Vitamin E Supplementation Restores Glutathione and Malondialdehyde to Normal Concentrations in Erythrocytes of Type 1 Diabetic Children

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OBJECTIVE — This study examined the relationship between cellular glutathione and vitamin E concentrations and the effect of vitamin E (α-tocopherol) supplementation on glutathione and lipid peroxidation product concentrations in the erythrocytes of type 1 diabetic patients.

RESEARCH DESIGN AND METHODS — We obtained written informed consent to participate in this study from diabetic patients (n = 29) and their age-matched nondiabetic siblings (n = 21) according to the guidelines of the Institutional Review Board on Human Experimentation. Diabetic patients were supplemented with a DL-α-tocopherol (vitamin E) capsule (100 IU/ orally) or placebo for 3 months in a double-blind clinical trial. Fasting blood samples were collected from each diabetic patient before the start of and after the 3 months of vitamin E or placebo supplementation. Glutathione, malondialdehyde (which is a product of lipid peroxidation), and α-tocopherol were determined using high-performance liquid chromatography. A total of 5 diabetic patients were excluded after randomization from the data analyses. Data were analyzed statistically using a paired Student's t test to compare 12 diabetic patients taking vitamin E with 12 diabetic patients receiving placebo supplementation and to compare diabetic patients with healthy nondiabetic subjects.

RESULTS — Erythrocytes of diabetic patients had 21% higher ($P < 0.001$) malondialdehyde and 15% lower ($P < 0.05$) glutathione concentrations than healthy subjects. Vitamin E in erythrocytes had a significant correlation with the glutathione concentrations in the erythrocytes (r = 0.46, $P < 0.02$). Vitamin E supplementation increased glutathione concentrations by 9% ($P < 0.01$) and lowered concentrations of malondialdehyde by 23% ($P < 0.001$) and of HbA_{1c} by 16% ($P < 0.02$) in erythrocytes of diabetic patients. No differences were evident in these parameters before versus after placebo supplementation.

CONCLUSIONS — Glutathione level is significantly related to vitamin E level, and supplementation with vitamin E (100 IU/day) significantly increases glutathione and lowers lipid peroxidation and HbA_{1c} concentrations in the erythrocytes of type 1 diabetic patients.

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Reduced glutathione is a major intracellular nonprotein sulfhydryl compound. Glutathione has many biologic functions, including maintenance of membrane protein sulfhydryl groups in the reduced form, the oxidation of which can otherwise cause altered cellular structure and function (1,2). Glutathione is also a cofactor for many enzymes such as glutathione peroxidase, which catalyzes detoxification of intracellular peroxides. Thus, maintenance of glutathione level is pivotal for cellular defense against oxidative injury and for cellular integrity (1,2).

Hyperglycemia in diabetes can generate free radicals, hydrogen peroxide, and reactive ketoaldehydes by the auto-oxidation of glucose or from glycated proteins (3–8). Many investigators have reported lower concentrations of glutathione in the erythrocytes, aorta, and lenses of diabetic patients compared with healthy subjects (9–19), except for some studies that reported similar glutathione concentrations (20). In addition, an increase in malondialdehyde concentration has been reported in erythrocytes and other tissues of diabetic animals and patients (21–28).

Lower glutathione and elevated lipid peroxidation concentrations are risk factors for the development of pathological states such as retinopathy, neuropathy, cataracts, and atherosclerosis (1–3). Vitamin E has been proposed to be the major lipid-soluble chain-breaking antioxidant and protects biologic membranes from lipid peroxidation (29). In vitro studies have shown that reduced glutathione can protect against peroxidation of lipids in cytosolic and particulate subfraction components of rat liver and other tissues (29). In some experiments, this protection depends on membrane α-tocopherol (30), whereas in others it does not (31). Mechanisms that have been proposed to explain the glutathione effect include the removal of species that initiate lipid peroxidation, scavenging of radicals by a glutathione-dependent protein (32), scavenging of peroxy radicals by glutathione (33), maintenance of membrane protein thiols by glutathione (34), the protection by glutathione and α-tocopherol of a glutathione S-transferase isozyme responsible for the reduction of lipid hydroperoxides (35), and a glutathione-dependent protein that recycles α-tocopherol from the α-tocopheroxy radical (31).

In vivo studies in animal models have shown inhibition of lipid peroxidation and increased glutathione concentrations in the

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Abbreviations: HPLC, high-performance liquid chromatography; TBA, thiobarbituric acid.
A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Age, duration of diabetes, HbA_{1c}, glutathioine, malondialdehyde, and α-tocopherol concentrations in erythrocytes of nondiabetic subjects and diabetic patients*

	Diabetic patients	Nondiabetic subjects	*P*
n	29	21	—
Age (years)	12.4 ± 0.7	10.9 ± 0.9	NS
Duration of diabetes (years)	5.3 ± 0.7	—	—
Blood HbA_{1c} (%)	8.44 ± 0.21	5.03 ± 0.14	<0.001
Glutathione erythrocytes (μmol/g Hb)	5.8 ± 0.2	6.4 ± 0.2	<0.05
Malondialdehyde erythrocytes (nmol)			
Per milliliter packed cell volume	1.46 ± 0.04	1.30 ± 0.04	<0.02
Per gram Hb	4.27 ± 0.13	3.67 ± 0.14	<0.03
Per micromole total lipid	0.29 ± 0.008	0.24 ± 0.006	<0.01
α-Tocopherol erythrocytes (nmol)			
Per milliliter packed cell volume	4.38 ± 0.13	4.11 ± 0.13	NS
Per gram Hb	12.88 ± 0.40	12.00 ± 0.40	NS
Per micromole total lipid	0.87 ± 0.03	0.75 ± 0.03	NS

Data are means ± SEM unless otherwise indicated. Note significant differences in the blood HbA_{1c} and in glutathione and malondialdehyde erythrocytes concentrations in erythrocytes of diabetic patients versus healthy nondiabetic subjects. Details of statistical analyses are discussed in RESEARCH DESIGN AND METHODS.

liver and heart of guinea pigs after dietary supplementation (150 mg/kg diet) with vitamin E (36,37). Similarly, subcutaneous treatment with vitamin E (10 mg/100 kg body wt) increased glutathione concentrations and reduced lipid peroxidation in renal tissues of adult rats (38). Increases in glutathione level were reported in the erythrocytes, aqueous humor, and lenses of rabbits supplemented orally with 30 mg vitamin E · day^{-1} · kg^{-1} body wt for 10 or 20 days (39). In humans, increases in glutathione level were shown in the erythrocytes and aqueous humor after high-dose oral supplementation of 1,000 IU vitamin E for 10 days in 20 subjects hospitalized for bilateral idiopathic senile cataract surgery (39). To our knowledge, no previous reports exist on the effects of vitamin E supplementation on cellular concentrations of glutathione and lipid peroxidation in diabetic patients.

This study was undertaken to test the hypothesis that vitamin E supplementation can increase cellular glutathione concentrations and lower membrane malondialdehyde levels in the erythrocytes of type 1 diabetic patients. Specific aims of this study were to examine any correlation between the endogenous vitamin E and glutathione concentrations in the erythrocytes and to determine using a placebo-controlled study whether oral vitamin E supplementation (100 IU/day) can increase glutathione and vitamin E and reduce malondialdehyde concentrations in the erythrocytes of type 1 diabetic patients.

RESEARCH DESIGN AND METHODS

— Informed written consent of all patients was obtained in accordance with the protocol approved by the Institutional Review Board on Human Experimentation. No specific criterion was used for inclusion or exclusion of patients in this study except that patients who had other disorders such as sickle cell disease or thyroid disorders or those taking other medications were excluded from the study. All patients agreed to participate in this study. Diabetic patients who were invited and agreed to participate in this study were asked to come to the clinic after overnight fasting and before taking any insulin. They were told to bring their insulin and syringes for use after drawing blood. All patients were provided with a free breakfast at the medical center cafeteria after the blood drawing. The routine examination of the patients was done after they ate their breakfast. At the end of the routine examination by the physician, patients visited the nurse in charge in the clinic in a random order. The nurse gave a bottle of vitamin E (100 IU) or placebo capsules alternatively to each diabetic patient to take orally and daily until the next visit (after 3 months). The diet of these patients was not controlled. Except for the nurse in charge, no one knew about the assignment of patients to vitamin E or placebo. Both the placebo and vitamin E capsules used in this study were similar in appearance, taste, texture, and smell. A total of 29 diabetic patients were enrolled in this study. A total of 21 nondiabetic subjects (healthy siblings) were also enrolled to serve as healthy control subjects, and data from healthy control subjects were used for the cross-sectional aspect of the study. Fasting blood samples were collected into tubes with and without EDTA before and after the vitamin E or placebo supplementation from each patient. Erythrocytes were separated and washed from the EDTA-treated blood (21). All analyses were conducted immediately after blood collection.

Glutathione was determined using high-performance liquid chromatography (HPLC) with a Lichrosphere (EM Separation Technology, Gibbstown, NJ) 100 NH_2 column, a gradient system using buffer A (80% methanol and 20% water) and buffer B (4 mol/l acetate, pH 4.5 in 64% methanol-water), and a ultraviolet/visible detector set at 365 nm (40). Vitamin E as α-tocopherol was measured by HPLC (23). Freshly obtained erythrocytes were stored in 2% pyrogallol in ethanol at −70°C. All samples were analyzed within 1 month of storage using a reverse-phase C-18 column (Waters, Milford, MA), a 95% methanol solvent system, and a uv/vis detector set at 292 nm (23). Lipid peroxidation was determined by measuring malondialdehyde, which is an end product of lipid peroxidation. Malondialdehyde can react with thiobarbituric acid (TBA), and the concentration of malondialdehyde-TBA complex was determined by its separation with an HPLC system (Waters) and a uv/vis detector set at 532 nm (41). Variation in the assay of the same sample on different days was <7%.

The HbA_{1c} value was measured by using columns and a kit from Bio-Rad (Richmond, CA), blood counts were measured by an autoanalyzer, and total phospholipid and cholesterol levels were measured as described previously (42). Total lipid concentration was determined to normalize α-tocopherol and malondialdehyde values by adding total phospholipid and total cholesterol concentrations in the erythrocytes. Before opening the code, 5 subjects were deleted for noncompliance, 2 were deleted because of lost bottles, 1 was deleted because of lost contact with the patient, 1 was deleted for taking another medication, and 1 was deleted because the patient was found to have a thyroid disorder. After the biochemical analyses and the breaking of the code, 12 diabetic patients were taking vitamin E, and 12 diabetic patients were taking placebo supplementation. All diabetic patients were under the care of the same

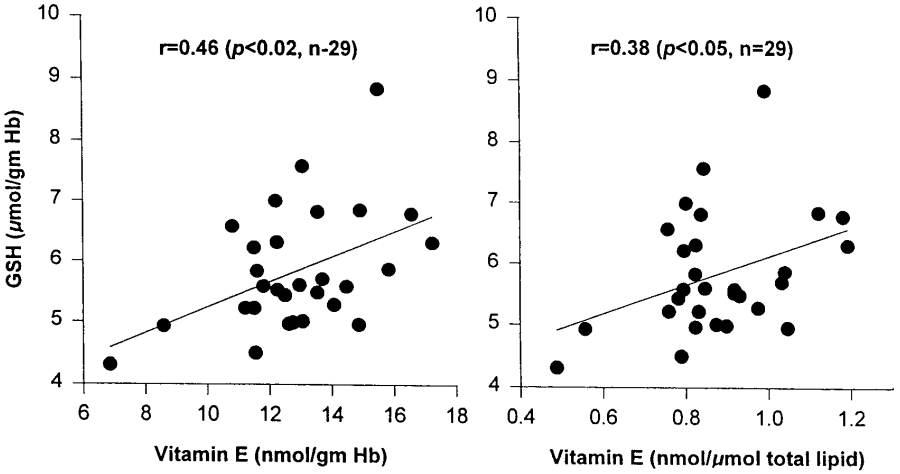

Figure 1—*Relationship between vitamin E (α-tocopherol) and GSH concentrations in erythrocytes of type 1 diabetic patients. Note a significant relationship between vitamin E and glutathione concentrations even after normalization of vitamin E to the lipid concentration. Pearson's correlation was performed using Sigma Stat Software.*

physician and did not have signs of any clinical complications. Fundoscopic exams were normal in all patients with no exudates or hemorrhage. No patient had proteinuria. Sensation to light touch was normal in all patients. Vitamin E (DL-α-tocopherol) and placebo capsules were supplied by Hoffmann-La Roche (Paramus, NJ). All data were tested for normality. Data were analyzed using the Mann-Whitney *U* test (for data that failed the normality test), Student's *t* test (for data that passed the normality test), Pearson's correlation coefficient, and the paired *t* test for comparison before and after vitamin E or placebo supplementation with Sigma Stat statistical software (Jandel Scientific, San Rafael, CA).

RESULTS — Table 1 shows data on age, duration of diabetes, HbA_{1c}, glutathione, malondialdehyde, and vitamin E (α-tocopherol) concentrations in erythrocytes of diabetic patients and nondiabetic subjects. We noted a similar age distribution among the diabetic and nondiabetic populations, but diabetic patients showed an elevated level of blood HbA_{1c} compared with nondiabetic subjects. Table 1 also shows that erythrocytes of diabetic patients have a lower level of glutathione ($P < 0.05$) and higher levels of malondialdehyde ($P < 0.03$) and HbA_{1c} ($P < 0.001$) compared with nondiabetic subjects. Malondialdehyde concentrations in diabetic patients were significantly elevated when expressed per volume or after normalization with lipid level. HbA_{1c} concentrations in diabetic patients were significantly correlated with malondialdehyde when expressed per volume ($r = 0.39$, $P < 0.05$) or after normalization with lipid level ($r = 0.38$, $P < 0.05$) or HbA_{1c} ($r = 0.39$, $P < 0.05$). No differences were evident in the vitamin E concentrations between the erythrocytes of diabetic patients and nondiabetic subjects. Figure 1 illustrates that glutathione level had a significant correlation with vitamin E level in the erythrocytes of diabetic patients. This relationship was significant regardless of whether vitamin E was expressed in micromoles per gram HbA_{1c} ($r = 0.46$, $P < 0.02$) or after the normalization with total lipid concentration ($r = 0.38$, $P < 0.05$). Glutathione concentrations did not show any relationship with the malondialdehyde per lipid or per milliliter cells, duration of diabetes, or age of diabetic patients. No difference was evident in the vitamin E concentrations between diabetic patients and nondiabetic subjects, nor was any relationship evident between malondialdehyde and vitamin E concentrations in diabetic patients.

Table 2 shows vitamin E concentrations of erythrocytes in diabetic patients before and after vitamin E (E) or placebo (P) supplementation. No difference was evident in the baseline level of vitamin E among both diabetic groups. However, the vitamin E level was significantly higher in patients supplemented with vitamin E (D_1 + E) compared with respective baseline values (D_1). No effect was evident on α-tocopherol concentrations in placebo-supplemented (D_2 + P) diabetic patients compared with baseline values (D_2) .

Figure 2 illustrates glutathione concentrations in diabetic patients before and after vitamin E or placebo supplementation. No difference was evident in the baseline level of glutathione among both diabetic groups. However, glutathione concentrations were significantly elevated ($P < 0.01$) in patients supplemented with vitamin E (6.3 ± 0.2 μmol/gm Hb) compared with respective baseline values (5.8 ± 0.3 μmol/gm Hb). No effect was evident on glutathione concentrations in placebo-supplemented diabetic patients (5.9 ± 0.2 μmol/g Hb) compared with baseline values (5.9 ± 0.4 μmol/g Hb).

Figure 3 shows data on malondialdehyde concentrations in the erythrocytes of diabetic patients before and after vitamin E or placebo supplementation. No difference was evident in the baseline level of malondialdehyde among both diabetic groups. However, malondialdehyde level was significantly lower ($P < 0.001$) in patients supplemented with vitamin E (0.23 ± 0.01 nmol/μmol total lipid) compared with respective baseline values (0.30 ± 0.02 nmol/μmol total lipid). No effect was evident on malondialdehyde concentrations in placebo-supplemented dia-

Table 2—*Effect of vitamin E (α-tocopherol) or placebo supplementation on α-tocopherol concentrations in erythrocytes of diabetic patients*

Groups	Vitamin E baseline (D_1)	After vitamin E supplementation (D_1 + E)	Placebo baseline (D_2)	After placebo supplementation (D_2 + P)
n	12	12	12	12
α-Tocopherol (nmol)				
Per milliliter	4.21 ± 0.16*	7.25 ± 0.27†	4.53 ± 0.24	3.94 ± 0.27
Per gram Hb	12.41 ± 0.59*	21.06 ± 0.74†	13.28 ± 0.69	11.45 ± 0.79
Per micromole total lipid	0.84 ± 0.04*	1.27 ± 0.04†	0.89 ± 0.05	0.80 ± 0.06

Data are *n* or means ± SEM. Data were analyzed using Student's paired *t* test. Differences in values between * and † are significant ($P < 0.0001$).

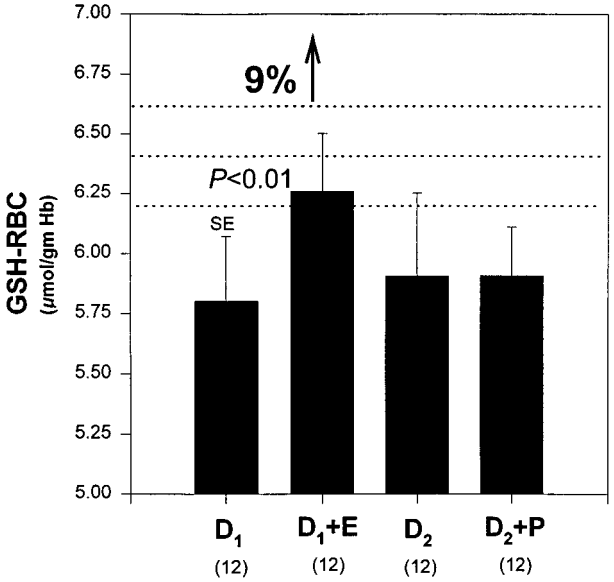

Figure 2—*GSH concentrations in erythrocytes (RBC) of diabetic patients before and after the vitamin E or placebo treatments. Note a significant increase in glutathione concentration after the vitamin E supplementation but not after placebo supplementation. Data were analyzed using Student's paired* t *test. - - -, Means ± SEM of normal values.*

betic patients (0.27 ± 0.01 nmol/μmol) compared with baseline values (0.28 ± 0.01 nmol/μmol).

Table 3 shows that HbA_{1c} concentrations were lower in patients supplemented with vitamin E but not in patients supplemented with placebo. Vitamin E or placebo supplementation did not show any effect on erythrocyte indexes. No difference was evident among vitamin E–supplemented versus placebo-supplemented diabetic patients in the distribution of age (12 ± 1 vs. 13 ± 1 years), duration of diabetes (5 ± 1 vs. 5 ± 1 years), duration of supplementation (13.3 ± 0.4 vs. 13.6 ± 0.4 weeks), and mean insulin dosage intake (0.9 ± 0.1 IU vs. 0.91 ± 0.09 IU).

CONCLUSIONS — Various studies have suggested that vitamin E may improve the metabolism of glucose by muscle cells and the circulation to the islets of Langerhans and other tissues (43,44). Different clinical trials in type 2 diabetic patients have shown that supplementation with vitamin E (100–2,000 IU/day) for a duration of 2–4 months results in either a decrease (45–47) or no effect (48,49) on blood HbA_{1c} levels, a decrease (50) or no effect (45–48) on blood glucose levels, decreased (45–47,50) or no effect (48) on triglyceride levels, lower plasma malondialdehyde concentrations (51), and reduced LDL oxidizability (48,49) compared with placebo treatments. To our knowledge, no study has examined the effect of vitamin E supplementation (100 IU/day) on glutathione and lipid peroxidation concentrations in the erythrocytes of diabetic patients.

Similar to previous studies, the present study also found elevated lipid peroxidation and lower glutathione concentrations in erythrocytes of diabetic patients compared with age-matched healthy control subjects. However, supplementation with vitamin E (100 IU/day for 3 months) significantly lowered malondialdehyde concentrations in the erythrocytes of diabetic patients. These diabetic patients did not have signs of any clinical complications, which suggests that elevated malondialdehyde and lower glutathione concentrations in erythrocytes in diabetes are not a result of the complications. The lack of any relationship of malondialdehyde with vitamin E or glutathione concentrations in erythrocytes before vitamin E supplementation to diabetic patients suggests that all of the antioxidants, not just vitamin E or glutathione, determine the level of oxidative stress in the blood. In contrast with a previous study (52), this study did not observe any differences in the vitamin E concentrations in the erythrocytes of diabetic patients versus healthy subjects.

The assay for malondialdehyde not accompanied by HPLC lacks specificity because of interference from nonmalondialdehyde material in the blood (41). However, this study has minimized such interference in the malondialdehyde assay by using HPLC. This study did not collect any data on dietary intake, so whether that has any influence on the results of this study is not known. An additional limitation of this

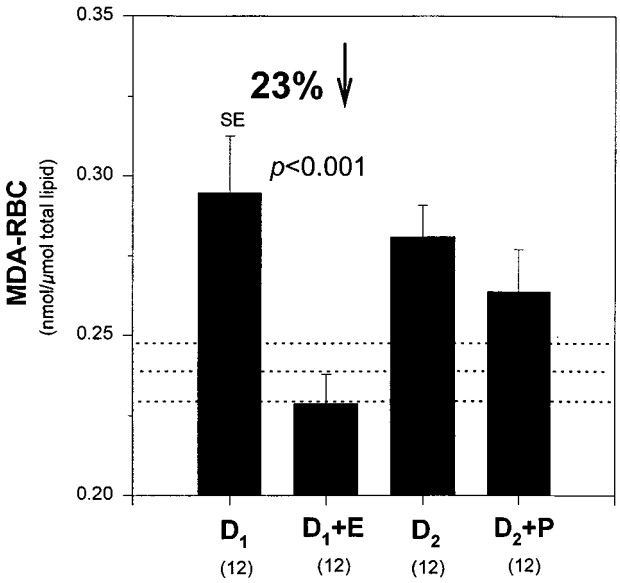

Figure 3—*MDA concentrations in erythrocytes (RBC) of diabetic patients before and after the vitamin E (α-tocopherol) or placebo treatments. Note a significant decrease in malondialdehyde concentration after the vitamin E supplementation but not after placebo supplementation. Data were analyzed using Student's paired* t *test. - - -, Means ± SEM of normal values.*

Table 3—*Effect of vitamin E (α-tocopherol) or placebo supplementation on HbA_{1c} and erythrocyte indexes in type 1 diabetic patients*

	Baseline (D_1)	Vitamin E supplementation (D_1 + E)	Baseline (D_2)	Placebo supplementation (D_2 + P)
n	12	12	12	12
HbA_{1c} (%)	8.50 ± 0.48*	7.15 ± 0.26†	8.65 ± 0.50	7.86 ± 0.35
Erythrocytes ($\times 10^6/mm^3$)	5.03 ± 0.09	5.06 ± 0.07	5.04 ± 0.07	4.97 ± 0.07
Hematocrit (%)	41.64 ± 0.81	42.03 ± 0.81	42.33 ± 0.75	42.06 ± 0.67
Hb (g/dl)	14.22 ± 0.35	14.45 ± 0.30	14.46 ± 0.26	14.47 ± 0.24

Data are means ± SEM. Data were analyzed using paired Student's *t* test. Differences in values between * and † are significant ($P < 0.02$).

study is that analysis did not involve an intent to treat the patients because patients were excluded from the analyses based on noncompliance, and assignment of patients to groups did not involve true randomization. Furthermore, the present clinical trial on vitamin E supplementation in diabetic patients is a short-term study with a limited patient population. Thus, additional clinical trials with larger patient populations are needed to replicate the results of this study.

This present study found a significant relationship between glutathione and vitamin E concentrations in the erythrocytes of diabetic patients. In addition, we have documented that vitamin E supplementation can increase cellular glutathione concentrations. This suggests that vitamin E can modulate cellular glutathione concentrations. This relationship could be because of sparing by vitamin E of glutathione utilization for the scavenging of lipid peroxidation reactions. The effect of vitamin E on lowering lipid peroxidation concentrations could be directly on scavenging of lipid peroxides, the stimulation of glutathione peroxidase activity in a fashion similar to the vitamin E stimulation of glutathione peroxidase activity in cultured cardiomyocytes (53), or the elevated level of its cofactor glutathione. We did not determine the glutathione peroxidase activity in erythrocytes in our patient population.

In addition to oxidant–antioxidant balance, the decreased level of glutathione in diabetic patients could be influenced by the decreased activity of enzymes such as γ-glutamylcysteine synthetase and glutathione reductase, possibly because of their glycation by hyperglycemia (12–14). Previous studies have reported that vitamin E in vitro (54) and vitamin E supplementation to diabetic patients can lower glycosylation of proteins (45–47). We did not measure the activity of γ-glutamylcysteine synthetase and glutathione reductase; therefore, whether vitamin E has any affect on these enzyme activities (which in turn may contribute to higher glutathione concentrations in erythrocytes after vitamin E supplementation to diabetic patients) is not known. Lack of any effect of vitamin E or placebo supplementation on erythrocyte indexes suggests that the decrease in HbA_{1c} is not likely to be because of a decrease in erythrocyte life span that can otherwise influence the blood HbA_{1c} level (55).

The present study demonstrates a significant relationship between the concentrations of vitamin E and glutathione in the erythrocytes of diabetic patients. Furthermore, vitamin E supplementation was shown to increase cellular glutathione and to lower malondialdehyde and HbA_{1c} concentrations. Thus, daily supplementation of vitamin E (100 IU/day) may reduce the incidence of vascular disease in type 1 diabetic patients.

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