 μg/ml) vitamin E levels, although reduced compared with the vitamin E treatment, remained significantly elevated ($P < 0.030$) compared with baseline. Serum lipids, however, tended to increase after vitamin E treatment. Thus, the ratio of vitamin E to lipid after withdrawal (0.87 ± 0.24), though elevated compared with baseline (0.80 ± 0.22), was not statistically significant. After vitamin E treatment, however, the vitamin E–to–lipid ratio (1.68 ± 0.79) remained significantly ($P < 0.001$) elevated.

Ocular characteristics

Visual acuity, visual function (D15 color hue test and Amsler Grid), and intra-ocular pressure was normal for all subjects, did not change over the course of the study, and were not significantly different between diabetic and nondiabetic groups. Changes in retinopathy level (from level 10 to 20 [$n = 4$] and from 20 back to 10 [$n = 2$]) were noted during the course of the study, but in all cases retinopathy did not progress beyond level 20. These changes were not statistically significant, nor were they unexpected for patients at this early stage of diabetes.

Retinal blood flow

The retinal hemodynamic parameters are summarized in Table 2. There were no significant differences in arterial or venous diameters. In diabetic patients at baseline, the retinal MCT (5.94 ± 2.09 s, $P = 0.021$) was significantly prolonged and retinal blood flow (29.1 ± 7.5 pixel²/s, $P = 0.030$) was significantly reduced compared with nondiabetic subjects. After 4 months of vitamin E treatment, there was a significant ($P = 0.002$) decrease in the retinal MCT (4.84 ± 1.02 s) and a significant ($P < 0.001$) increase in retinal blood flow (34.5 ± 7.8 pixel²/s) in diabetic patients with normalization of both parameters compared with nondiabetic patients. In the withdrawal group, MCT and blood flow normalization was maintained (4.62 ± 0.59 s and 35.8 ± 7.7 pixel²/s, respectively, $P = 0.010$ and $P = 0.006$ compared with baseline). In nondiabetic subjects, there were no significant changes in MCT or retinal blood flow after vitamin E treatment or after withdrawal.

Further analysis was performed on diabetic patients grouped by retinal blood flow quartiles. Patients in the lowest quartile showed the greatest retinal blood flow increase (9.4 ± 3.2 pixel²/s) after vitamin E treatment (19.9 ± 3.5 to 28.9 ± 3.0 pixel²/s) (Fig. 1). The increases in retinal blood flow in response to vitamin E treatment became

Table 2—*Retinal hemodynamic parameters*

	Baseline	Placebo	Vitamin E	Withdrawal
Diabetic group				
n	36	16	36	20
Intra-ocular pressure (mmHg)	14.2 ± 2.4	13.9 ± 2.8	13.7 ± 2.8	14.8 ± 2.4
Systolic blood pressure (mmHg)	114.7 ± 11.1	112.8 ± 11.4	115.4 ± 12.8	118.1 ± 13.9
Diastolic blood pressure (mmHg)	73.3 ± 9.8	71.0 ± 9.5	74.2 ± 10.4	77.4 ± 9.1
MCT (s)	5.94 ± 2.09*	5.92 ± 1.34†	4.84 ± 1.02‡	4.62 ± 0.59§
Arterial diameter (pixel)	7.6 ± 0.7	7.6 ± 0.6	7.4 ± 0.7	7.4 ± 0.6
Venous diameter (pixel)	9.6 ± 0.8	9.5 ± 0.7	9.4 ± 0.7	9.6 ± 0.8
Retinal blood flow (pixel²/s)	29.1 ± 7.5*	29.3 ± 6.3*	34.5 ± 7.8‡	35.8 ± 7.7§
Nondiabetic group				
n	9	4	9	5
Intra-ocular pressure (mmHg)	14.6 ± 2.4	14.7 ± 1.9	13.0 ± 2.0	13.3 ± 2.0
Systolic blood pressure (mmHg)	119.2 ± 13.4	116.8 ± 14.3	117.5 ± 9.6	115.2 ± 10.6
Diastolic blood pressure (mmHg)	75.0 ± 13.3	77.8 ± 10.0	78.1 ± 10.5	72.6 ± 6.2
MCT (s)	4.50 ± 0.77	4.60 ± 0.95	4.06 ± 0.87	4.73 ± 1.1
Arterial diameter (pixel)	7.3 ± 0.5	7.1 ± 0.5	7.1 ± 0.3	7.2 ± 0.3
Venous diameter (pixel)	9.6 ± 0.7	9.8 ± 1.0	9.5 ± 0.7	9.3 ± 0.7
Retinal blood flow (pixel²/s)	35.2 ± 6.4	35.0 ± 10.4	38.3 ± 11.0	33.8 ± 6.9

Data are means ± SD unless otherwise indicated. *$P < 0.05$ and †$P < 0.01$ compared with baseline nondiabetic patient group; ‡$P < 0.002$ and §$P < 0.04$ compared with diabetic patient baseline and placebo groups. The placebo column includes patients randomly assigned to placebo for the first 4 months and vitamin E for the final 4 months of the study. The vitamin E column includes all patients after 4 months of vitamin E treatment regardless of initial assignment. The withdrawal column includes patients randomly assigned to vitamin E for the first 4 months and placebo for the final 4 months of the study.

progressively less in the higher quartiles (25–49%: 6.0 ± 6.6; 50–74%: 6.0 ± 6.1; 75–100%: 1.1 ± 5.3 pixel²/s). However, in each of the three lower quartiles, there was a significant ($P < 0.030$) increase in retinal blood flow after vitamin E treatment. Patients in the highest quartile had blood flows comparable with nondiabetic subjects. There were no significant changes in the magnitude of the serum vitamin E increase between the four quartiles (5.9 ± 3.8, 8.2 ± 5.5, 6.2 ± 4.1, 6.0 ± 7.6 µg/ml, respectively). The level of glycemic control (HbA_{1c} 8.6 ± 1.8%) in the lowest quartile tended to be poorer than in the three higher quartiles (8.0 ± 1.8, 7.9 ± 1.6, 7.5 ± 1.3%). Regression analysis, however, showed no statistically significant (P = 0.14) association between baseline HbA_{1c} levels and retinal blood flown although there was a trend of decreasing retinal blood flow with poorer glycemic control (greater HbA_{1c}).

Renal function

Urinary albumin excretion was in the normal clinical range for both the diabetic and nondiabetic groups. Albumin excretion rates (AERs) >30 µg/min were noted in four patients during the course of the study; two of these patients reverted to normal AERs at subsequent study visits. These changes were not statistically significant, nor were they unexpected for patients at this early stage of diabetes. There were no significant changes in AER during the course of the study (baseline: 8.9 ± 11.7, placebo: 5.5 ± 8.5, vitamin E: 8.9 ± 15.9, withdrawal: 6.6 ± 11.6 µg/min). At baseline, creatinine clearance in the diabetic subjects (141.9 ± 32.9 ml · min^{-1} · 1.73 m^{-2}) tended to be greater than in the nondiabetic subjects (129.1 ± 23.3 ml · min^{-1} · 1.73 m^{-2}, P = 0.21). After vitamin E treatment, creatinine clearance in the diabetic patients (127.5 ± 40.1 ml · min^{-1} · 1.73 m^{-2}) decreased significantly (P = 0.039) compared with baseline. During withdrawal, creatinine clearance in the diabetic patients reverted to baseline levels (142.4 ± 52.3 ml · min^{-1} · 1.73 m^{-2}). In nondiabetic subjects, there were no significant differences in creatinine clearance during the course of the study.

Diabetic patients were divided into quartiles according to their baseline creatinine clearances. Patients in the highest quartile (Fig. 2) showed a significant (P = 0.005) reduction in creatinine clearance after vitamin E treatment (185.8 ± 5.7 at baseline to 130.1 ± 42.7 ml · min^{-1} · 1.73 m^{-2}). Patients in the three lower quartiles showed no significant changes after vitamin E treatment, although creatinine clearances tended to decrease. Additionally, the level of glycemic control tended to be worse in the highest quartile (HbA_{1c} 8.4 ± 1.3%) compared with the lowest quartile (HbA_{1c} 7.7 ± 1.8%). These differences

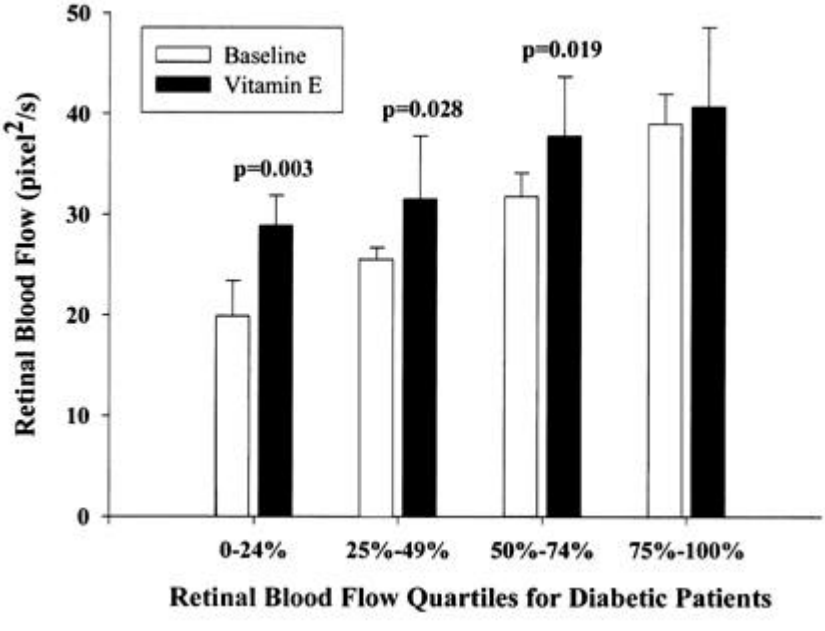

Figure 1—*Vitamin E treatment effect on retinal blood flow in diabetic patients in quartiles of retinal blood flow as determined from baseline.*

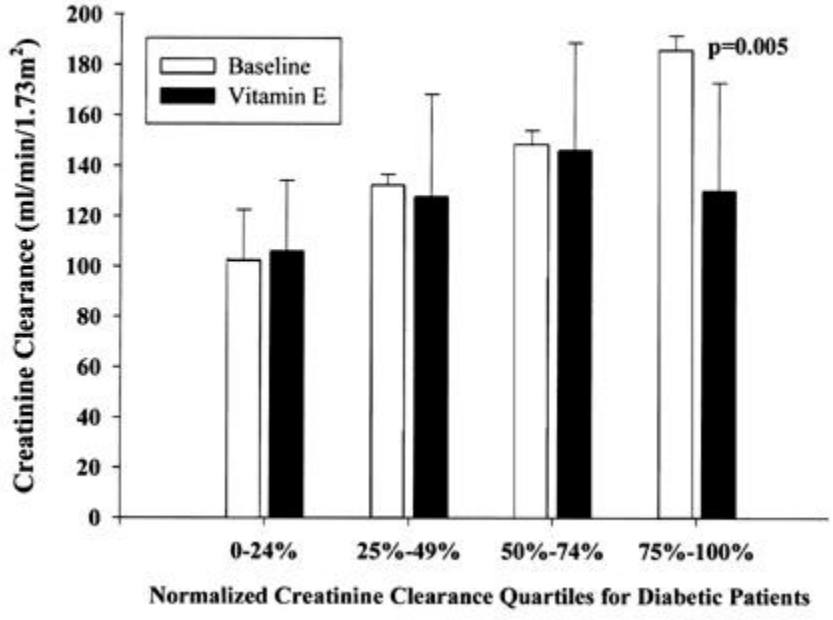

Figure 2—*Vitamin E treatment effect on creatinine clearance in diabetic patients in quartiles of creatinine clearance as determined from baseline.*

were not statistically significant. Regression analysis showed a trend for increased creatinine clearances with poorer glycemic control (higher HbA_{1c}), but this was not statistically significant.

PAI-1 measurements

PAI-1 was assayed in 23 of the diabetic patients for whom sequential blood samples were available. After vitamin E treatment, there was a significant (P = 0.048) decrease in PAI-1 levels (pretreatment: 67.3 ± 44.2, posttreatment: 53.1 ± 35.7ng/ml) (Fig. 3). The decrease in PAI-1 values was maintained during withdrawal (48.2 ± 24.4 ng/ml), but this change was not significant (P = 0.09). Regression analyses showed a significant (P = 0.012) association between baseline PAI-1 levels and triglyceride levels (slope = 0.33 ± 0.12, r = 0.54) and a nonsignificant trend for increased PAI-1 levels with poorer glycemic control. After vitamin E treatment, the association between PAI-1 levels and triglycerides was lost.

Adverse events

One diabetic patient randomized to vitamin E developed a low thyroid result (T4 = 3.9 µg/dl) and was discontinued from the study. The patient was referred to the primary care physician, and follow-up thyroid tests reverted to normal values. All reported adverse events (four dizziness, one headache, one nausea, two breast pain, one fatigue, and one diarrhea) were considered not serious, and no discernible association with vitamin E treatment was evident.

During the course of the study, diabetic and nondiabetic group comparisons showed no significant differences in clinical laboratory values, liver function, lipids, and blood chemistries (data not shown) both within and between groups, and all values were within the normal clinical ranges for all study visits. For example, diabetic patient serum creatinine (baseline: 75.9 ± 12.5, placebo: 72.1 ± 8.5, vitamin E: 74.9 ± 13.5, withdrawal: 74.4 ± 10.4 µmol/l) was not affected by vitamin E treatment.

CONCLUSIONS — These study results show that short-duration, high-dose vitamin E treatment in type 1 diabetic patients with <10 years' duration of diabetes and no or minimal diabetic retinopathy and/or microalbuminuria significantly normalized retinal blood flow and renal hyperfiltration. This physiological normalization in the early stages of diabetes could ameliorate the risk for development of retinal or renal complications. Retinal blood-flow changes were used to assess hemodynamic function and served as the end point for demonstrating the effectiveness of vitamin E treatment. The average baseline retinal blood flow of the type 1 diabetic patients was 17.3% lower than in the nondiabetic subjects, in agreement with prior studies (18,19). After 4 months of 1,800 IU/day vitamin E in these diabetic patients, retinal blood flow was significantly increased, with 88% normalization compared with nondiabetic patients. The retinal blood flow normalization was attained despite an unchanged level of glycemic control during the course of the study. Interestingly, retinal blood flow normalization by vitamin E treatment was most marked in the diabetic patients with the

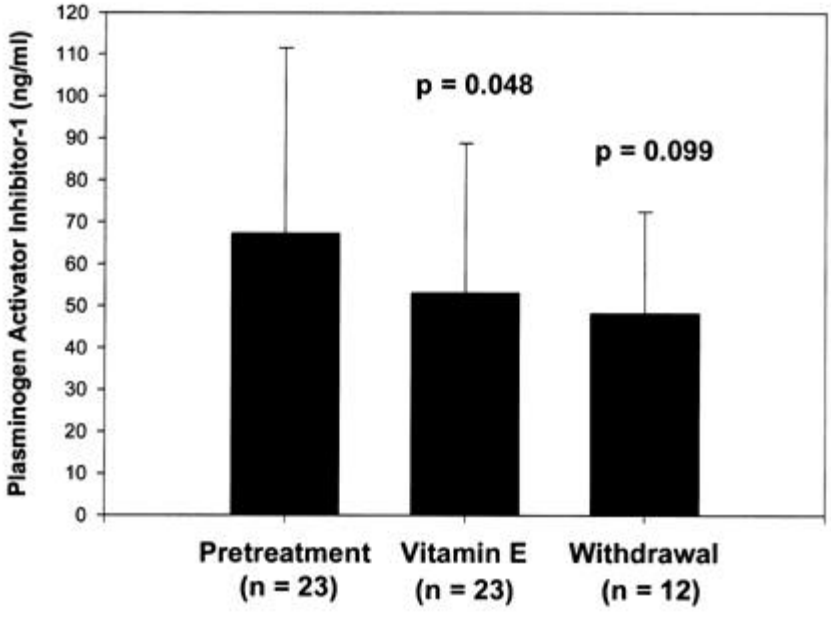

Figure 3—*Vitamin E treatment effect on serum PAI-1 levels in diabetic patients.*

lowest retinal blood flows and the poorest glycemic control, whereas diabetic patients with better glycemic control and retinal blood flows comparable with nondiabetic subjects showed no significant changes. Furthermore, retinal blood flow normalization was maintained in the diabetic patients after withdrawal (vitamin E first followed by placebo). This sustained normalization was probably associated with significant accumulation of vitamin E in lipid-rich body-tissue compartments (rat retinal and kidney vitamin E levels were ~10 and 2 times higher, respectively [14,16], than plasma levels). This stored vitamin E could subsequently be released during withdrawal, as was reflected in the still significantly elevated plasma vitamin E levels.

Renal function was assessed with AER and creatinine clearance determinations. After vitamin E treatment, type 1 diabetic patients showed an average 11% decrease in creatinine clearance to levels comparable to nondiabetic patients. As with retinal blood flow, diabetic patients with the highest creatinine clearances and poorest glycemic control showed the most marked normalization in response to vitamin E treatment. However, in contrast to the sustained normalization of retinal blood flow, creatinine clearance reverted to the baseline values after withdrawal.

Renal function analysis in the Diabetes Control and Complications Trial (DCCT) showed a significant association between HbA_{1c} and baseline creatinine clearance (24) and between good glycemic control and risk reduction for development of microalbuminuria (20,23). The baseline findings from our study are in agreement with the DCCT. In addition, the normalization of creatinine clearance in our study suggests that vitamin E could exert a beneficial effect on renal function by reducing hyperfiltration and possibly decreasing hemodynamically mediated renal injury, furthering the beneficial effect of improved glycemic control.

The mechanism of vitamin E action in normalizing retinal blood flow and renal hyperfiltration, based on prior animal studies, is associated with a normalization of the DAG/PKC (9,10,14–16) pathway through activation of DAG kinase (14,15). The hemodynamic results from the current clinical study are consistent with these results. Vitamin E may also exert a beneficial effect through antioxidant actions or through improvement in red blood cell deformability. The level of oxidative stress was not measured in this study. Results from animal studies, however, showed that the vitamin E dose required to ameliorate vascular dysfunction was greater than that needed to reduce oxidative stress (25).

Increased PAI-1 has been implicated in the development of vascular complications in diabetes (26), and a number of factors, including triglycerides, renal dysfunction and oxidative stress, contribute to elevated PAI-1 levels in type 1 diabetic patients (27,28). The results from our study indicate that vitamin E treatment in type 1 diabetic patients reduced plasma PAI-1 levels, thus potentially reducing the risks for vascular complications.

The adverse events reported in this study were mild and distributed evenly between the placebo (five events) and vitamin E groups (five events). One patient was discontinued from the study because of a low thyroid (T4) result after 1 month of vitamin E treatment. Vitamin E treatment had no significant effect on standard clinical laboratory results. There was, however, a nonsignificant trend of increasing cholesterol with vitamin E treatment, in agreement with other studies (29). Generally, high-dose vitamin E treatment has been associated with few side effects (30–32) even at doses as high as 3,200 IU/day for 9 weeks.

There has been considerable interest in the use of vitamin E as an antioxidant agent for potential benefits in coronary disease and neoplasms. Multicenter clinical trial results have shown a significant risk reduction for coronary heart disease in women (33) and men (34) with increased vitamin E intake and a 32% decrease in the incidence of prostate cancer in subjects taking vitamin E supplements (35). Studies in the eye suggest that vitamin E may be beneficial for age-related macular degeneration (36) and retinopathy of prematurity (37). However, there have been no clinical studies to investigate the effect of vitamin E on diabetic retinopathy.

In our study, vitamin E was beneficial in normalizing retinal and renal hemodynamics without changing glycemic control, and it was most beneficial in those cases where glycemic control was poorest and retinal and renal hemodynamic abnormalities were greatest. Thus, vitamin E may potentially provide additional risk reductions for the development of retinopathy or nephropathy in addition to those achievable through intensive insulin therapy alone (34). Vitamin E is a low-cost and readily available compound associated with few known side effects; thus, its use could have a dramatic socioeconomic impact if found to be efficacious in delaying the onset of diabetic retinopathy and/or nephropathy. Since there are no other generally acceptable surrogate ophthalmic end points for diabetic retinopathy amenable to evaluating vitamin E treatment, the performance of a randomized placebo-controlled double-masked clinical trial appropriately powered to evaluate the efficacy of oral vitamin E in preventing the onset and/or slowing the progression of retinopathy and renal disease in type 1 diabetes patients should be seriously considered at this time. However, until a clinical therapeutic benefit has been unequivocally documented by such a trial, the results of the current study should not be misconstrued as an indication for the widespread use of vitamin E for the treatment of diabetic retinopathy.

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