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Vitamin E Reduction of Protein Glycosylation in Diabetes

New Prospect for Prevention of Diabetic Complications?

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Objective: This study evaluated the possibility of inhibiting protein glycosylation in vivo with vitamin E. Research Design and Methods: Two groups of 10 insulin-requiring diabetic patients, matched for duration of disease and metabolic control, received daily vitamin E supplementation of 1200 and 600 mg, respectively, for 2 mo. A third group of 10 diabetic patients, matched for duration of disease and metabolic control, served as the control group and received placebo. Fasting plasma glucose, mean daily plasma glucose, fasting labile HbA_1, and glycosylated proteins were measured in the basal state and after 1 and 2 mo of treatment. In addition, hyperglycemic clamp studies were performed in basal state and after 1 mo of vitamin E administration in all patients. Results: Glycemic indices did not show any significant changes during the study, whereas fasting labile HbA_1 and glycosylated proteins decreased significantly after 1 and 2 mo in patients on vitamin E administration. Stable HbA_1 decreased after 2 mo. Mean glycemic incremental area in the hyperglycemic clamp procedure was similar before and after treatment, whereas a significant reduction in mean labile HbA_1 incremental area was found after vitamin E supplementation. A significant difference was also found in both fasting and incremental labile HbA_1 levels, stable HbA_1, and glycosylated proteins between the two groups of diabetic patients on the two doses of vitamin E; the diabetic patients who received the higher dose of vitamin E showed the greater reduction. No significant changes in these parameters were observed in diabetic patients on placebo administration. Conclusions: These results demonstrate that vitamin E administration may reduce protein glycosylation in diabetic subjects independently of changes in plasma glucose, an effect

that may be due to the inhibition of labile glycosylation, the first step of the Maillard reaction. Long-term studies will help establish the usefulness of vitamin E administration for the prevention of diabetic complications. *Diabetes Care* 14:68–72, 1991

Nonenzymatic glycosylation (NEG) of many proteins occurs in diabetes mellitus, and it has been suggested that NEG may play a critical role in the long-term tissue complications of diabetes (1,2). As currently accepted, the initial step of NEG is the formation of a Schiff base between the free aldehyde group of glucose in its open-chain form and amino groups from amino acid residues in a protein, the NH_2-terminal and ϵ-amino group of lysine being important binding sites. This rapid and reversible reaction contrasts with the subsequent slow rearrangement of the Schiff base to a more stable ketoamine linkage (the Amadori rearrangement), which is further transformed to browning products (3). This browning is the process recognized since 1912 as the discoloration of stored and heated foods (Maillard reaction; 4).

Workers familiar with food technology are well aware of the possiblity that the Maillard reaction can also occur between the amino substances and reductones (5). Moreover, it is well acknowledged in the food industry that inhibition of the Maillard reaction can be obtained by adding various reducing agents (6). More recently, the inhibiting effect of the potent reducing agent vitamin E on protein glycosylation in vitro has been reported (7).

The aim of this study was to evaluate the effect of vitamin E supplementation on labile and stable glycosylation in vivo in diabetic patients.

RESEARCH DESIGN AND METHODS

Thirty insulin-requiring diabetic patients were divided into three groups matched for age, duration of disease, and metabolic control (Tables 1 and 2). All patients gave informed consent to participate in the study, whose protocol was approved by the ethical committee of our institution. Apart from insulin, no other medication was allowed. All patients were asked to attempt to maintain weight and to not modify insulin dosages throughout the study. They were on a standard diabetic diet with at least 200 g of carbohydrate/day (50% carbohydrate, 30% fat, and 20% protein).

Two groups of diabetic patients were given vitamin E supplementation at 600 and 1200 mg/day, respectively; the third group, on placebo administration, served as the control group. The assignment of a group to a drug treatment was randomized and unknown to the physician. The patients were seen on a weekly basis, and metabolic evaluation was made throughout the study. Glycemic indices (fasting plasma glucose and mean daily plasma glucose levels, calculated as the mean of glucose values before and after meals) and glycosylation indices (fasting labile HbA_1, stable HbA_1, and glycosylated proteins) were measured in the basal state and after 1 and 2 mo of treatment (end point). All patients also submitted to a hyperglycemic clamp study for evaluation of plasma glucose and labile HbA_1. The test was performed in the basal state and after the 1st mo of treatment (vitamin E or placebo).

On the morning of the test, the patients were allowed to rest for 90 min, and acute hyperglycemia was induced with a pulse of 0.33 g glucose/kg body wt. Plasma glucose was maintained between 19 and 22 mM with adjustment of glucose infusion on the basis of bedside plasma glucose determinations every 5 min. Plasma glucose was measured on each occasion with a Beckman Analyzer. Labile and stable HbA_1 were determined in triplicate according to the method of Compagnucci et al. (8), by ion-exchange microcolumns (Helena, Beaumont, TX), at constant temperature (18°C). The intra-assay and interassay coefficients of variation (C.V.s) were 3.8 and 5.2% for stable HbA_1 and 4.5 and 6.7% for labile HbA_1, respectively. Glycosylated proteins were evaluated by the thiobarbituric acid method (9). Protein glycosylation was calculated as nanomoles of 5-(hydroxymethyl)-2-furaldehyde per milligram of protein with pure 5-(hydroxymethyl)-2-furaldehyde (Sigma, St. Louis, MO) as standard. All estimations were performed in triplicate, and the intra-assay and interassay C.V.s were 4.5 and 6.2%, respectively.

Stable HbA_1 and glycosylated proteins were also evaluated by affinity-gel chromatography, a sensitive method that is independent of temperature, hemoglobin variants, and free-glucose adducts (10) and is not affected by the presence of vitamin E in the sample (7). The intra-assay and interassay C.V.s for this method

TABLE 1
Clinical characteristics of diabetic patient groups

	Age (yr)	Sex (M/F)	Body mass index (kg/m²)	Duration of diabetes (yr)	Fasting C-peptide (ng/ml)	Insulin dose (U/day)
Vitamin E						
1200 mg/day	41 ± 1.5	6/4	27.3 ± 0.4	6.4 ± 1.0	0.5 ± 0.01	38.2 ± 3.3
600 mg/day	42 ± 1.0	3/7	28.6 ± 0.4	5.5 ± 1.4	0.6 ± 0.01	40.2 ± 4.8
Placebo	40 ± 1.3	4/6	28.5 ± 0.5	5.8 ± 0.7	0.5 ± 0.01	44.0 ± 5.4

Values are means ± SE.

TABLE 2
Values in basal state and after 1 and 2 mo of vitamin E administration in diabetic patient groups

	Plasma glucose (mM)	Mean daily plasma glucose (mM)	Stable HbA_1 (%)		Glycosylated protein		Serum vitamin E (μg/ml)	Mean glycemic incremental area (mM/min)	Mean labile HbA_1 incremental area (%/min)
			Fasting labile HbA_1 (%)	Ion-exchange chrom	Affinity-gel chrom	nmol HMF/mg protein	% by affinity-gel chrom		
Normal range			0.05–0.10	3–5	3–5	0.03–0.07	2–5		
Vitamin E									
1200 mg/day									
Basal	8.3 ± 1.2	10.2 ± 1.4	0.33 ± 0.03	11.8 ± 0.7	11.4 ± 0.7	1.80 ± 0.06	13.4 ± 1.0	9.9 ± 0.3	8.8 ± 0.3	1.16 ± 0.01
1st mo	9.3 ± 0.9	10.5 ± 1.3	0.20 ± 0.02*	10.6 ± 1.1	10.7 ± 0.8	1.20 ± 0.03*	7.3 ± 0.9*	29.1 ± 0.5*	9.1 ± 0.4	0.48 ± 0.01*
2nd mo	8.8 ± 1.2	10.4 ± 1.4	0.21 ± 0.02*	7.8 ± 0.5*	7.1 ± 0.6*	1.24 ± 0.05*	7.2 ± 0.8*	31.2 ± 0.6*		
600 mg/day										
Basal	8.5 ± 1.2	9.9 ± 1.2	0.37 ± 0.02	11.5 ± 0.5	11.0 ± 0.7	1.78 ± 0.06	13.6 ± 1.0	10.1 ± 0.4	8.9 ± 0.3	1.18 ± 0.02
1st mo	8.9 ± 1.1	10.4 ± 1.1	0.26 ± 0.02*	11.0 ± 0.9	11.1 ± 0.7	1.40 ± 0.04*	9.0 ± 0.9*	19.2 ± 0.5*	8.8 ± 0.2	0.83 ± 0.01*
2nd mo	9.1 ± 1.3	10.6 ± 1.5	0.25 ± 0.02*	8.9 ± 0.5*	8.1 ± 0.5*	1.41 ± 0.04*	8.9 ± 0.8*	21.4 ± 0.5*		
Placebo										
Basal	8.8 ± 0.8	10.3 ± 1.3	0.38 ± 0.04	11.60 ± 0.07	11.5 ± 0.7	1.81 ± 0.06	13.4 ± 1.0	9.8 ± 0.3	9.83 ± 0.20	1.18 ± 0.01
1st mo	8.6 ± 1.3	10.4 ± 1.6	0.35 ± 0.03	11.5 ± 0.6	11.4 ± 0.6	1.79 ± 0.06	13.2 ± 0.9	10.0 ± 0.4	9.2 ± 0.2	1.19 ± 0.02
2nd mo	8.7 ± 1.2	10.3 ± 1.2	0.36 ± 0.03	11.4 ± 0.6	11.4 ± 0.7	1.80 ± 0.05	13.2 ± 1.0	9.8 ± 0.2		

Values are means ± SE. HMF, 5-(hydroxymethyl)-2-furaldehyde; chrom, chromatography.
*$P < 0.01$ vs. basal values.

were 1.9 and 2.4% for hemoglobin and 1.8 and 2.2% for protein, respectively.

Serum vitamin E was estimated according to the method of Baker and Frank (11). C-peptide was determined as previously described (12). Statistical comparisons were made by analysis of variance; the correlation between the methods for the assay of glycosylated products was performed by simple linear regression analysis.

RESULTS

Serum vitamin E concentrations significantly increased after 1 mo of vitamin E treatment and were similar after 2 mo (Table 2). Diabetic patients taking 1200 mg vitamin E/day had serum vitamin E concentrations significantly higher than patients taking 600 mg/day (Table 2). Fasting plasma glucose and mean daily plasma glucose were not significantly different throughout the study (Table 2). After only 1 mo, vitamin E administration significantly reduced fasting labile HbA_1 levels. Similarly, glycosylated protein levels were also significantly reduced (Table 2). Stable HbA_1 levels were significantly reduced after 2 mo of treatment only (Table 2).

Mean glycemic incremental area in the hyperglycemic clamp procedure was similar before and after treatment, whereas a significant reduction in mean labile HbA_1 incremental area was found after vitamin E supplementation (Table 2, Fig. 1).

A significant difference was also found in both fasting and incremental labile HbA_1 levels, stable HbA_1, and glycosylated proteins, between the two groups of diabetic patients on the two doses of vitamin E supplementation: the diabetic patients who received the higher dose of vitamin E showed the greater reduction. No significant changes in these parameters were observed in diabetic patients on placebo administration (Table 2). A strong correlation existed between the methods used to assay HbA_1 ($r = 0.98$) and glycosylated proteins ($r = 0.97$).

CONCLUSIONS

This study demonstrates for the first time that vitamin E administration in diabetic patients reduces protein glycosylation in vivo. This phenomenon is dose related, being more evident in the diabetic patients who received 1200 mg vitamin E/day. In our patients, mean fasting plasma glucose and mean daily plasma glucose remained unchanged during the study, whereas the indices of protein glycosylation significantly decreased.

A direct correlation between plasma glucose and labile HbA_1 has been reported, suggesting a direct link between blood glucose levels and labile HbA_1 glycosylation (13). The significant decrease of both fasting labile HbA_1 and mean labile HbA_1 incremental area during the hyperglycemic clamp in the presence of unchanged glycemic values before and after vitamin E

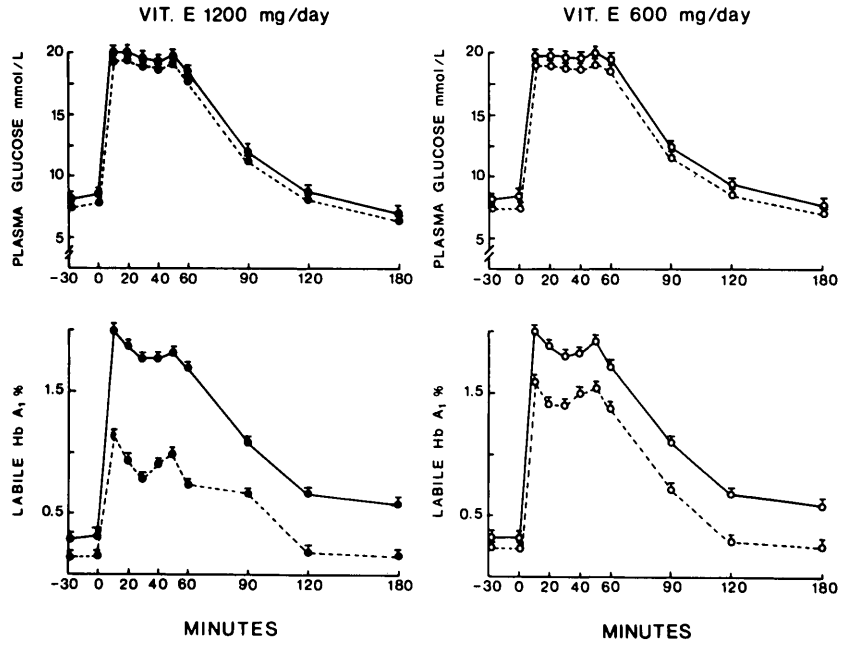

FIG. 1. Effects of vitamin E (VIT.E) administration on labile HbA_1 increments during hyperglycemic clamp in diabetic patients before (●——●) and after (●- - -●) 1200 mg vitamin E/day, and before (○——○) and after (○- - -○) 600 mg vitamin E/day.

treatment supports the evidence that vitamin E interferes with protein glycosylation at an early step in the Maillard reaction. The mechanism of inhibition remains uncertain. Monosaccharide auto-oxidation (a transition-metal–catalyzed process that generated H_2O_2 and ketoaldehydes) appears to contribute to protein modification by glucose (14). The inhibition of glucose auto-oxidation reduces the covalent linking of glucose to serum albumin and thus reduces total protein glycosylation (14). It may be hypothesized that vitamin E, a potent antioxidant, may interfere with glucose oxidation. This hypothesis might provide a satisfactory explanation for the inhibition of NEG by vitamin E.

Interestingly, an inhibiting effect of vitamin C on glycosylation of protein (15), collagen, and glomerular basement membrane (16) both in vitro and in vivo has been preliminarily reported. Note that both vitamin C and vitamin E are potent antioxidants.

The rate and extent of NEG of proteins are functions of both glucose concentration and duration of exposure to glucose, thus, it is not surprising that protein glycosylation was significantly reduced after 1 mo of vitamin E administration, whereas stable HbA_1 level decreased only after 2 mo, the protein and hemoglobin half-life being different. NEG is enhanced during hyperglycemia; consequently, its role in the pathogenesis of the diabetic complications has been suggested (1,2). Consequently, the inhibition of NEG could be of great interest to prevent these complications.

To this aim, various compounds have been proposed, including aspirin (17), lysine (18), or aminoguanidine (19). The usefulness of aspirin has been largely questioned (20,21), whereas lysine administration is not acceptable because of the toxic effects of hyperlysinemia. Aminoguanidine treatment could be of interest; however, this substance has effects only on formation of advanced glycosylation product formation (19). Vitamin E seems to be able to interfere at the very first step of the Maillard reaction, thus, its administration could be particularly advantageous.

This study demonstrates the possibility of inhibiting NEG with vitamin E in vivo. Furthermore, long-term studies are needed to demonstrate the usefulness of vitamin E supplementation for the prevention of diabetic complications.

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