

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

SUSHIL K. JAIN, PHD  
ROBERT McVIE, MD  
TINEY SMITH, RN

**OBJECTIVE** — This study examined the relationship between cellular glutathione and vitamin E concentrations and the effect of vitamin E ( $\alpha$ -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- $\alpha$ -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  $\alpha$ -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<sub>1c</sub> 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<sub>1c</sub> concentrations in the erythrocytes of type 1 diabetic patients.

*Diabetes Care* 23:1389–1394, 2000

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 detox-

ification 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  $\alpha$ -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  $\alpha$ -tocopherol of a glutathione S-transferase isozyme responsible for the reduction of lipid hydroperoxides (35), and a glutathione-dependent protein that recycles  $\alpha$ -tocopherol from the  $\alpha$ -tocopheroxy radical (31).

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

From the Department of Pediatrics, Louisiana State University Health Sciences Center, Shreveport, Louisiana.

Address correspondence and reprint requests to Sushil K. Jain, PhD, Department of Pediatrics, Louisiana State University Health Sciences Center, 1501 Kings Highway, Shreveport, LA 71130. E-mail: sjain@lsu.edu.

Received for publication 4 November 1999 and accepted in revised form 23 May 2000.

**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<sub>1c</sub>, glutathione, malondialdehyde, and  $\alpha$ -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 <sub>1c</sub> (%)	8.44 ± 0.21	5.03 ± 0.14	<0.001
Glutathione erythrocytes ( $\mu$ mol/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
$\alpha$ -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<sub>1c</sub> 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<sup>-1</sup> · kg<sup>-1</sup> 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.

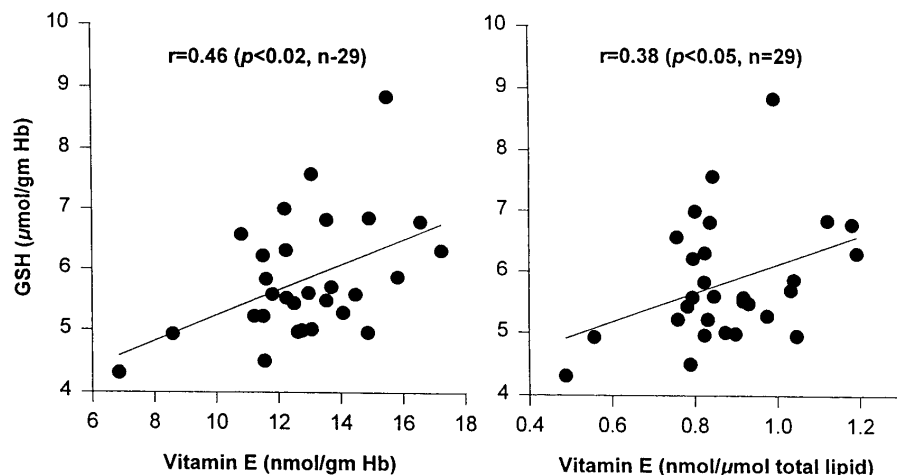
#### RESEARCH DESIGN AND

**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<sub>2</sub> 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  $\alpha$ -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<sub>1c</sub> 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  $\alpha$ -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 ( $\alpha$ -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- $\alpha$ -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<sub>1c</sub>, glutathione, malondialdehyde, and vitamin E ( $\alpha$ -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<sub>1c</sub> 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<sub>1c</sub> ( $P < 0.001$ ) compared with nondiabetic subjects. Malondialdehyde concentrations in diabetic patients were significantly elevated when expressed per volume or after normalization with lipid level. HbA<sub>1c</sub> concentrations in diabetic patients were signifi-

cantly 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<sub>1c</sub> ( $r = 0.39$ ,  $P < 0.05$ ). 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<sub>1c</sub> ( $r = 0.46$ ,  $P < 0.02$ ) 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  $\alpha$ -tocopherol concentrations in placebo-supplemented ( $D_2 + P$ ) diabetic patients compared with baseline values ( $D_2$ ).

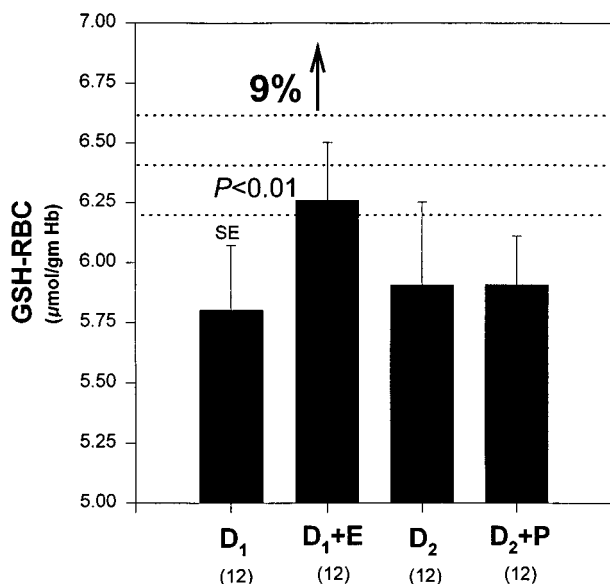
Figure 2 illustrates glutathione concentrations in diabetic patients before and after vitamin E or placebo supplementation. No difference was evident in the baseline level of glutathione among both diabetic groups. However, glutathione concentrations were significantly elevated ( $P < 0.01$ ) in patients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

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 ( $0.23 \pm 0.01$   $\text{nmol}/\mu\text{mol total lipid}$ ) compared with respective baseline values ( $0.30 \pm 0.02$   $\text{nmol}/\mu\text{mol total lipid}$ ). No effect was evident on malondialdehyde concentrations in placebo-supplemented dia-

**Table 2**—Effect of vitamin E ( $\alpha$ -tocopherol) or placebo supplementation on  $\alpha$ -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 ( $D_2 + P$ )
<i>n</i>	12	12	12	12
$\alpha$ -Tocopherol (nmol)				
Per milliliter	$4.21 \pm 0.16^*$	$7.25 \pm 0.27^\dagger$	$4.53 \pm 0.24$	$3.94 \pm 0.27$
Per gram Hb	$12.41 \pm 0.59^*$	$21.06 \pm 0.74^\dagger$	$13.28 \pm 0.69$	$11.45 \pm 0.79$
Per micromole total lipid	$0.84 \pm 0.04^*$	$1.27 \pm 0.04^\dagger$	$0.89 \pm 0.05$	$0.80 \pm 0.06$

Data are *n* or means  $\pm$  SEM. Data were analyzed using Student's paired *t* test. Differences in values between \* and  $\dagger$  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  $\pm$  SEM of normal values.

betic patients ( $0.27 \pm 0.01$  nmol/ $\mu$ mol) compared with baseline values ( $0.28 \pm 0.01$  nmol/ $\mu$ mol).

Table 3 shows that HbA<sub>1c</sub> 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 ( $12 \pm 1$  vs.  $13 \pm 1$  years), duration of diabetes ( $5 \pm 1$  vs.  $5 \pm 1$  years), duration of supplementation ( $13.3 \pm 0.4$  vs.  $13.6 \pm 0.4$  weeks), and mean insulin dosage intake ( $0.9 \pm 0.1$  IU vs.  $0.91 \pm 0.09$  IU).

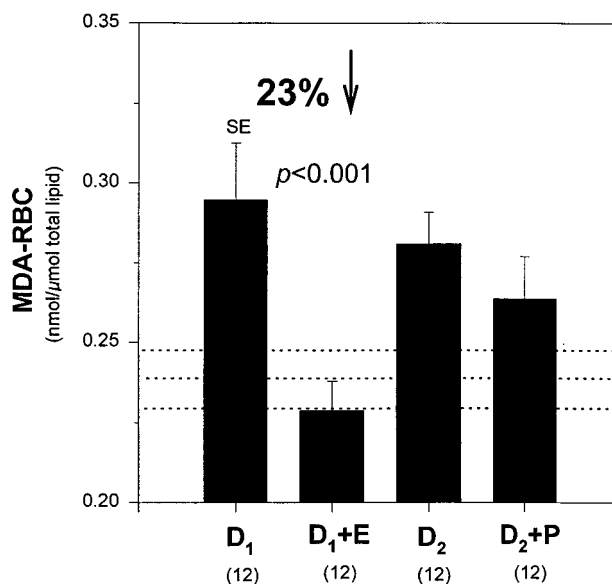
**CONCLUSIONS** — Various studies have suggested that vitamin E may improve the metabolism of glucose by muscle cells and the circulation to the islets of Langerhans and other tissues (43,44). Different clinical trials in type 2 diabetic patients have shown that supplementation with vitamin E (100–2,000 IU/day) for a duration of 2–4 months results in either a decrease (45–47) or no effect (48,49) on blood HbA<sub>1c</sub> 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 ( $\alpha$ -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  $\pm$  SEM of normal values.

**Table 3—Effect of vitamin E ( $\alpha$ -tocopherol) or placebo supplementation on HbA<sub>1c</sub> and erythrocyte indexes in type 1 diabetic patients**

	Baseline (D <sub>1</sub> )	Vitamin E supplementation (D <sub>1</sub> + E)	Baseline (D <sub>2</sub> )	Placebo supplementation (D <sub>2</sub> + P)
n	12	12	12	12
HbA <sub>1c</sub> (%)	8.50 ± 0.48*	7.15 ± 0.26†	8.65 ± 0.50	7.86 ± 0.35
Erythrocytes (×10 <sup>6</sup> /mm <sup>3</sup> )	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  $\gamma$ -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  $\gamma$ -glutamylcysteine synthetase and glutathione reductase; therefore, whether vitamin E has any effect 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<sub>1c</sub> is not likely to be because of a decrease in erythrocyte life span that can otherwise influence the blood HbA<sub>1c</sub> 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<sub>1c</sub> concentrations. Thus, daily supplementation of vitamin E (100 IU/day) may reduce the incidence of vascular disease in type 1 diabetic patients.

**Acknowledgments** — This study was supported in part by research awards from the American Heart Association (Southeast Affiliate) and a grant-in-aid from Henkel, Kankakee, IL.

#### References

- Comperti M: Glutathione depleting agents and lipid peroxidation. *Chem Phys Lipid* 45: 143–169, 1987
- Giugliano D, Paolisso G, Ceriello A: Oxidative stress and diabetic vascular complications. *Diabetes Care* 19:257–267, 1996
- Baynes JW: Role of oxidative stress in development of complications in diabetes. *Diabetes* 40:405–411, 1991
- Jain SK: Hyperglycemia can cause membrane lipid peroxidation and osmotic fragility in human red blood cells. *J Biol Chem* 264:21340–21345, 1989
- Tesfamariam B, Cohen RA: Free radicals mediate endothelial cell dysfunction caused by elevated glucose. *Am J Physiol* 263:H321–H326, 1992
- Ceriello A, Bortolotti N, Pirisi M, Crescentini A, Tonutti L, Motz E, Russo A, Giacomello R, Stel G, Taboga C: Total plasma antioxidant capacity predicts thrombosis-prone status in NIDDM patients. *Diabetes Care* 20:1589–1593, 1997
- Woff SP, Jiang ZY, Hunt JV: Protein glycation and oxidative stress in diabetes mellitus and ageing. *Free Radic Biol Med* 10:339–352, 1991
- Mullarkey CJ, Edelstein D, Brownlee M: Free radical generation by early glycation products: a mechanism for accelerated atherogenesis in diabetes. *Biochem Biophys Res Commun* 173:932–939, 1990
- Gandhi CR, Roy-Chowdhury D: Effect of diabetes mellitus on sialic acid and glutathione content of human erythrocytes of different ages. *Indian J Exp Biol* 17:585–587, 1979
- Bono AA, Caimi A, Catania A, Samo I, Pandolfo I: Red cell peroxide metabolism in diabetes mellitus. *Horm Metab Res* 19:264–266, 1987
- Uzel N, Sivas A, Oz H: Erythrocyte lipid peroxidation and glutathione peroxidase activities in patients with diabetes. *Horm Metab Res* 19:89–90, 1987
- Stahlberg MR, Hietanen E: Glutathione and glutathione-metabolizing enzymes in the erythrocytes of healthy children with insulin-dependent diabetes mellitus, juvenile rheumatoid arthritis, celiac disease and acute lymphoblastic leukemia. *Scand J Clin Invest* 51:125–130, 1991
- Fujiwara Y, Kondo T, Murakami K, Kawakami Y: Decrease of the inhibition of lipid peroxidation by glutathione-dependent system in erythrocytes of non-insulin dependent diabetic patients. *Klin Wochenschr* 67:336–341, 1989
- Murakami K, Kondo T, Ohtsuka Y, Fujiwara Y, Shimada M, Kawakami Y: Impairment of glutathione metabolism in erythrocytes from patients with diabetes mellitus. *Metabolism* 38:753–758, 1989
- Jain SK, McVie R: Effect of glycemic control, race (white versus black), and duration of diabetes on reduced glutathione content in erythrocytes of diabetic patients. *Metabolism* 43:306–309, 1994
- Mitton KP, Dean PA, Dzialoszynski T, Xiong H, Sanford SE, Trevithick JR: Modelling cortical cataractogenesis. 13. Early effects on lens ATP/ADP and glutathione in the streptozotocin rat model of the diabetic cataract. *Exp Eye Res* 56:187–198, 1993
- Tagami S, Kondo T, Yoshida K, Hirokawa J, Ohtsuka Y, Kawakami Y: Effect of insulin on impaired antioxidant activities in aortic endothelial cells from diabetic rabbits. *Metabolism* 41:1053–1058, 1992

18. Kaji H, Kurasaki M, Ito K, Saito T, Saito K, Niioka T, Kojima Y, Ohsaki Y, Ide H, Tsuji M, Kondo T, Kawakami Y: Increased lipoperoxide value and glutathione peroxidase activity in blood plasma of type 2 (non-insulin-dependent) diabetic women. *Klin Wochenschr* 63:765-768, 1985
19. Selvam R, Anuradha CV: Lipid peroxidation and antiperoxidative enzyme changes in erythrocytes in diabetes mellitus. *Indian J Biochem Biophys* 25:268-272, 1988
20. Sinclair AJ, Girling AJ, Gray L, Lunec J, Barnett AH: An investigation of the relationship between free radical activity and vitamin C metabolism in elderly diabetic subjects with retinopathy. *Gerontology* 38:268-274, 1992
21. Jain SK, McVie R, Duett J, Herbst JJ: Erythrocyte membrane lipid peroxidation and glycosylated hemoglobin in diabetes. *Diabetes* 38:1539-1543, 1989
22. Rajeswari P, Natarajan R, Nadler JL, Kumar D: Glucose induces lipid peroxidation and inactivation of membrane associated ion transport enzymes in human erythrocytes in vivo and in vitro. *J Cell Physiol* 149:100-109, 1991
23. Jain SK, Levine SN, Duett J, Hollier B: Reduced vitamin E and increased lipofuscin products in erythrocytes of diabetic rats. *Diabetes* 40:1241-1244, 1991
24. Jain SK, Levine SN, Duett J, Hollier B: Elevated lipid peroxidation levels in red blood cells of streptozotocin-treated diabetic rats. *Metabolism* 39:971-975, 1989
25. Sundram RK, Bhaskar A, Vijayalingam S, Viswanathan M, Mohan R, Shanmugasundaram KR: Antioxidant status and lipid peroxidation in type II diabetes mellitus with and without complications. *Clin Sci* 90:255-260, 1996
26. Santini SA, Marra G, Giardina B, Cotroneo P, Mordente A, Martorana GE, Manto A, Ghirlanda G: Defective plasma antioxidant defenses and enhanced susceptibility to lipid peroxidation in uncomplicated IDDM. *Diabetes* 46:1853-1858, 1997
27. Ozben T, Nacitarhan S, Tuncer N: Plasma and urine malondialdehyde levels in non-insulin dependent diabetic patients with and without microalbuminuria. *Int J Clin Lab Res* 25:162-164, 1995
28. Horie K, Miyata T, Maeda K, Miyata S, Sugiyama S, Sakai H, Strihou CY, Monnier VM, Witztum JL, Kurokawa K: Immunohistochemical colocalization of glycoxidation products and lipid peroxidation products in diabetic renal glomerular lesions: implication for glycoxidative stress in the pathogenesis of diabetic nephropathy. *J Clin Invest* 100:2995-3004, 1997
29. Scholz RW, Reddy PV, Wynn MK, Graham KS, Liken AD, Gumprich E, Reddy CC: Glutathione-dependent factors and inhibition of rat liver microsomal lipid peroxidation. *Free Radic Biol Med* 23:815-828, 1997
30. Reddy CC, Scholtz RW, Thomas CE, Massaro EJ: Vitamin E dependent reduced glutathione inhibition of rat liver microsomal lipid peroxidation. *Life Sci* 31:571-576, 1982
31. Burk R: Glutathione dependent protection by rat liver microsomal protein against lipid peroxidation. *Biochim Biophys Acta* 757:21-28, 1983
32. Hill K, Burk RF: Role of vitamin E and selenium on glutathione-dependent protection against microsomal lipid peroxidation. *Biochem Pharmacol* 33:1065-1068, 1984
33. Barclay LR: The cooperative antioxidant role of glutathione with a lipid-soluble and a water-soluble antioxidant during peroxidation of liposomes initiated in the aqueous phase and in the lipid phase. *J Biol Chem* 263:16138-16142, 1988
34. Palamanda JR, Kehrer JP: Involvement of vitamin E and protein thiols in the inhibition of microsomal lipid peroxidation. *Lipids* 28:427-431, 1993
35. Tampo Y, Yonaha M: Vitamin E and glutathione are required for preservation of microsomal glutathione S-transferase from oxidative stress in microsomes. *Pharmacol Toxicol* 66:259-265, 1990
36. Cadenas S, Rojas C, Perez-Campo R, Lopez-Torres M, Barja G: Vitamin E protects guinea pig liver from lipid peroxidation without depressing levels of antioxidants. *Int J Biochem Cell Biol* 27:1175-1181, 1995
37. Rojas C, Cadenas S, Lopez-Torres M, Perez-Campo R, Barja G: Increase in heart glutathione redox ratio and total antioxidant capacity and decrease in lipid peroxidation after vitamin E dietary supplementation in guinea pigs. *Free Radic Biol Med* 21:907-915, 1996
38. Fleck C, Haubold D, Hillman T, Braunlich H: Influence of vitamin E treatment on glutathione system after renal ischemia in immature and adult rats. *Exp Toxicol Pathol* 49:81-86, 1997
39. Costagliola C, Iuliano G, Menzione M, Rinaldi E, Vito P, Auricchio G: Effect of vitamin E on glutathione content in red blood cells, aqueous humor and lens of humans and other species. *Exp Eye Res* 43:905-914, 1986
40. Reed DJ, Babson JR, Beatty PW, Brodie AE, Ellis WW, Potter DW: High performance liquid chromatography analysis of nanomole levels of glutathione, glutathione disulfide, and related thiols and disulfides. *Anal Biochem* 106:55-62, 1980
41. Esterbauer H, Lang J, Zdravec S, Slater T: Detection of malonaldehyde by high-performance liquid chromatography. *Methods Enzymol* 105:319-328, 1984
42. Jain SK, McVie R, Duett J, Meachum ZA, Herbst JJ: The effect of glycemic control and duration of diabetes on cholesterol and phospholipid classes in erythrocytes of type 1 diabetes. *Metabolism* 41:285-289, 1992
43. Vogelsang A: Cumulative effect of alpha-tocopherol on the insulin requirements in diabetes mellitus. *Med Record* 161:363-365, 1948
44. Paolisso G, DiMaro G, Galzerano D, Cacciapuoti F, Varricchio G, Varricchio M, D'Onofrio F: Pharmacological doses of vitamin E and insulin action in elderly subjects. *Am J Clin Nutr* 59:1291-1296, 1994
45. Ceriello A, Giugliano D, Quattraro A, Donzella C, Dipalo G, Lefebvre PJ: Vitamin E reduction of protein glycosylation in diabetes. *Diabetes Care* 14:68-72, 1991
46. Paolisso G, D'Amore A, Galzerano D, Balbi B, Giugliano D, Varricchio M, D'Onofrio F: Daily vitamin E supplements improve metabolic control but not insulin secretion in elderly type II diabetic patients. *Diabetes Care* 16:1433-1437, 1993
47. Jain SK, McVie R, Jaramillo JJ, Palmer M, Smith T: Effect of modest vitamin E supplementation on blood glycosylated hemoglobin and triglyceride levels and red cell indices in type 1 diabetic patients. *J Am Coll Nutr* 15:458-461, 1996
48. Reaven PD, Herold DA, Barnett J, Edelman S: Effects of vitamin E on susceptibility of low-density lipoprotein and low-density lipoprotein subfractions to oxidation and on protein glycation in NIDDM. *Diabetes Care* 18:807-816, 1995
49. Fuller CJ, Chandalia M, Garg A, Grundy SM, Jialal I: RRR-alpha-tocopherol acetate supplementation at pharmacological doses decreases low-density-lipoprotein oxidative susceptibility but not protein glycation in patients with diabetes mellitus. *Am J Clin Nutr* 63:753-759, 1996
50. Bierenbaum ML, Noonan FJ, Machlin LJ, Machlin S, Stier A, Watson PB, Naso AM, Fleischman AI: The effect of supplemental vitamin E on serum parameters in diabetic patients, postcoronary and normal subjects. *Nutr Rep Int* 31:1171-1180, 1985
51. Jain SK, McVie R, Jaramillo JJ, Palmer M, Smith T, Meachum ZD, Little RL: The effect of modest vitamin E supplementation on lipid peroxidation products and other cardiovascular risk factors in diabetic patients. *Lipids* 31 (Suppl.):S87-S90, 1996
52. Cinaz P, Hasanoglu A, Bideci A, Biberoglu G: Plasma and erythrocyte vitamin E levels in children with insulin dependent diabetes mellitus. *J Pediatr Endocrinol Metab* 12:193-196, 1999
53. Li RK, Cowan DB, Mickle DA, Weisel RD, Burton GW: Effect of vitamin E on human glutathione peroxidase (GSH-PX1) expression in cardiomyocytes. *Free Radic Biol Med* 21:419-426, 1996
54. Jain SK, Palmer M: The effect of oxygen radicals metabolites and vitamin E on glycosylation of proteins. *Free Radic Biol Med* 22:593-596, 1997
55. Nathan DM, Singer DE, Hurxthal K, Goodson JD: The clinical information value of the glycosylated hemoglobin assay. *N Engl J Med* 310:341-346, 1984