Daily Vitamin E Supplements Improve Metabolic Control But Not Insulin Secretion in Elderly Type II Diabetic Patients

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OBJECTIVE — To investigate the potential metabolic benefits deriving from daily vitamin E administration in type II diabetic patients.

RESEARCH DESIGN AND METHODS — Twenty-five type II diabetic patients were invited to randomly take placebo or vitamin E (d-α-tocopherol; 900 mg/day) along a similar 3-mo period in a double-blind, crossover procedure. A wash-out period of 30 days separated the two treatment periods. At the end of each treatment period blood samples were drawn for plasma metabolites determination, and an intravenous glucose tolerance test (25 g of glucose as bolus in 3 min) was performed. During this study oral hypoglycemic agents were not discontinued or changed in their dosage.

RESULTS — Chronic vitamin E administration reduced plasma glucose (8.3 ± 0.3 vs. 7.5 ± 0.2 mM, $P > 0.05$), triglycerides (2.27 ± 0.08 vs. 1.67 ± 0.09 mM, $P < 0.02$), free fatty acids (786 ± 116 vs. 483 ± 64 mM), total cholesterol (6.74 ± 0.09 vs. 5.50 ± 0.10 mM, $P < 0.05$), low-density lipoprotein cholesterol (4.73 ± 0.11 vs. 3.67 ± 0.07 mM, $P < 0.04$), and apoprotein B (1.7 ± 0.3 vs. 1.0 ± 0.1 g/L) levels but did not affect β-cell response to glucose. HbA_1 levels (7.8 ± 0.3 vs. 7.1 ± 0.5%, $P < 0.05$) were also significantly lowered after chronic vitamin E administration.

CONCLUSIONS — Daily vitamin E supplements seem to produce a minimal but significant improvement in the metabolic control in type II diabetic patients. More studies are necessary before conclusions can be drawn about the safety of vitamin E during long-term administration.

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Received for publication 19 October 1992 and accepted in revised form 29 July 1993.

Type II diabetes, non-insulin-dependent diabetes mellitus; GSH, glutathione; GSSG, oxidized glutathione; OHA, oral hypoglycemic agents; TG, triglyceride; FFA, free fatty acids; IVGTT, intravenous glucose tolerance test; RIA, radioimmunoassay; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein; CV, coefficient of variation; apoA, apoprotein A; apoB, apoprotein B; SOD, superoxide dismutase; FPG, fasting plasma glucose; LBM, lean body mass; BMI, body mass index; sBP, systolic blood pressure; dBP, diastolic blood pressure.

Previous studies have shown a relationship between oxidative stress and glucose homeostasis (1–3). In particular, it has been demonstrated that changes in plasma reduced GSH/GSSG levels ratio may affect both the β-cell response to glucose and insulin action (2). Chronic vitamin E administration, an antioxidant agent that enhances plasma and red blood cells levels of GSH (4), has also been demonstrated to improve nonoxidative glucose metabolism in type II diabetic patients (3). However, no studies have dealt with the possible effects of daily vitamin E supplements on plasma lipid levels.

In light of such evidence, this study investigated the relationship between chronic vitamin E administration and lipid metabolism in elderly, type II diabetic patients.

RESEARCH AND DESIGN METHODS

— We studied 25 type II diabetic patients (mean ± SE age 71.3 ± 0.8 yr). The patients were slightly overweight, free from micro- and macroangiopathy (evaluated by laboratory, clinical, and instrumental analysis including a treadmill test, renal scintigraphy with determination of glomerular filtration rate of each kidney, ophthalmoscopy, and tests for the diagnosis of autonomic neuropathy), had normal renal function (microalbuminuria <20 μg/24 h and plasma creatinine levels <100 μM), and had a mean diabetes duration of 8.4 ± 0.3 yr. In addition all patients were in adequate metabolic control (HbA_1 7.8 ± 0.3%; normal values 6.0 ± 0.5%), were using all OHAs ($n = 13$ glipizide; $n = 6$ tolbutamide; and $n = 6$ glyburide), which were not discontinued or changed in dosage throughout the study, and were eating a weight-maintaining diet containing at least 250 g/day of carbohydrate and 14.1 ± 0.6 mg/day of vitamin E. More detailed data concerning our patients are presented in Table 1. The study was approved by the University of Naples Eth-

Table 1—*Clinical characteristics of the diabetic patients at baseline, at the end of placebo, and after vitamin E administration*

	Baseline	End of placebo	Vitamin E administration
BMI (kg/m^2)	27.4 ± 0.3	27.3 ± 0.5	27.3 ± 0.4
Body weight (kg)	78.1 ± 0.7	77.4 ± 0.6	77.5 ± 0.6
Body weight change (kg)	—	−0.6 ± 0.6	−0.6 ± 0.5
LBM (kg)	53.5 ± 0.4	53.3 ± 0.6	53.4 ± 0.7
sBP (mmHg)	164 ± 9.1	165 ± 8.8	166 ± 9.3
dBP (mmHg)	85 ± 2.1	88 ± 2.0	87 ± 2.4

Data are means ± SE. No statistically significant differences were found at any time of study.

ical Committee, and informed consent was obtained from the patients.

Experimental design

The study was designed as randomized, cross-over, and double-blind. Patients participated in a 4-wk prestudy period before being invited to randomly take placebo (sodium citrate) and vitamin E (900 mg/day; d-α-tocopherol Ephynal, Roche, Italy). Of the 25 patients, 13 started with placebo, while the others started with vitamin E. Each treatment period (placebo or vitamin E) lasted 3 mo, and a wash-out period of 30 days separated each treatment period. After an overnight fast of at least 12 h at the end of the follow-up (baseline data) and treatment periods, blood samples for determination of plasma glucose, TGs, FFA, total cholesterol, and apoA and apoB levels were drawn; an IVGTT (25 g of glucose as bolus in 3 min) was performed.

Blood sampling

Blood samples were drawn at −20, 0, 3, 5, 7, 10, 15, 30, and 60 min during the IVGTT. Blood samples for plasma insulin were collected in 5-ml heparinized tubes containing 0.5 ml of an EDTA solution (KYR, Lepetit, Milan, Italy) (5000 U/ml and disodium-EDTA 1.2 g/L). Samples for plasma glucose were collected in tubes containing a trace of sodium fluoride. Samples for GSH or GSSG levels were collected according to Butler et al (5).

Analytical methods

Plasma glucose was determined immediately after the end test by the glucose-oxidase method (Auto Analyzer, Beckman, Fullerton, CA). All other blood samples were centrifuged (1500 g/15 min at 5°C) after each experiment, and the plasma was stored at −20°C until assayed. Plasma insulin levels were determined by RIA as reported previously (6).

GSH (normal values 0.80 ± 0.08 μM) and GSSG levels were determined using an enzymatic assay (7) that allows a recovery of GSH >90% and has no appreciable interference with other thiols present in the plasma or in the reactive mixture. Plasma vitamin E concentration was estimated according to the method of Baker and Frank (8). Plasma total and HDL cholesterol, TGs, and apoproteins levels were determined by standard enzymatic assay (6). LDL cholesterol was calculated by a modified Friedwald formula (9). Plasma FFA levels were determined by spectrophotometric method (Boehringer, Milan, Italy).

Stable HbA_1 levels were determined in triplicate according to the method of Compagnucci et al. (10) by ion-exchange microcolumns at constant temperature (18°C). The intra- and interassay CVs were 3.9 and 5.3%, respectively. O_2^- production was determined through the reduction of ferricytochrome C (Sigma, St. Louis, MO) followed spectrophotometrically at 550 nm (11). A 0.25-mM solution of ferricytochrome C in 0.1 M sodium carbonate buffer, pH 10.40, was prepared. Of this solution, 1 ml was mixed with 0.1 of normal or diabetic serum in the presence or absence of 55 U of Cu-Zn-SOD. After mixing by gentle stirring, the solution was incubated for 15 min in the cuvette of the spectrophotometer at 37°C, and the variation of absorbance was recorded at 550 nm. The molar extinction coefficient of ferricytochrome C was taken as 1.55 mmol/min (12). The part of ferricytochrome C reduction that was inhibited by SOD was considered as O_2 generated in the serum. The intra- and interassay CVs for this method were 5.4 and 6.5%, respectively.

Because vitamin E enhances plasma GSH, which might react with disulfide bridges of insulin and cause underestimation of the amount of insulin released, the effects generated by GSH were investigated on the standard curve of the insulin RIA. Different concentrations of insulin and GSH were incubated for 10 min in the incubation medium. In the presence of 0.1 mM GSH, the calibration curve for the insulin assay slightly, but not significantly, shifted downward.

Statistical analysis

Compliance to drug treatment was assessed by capsule counting at each visit and was expressed as the percentage of the capsule consumed times the number of days of therapy. LBM was determined according to Segal et al (13). Net changes (such as the difference between the values found after placebo and vitamin E administration periods) in plasma TGs, LDL cholesterol, O_2^- production, GSSG/GSH ratio, and Conard's *K* value were calculated as reported elsewhere (14). Acute and total insulin responses were calculated as the incremental area from 0 to 10 min and from 0 to 60 min, respectively. Conard's *K* value was calculated as the least-squares slope of the log absolute glucose concentration between 10 and 30 min after glucose bolus.

Statistical comparisons were

Table 2—*Changes in fasting plasma metabolites levels at baseline at the end of placebo, and vitamin E administration*

	Baseline	End of placebo	*P* value	Vitamin E administration
Glucose (mM)	8.2 ± 0.4	8.3 ± 0.3	<0.05	7.5 ± 0.2
TGs (mM)	2.24 ± 0.06	2.27 ± 0.08	<0.02	1.67 ± 0.09
FFA (mM)	780 ± 110	786 ± 116	<0.05	483 ± 64
Total cholesterol (mM)	6.72 ± 0.07	6.74 ± 0.09	<0.05	5.50 ± 0.10
LDL cholesterol (mM)	4.76 ± 0.12	4.73 ± 0.11	<0.04	3.67 ± 0.07
HDL Cholesterol (mM)	1.17 ± 0.07	1.16 ± 0.08	—	1.19 ± 0.07
LDL/HDL cholesterol	4.07 ± 0.07	4.07 ± 0.05	<0.03	3.08 ± 0.06
apoA (g/L)	2.2 ± 0.4	2.1 ± 0.2		2.0 ± 0.1
ApoB (g/L)	1.8 ± 0.4	1.7 ± 0.3	<0.05	1.0 ± 0.1

Data are means ± SE. Only statistically significant differences between placebo and vitamin E periods were found.

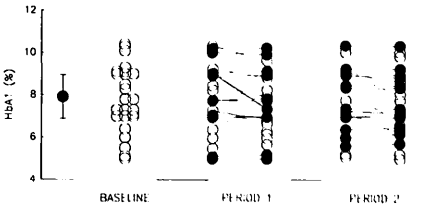

Figure 1—*Baseline values and individual changes in plasma HbA_1 levels before and after placebo (●——●) and vitamin E (O----O) administration at the different study periods. Period 1, 12 placebo patients and 13 vitamin E patients; period 2, 13 placebo patients and 12 vitamin E patients.*

made by paired two-tailed Student's *t* test. For variables not normally distributed (glucose and TGs) the Student's *t* test was confirmed by nonparametric test (Wilcoxon's test). Simple linear regression analysis was conducted by standard technique. $P \leq 0.05$ was considered statistically significant. All statistical analysis was made on IBM computer using SOLO software package (BMDP, Cork, Ireland). Data are expressed as mean ± SE.

RESULTS— In the baseline versus the placebo period no significant changes were found in any of the parameters studied (Tables 1–3). Vitamin E administration significantly lowered FPG, TGs, FFA, total cholesterol, LDL cholesterol, and apoB (Table 2). Furthermore, daily vitamin E supplements increased plasma vitamin E levels and decreased plasma O_2 production and GSSG/GSH ratio (Table 3); HbA_1 levels were also significantly lowered after vitamin E administration, as demonstrated by the mean values (Table 3) and the individual changes (Fig. 1) at all study times.

IVGTT

As indicated in Fig. 2, FPG levels (8.3 ± 0.3 vs. 7.5 ± 0.2 mM, $P < 0.05$), but not insulin levels (78 ± 5 vs. 79 ± 6 pM, NS), were significantly lower after vitamin E administration. After glucose pulse, plasma glucose levels were lower after vitamin E than placebo administration. By contrast, no significant changes in plasma insulin levels were found. In fact, acute (28.7 ± 0.9 vs. 29.8 ± 0.8 pM × min, NS) and total (0.91 ± 0.03 vs. 0.89 ± 0.04 nM × min, NS) insulin areas were also similar after placebo and vitamin E administration, respectively. Conard's *K* value was significantly higher (0.65 ± 0.03 vs. 0.98 ± 0.06%, $P < 0.04$) after daily vitamin E administration.

When dividing the patients into three groups in relation to the different OHA used, no significant differences were found versus placebo in any variable investigated. Nevertheless, in the patients treated with glipizide ($n = 13$), a positive trend ($t = 1.87$, $P < 0.08$) towards a significant effect of vitamin E versus placebo was observed. Finally, the patients were divided into two groups, patients who initially used vitamin E and patients who initially used placebo, and again no significant differences were found in any of the parameters studied.

Correlations

Net increase in plasma vitamin E correlated with the net decline in O_2^- production ($r = 0.41$, $P < 0.05$), GSSG/GSH ratio decline ($r = 0.46$, $P < 0.02$), plasma TGs decline ($r = 0.39$, $P < 0.05$), FFA decline ($r = 0.40$, $P < 0.05$), LDL-cholesterol decline ($r = 0.41$, $P < 0.05$), net increase in Conard's *K* value ($r = 0.43$, $P < 0.04$), and net decline in HbA_1 ($r = 0.40$, $P < 0.05$).

After vitamin E administration

Table 3—*Changes in fasting plasma vitamin E, O_2^- production, GSSG/GSH ratio, and HbA_1 levels at baseline, at the end of placebo, after vitamin E administration*

	Baseline	End of placebo	*P* value	Vitamin E administration
Vitamin E (μg/ml)	7.0 ± 0.6	7.3 ± 0.4	<0.001	35.4 ± 0.5
O_2^- (μmol × min)	0.44 ± 0.09	0.46 ± 0.08	<0.03	0.19 ± 0.10
GSSG/GSH ratio	1.16 ± 0.08	1.18 ± 0.07	<0.04	0.70 ± 0.09
HbA_1 (%)	7.7 ± 0.5	7.8 ± 0.3	<0.05	7.1 ± 0.5

Data are means ± SE. Only statistically significant differences between placebo and vitamin E periods were found.

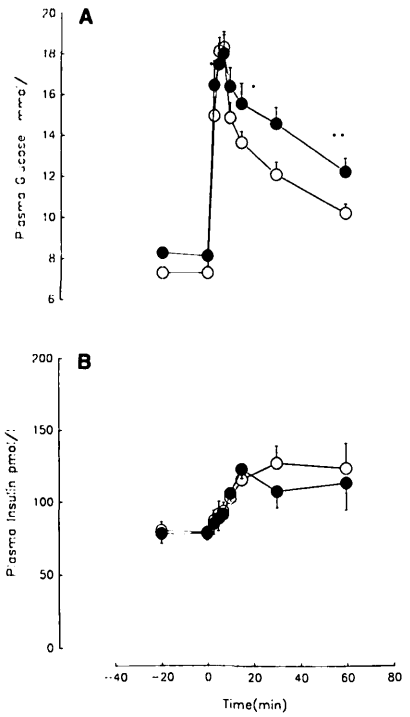

Figure 2—*Changes in plasma glucose (A) and insulin (B) levels after glucose bolus (25 g in 3 min) after placebo (●——●) and Vitamin E (○----○) administration in type II diabetic patients (n = 25). Data are means ± SE. Statistically significant differences were: *P < 0.05; **P < 0.03.*

net decline in plasma O_2^- production was also significantly correlated with the net decline in plasma TGs ($r = 0.39$, $P < 0.05$), a decline in FFA ($r = 0.39$, $P < 0.05$), a decline in LDL-cholesterol ($r = 0.40$, $P < 0.05$), and an increase in Conard's *K* values ($r = 0.41$, $P < 0.05$). In the same experimental conditions the net decline in plasma GSSG/GSH ratio correlated with the net increase in Conard's *K* values ($r = 0.48$, $P < 0.03$). No parameter studied correlated with the body weight change after placebo and vitamin E administration. No adverse effects caused by chronic vitamin E administration were observed. In particular, no changes in liver and renal function tests were found. Patients' compliance to vitamin E was 93 ± 0.3%. No patient dropped out of the study.

CONCLUSIONS— In type II diabetic patients an exaggerated free radical activity and lipid peroxidation has occurred (1). Such enhanced oxidative stress has been correlated to a poor metabolic control and to the genesis of microangiopathy (1,2,15). Nevertheless, no studies have dealt with a possible effect of free radical production on metabolic control.

Vitamin E, a potent antioxidant agent, exerts a protective role as a free radical scavanger through a nonenzymatic mechanism outside of cells (16,17). Modulation of the activities of intracellular enzymes is an indirect consequence of this effect, and it could be brought about by reducing the burden of the GSH system, which could be considered a second line of defense (16,17). In fact, if vitamin E had exerted any modulating effect on the enzyme glutathione-synthetase, more GSSG should have been found within red blood cells, because GSH rapidly undergoes autoxidation to GSSG (18). Moreover, because the erythrocyte membrane is permeable to GSSG but not to GSH (17), a consequent increase in the GSSG levels should have been measured outside of the red blood cells. Nevertheless, an augmented removal of GSSG from the circulation could be an additional factor that cannot be excluded. Thus, the two systems are closely related, but they probably act independently.

In this study, we confirm that daily vitamin E supplements have a protective role against oxidative stress in diabetic patients. Moreover, we have demonstrated that chronic vitamin E administration may be a useful tool to improve the metabolic control. In particular, daily vitamin E supplements were associated with a significant decline in plasma GHb, a decline in lipid levels, and an increase in Conard's *K* value. Such results are in agreement with a previous study showing, in a similar type of patients, an improvement in insulin action after chronic vitamin E administration (3).

The beneficial effects of vitamin E on plasma glucose and lipid levels seem linked to the changes in plasma GSSH/GSH ratio occurring after its oral administration. A significant increase in the plasma GSSG/GSH ratio might contribute to enhanced lipid peroxidation. Such a phenomenon might affect the physical-chemical integrity of the plasma membrane with a secondary increase in membrane microviscosity (2,19). Thus, one can hypothesize that daily oral vitamin E supplements, which restore more appropiate plasma GSH levels, might improve the physical state of the plasma membrane and its related activities such as insulin sensitivity.

Such a pathophysiological mechanism, if not proven, seems strengthened by the correlation between the plasma GSSG/GSH ratio and Conard's *K* value found in our study. From a physiological viewpoint, vitamin A is transported in lipoproteins, and a close positive correlation between serum TG and retinol levels is observable. By contrast, in our study we found an inverse relationship between plasma vitamin E and serum lipids. We hypothesize that such an apparent discrepancy might be explained and related to the way vitamin E is transported (20).

Improved insulin sensitivity after vitamin E administration, with a consequently greater inhibition of lipid oxidation (21), might explain the significant decline in plasma TGs and FFA found in this study. Furthermore, a reduction in plasma FFA might also contribute per se to the improvement in Conard's *K* values and in the metabolic control (21). As far as a vitamin E-induced decline in plasma total cholesterol and LDL cholesterol is concerned, one can hypothesize that the antioxidant power of vitamin E itself could preserve LDL-cholesterol from the peroxidation phenomenon (22). Furthermore, vitamin E per se might have a direct effect on plasma cholesterol levels because it contributes to inhibiting cholesterol biosynthesis (23,24). A hepatic overproduction of VLDLs and a decreased activity of lipoprotein lipase might be further attributable to the enhanced plasma cholesterol and TG levels.

Regarding the relationship between plasma vitamin E on plasma insulin levels, our study failed to demonstrate

any effect of vitamin E on β-cell secretion in fasting conditions or after glucose bolus. Note that relatively low plasma insulin levels, compared with BMI, were found in our patients. Such an apparent discrepancy might be attributable to the patients age and duration of the disease with a consequent decline in the secretory reserve of the endocrine pancreas. The significant decline in HbA_1 levels after chronic vitamin E administration might be caused by the improvement in the metabolic control. Nevertheless, a direct effect of vitamin E per se, as an antioxidant agent able to reduce protein glycosylation (15), cannot be excluded.

In this study a pharmacological dose of vitamin E has been used. Because no studies have investigated the possible effect of body storage of vitamin E in connection with long-term supplementation, we stress that vitamin E should not be routinely taken without careful monitoring of liver and renal funtions at the dosage used in our study. Finally, our study failed to demonstrate significant differences in the effects of vitamin E on plasma lipid levels and GHb in relation to the different OHA used by the patients. Such a negative finding might be explained by the small number of patients per group.

In conclusion, chronic vitamin E administration seems to produce a minimal but significant improvement in the metabolic control in type II diabetic patients. Nevertheless, further studies are needed to clarify the safety and the exact pharmacological mechanisms necessary to achieve such a result.

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