Chronic administration of pharmacologic doses of vitamin E improves the cardiac autonomic nervous system in patients with type 2 diabetes[1–3]

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ABSTRACT

Background: Type 2 diabetes is associated with elevated oxidative stress and declines in antioxidant defense. The disease is also characterized by an imbalance in the ratio of cardiac sympathetic to parasympathetic tone. Antioxidants, vitamin E in particular, may have beneficial effects on the cardiac autonomic nervous system through a decline in oxidative stress.

Objective: We investigated the possible effects of vitamin E on the cardiac autonomic nervous system, as assessed by analysis of heart rate variability, in patients with type 2 diabetes and cardiac autonomic neuropathy.

Design: In a double-blind randomized controlled trial, 50 patients with type 2 diabetes were assigned to treatment with vitamin E (600 mg/d) or placebo for 4 mo.

Results: The anthropometric characteristics of the patients remained unchanged throughout the study. Chronic vitamin E administration was associated with decreases in concentrations of glycated hemoglobin ($P < 0.05$), plasma insulin ($P < 0.05$), norepinephrine ($P < 0.03$), and epinephrine ($P < 0.02$); a lower homeostasis model assessment index ($P < 0.05$); and improved indexes of oxidative stress. Furthermore, vitamin E administration was associated with increases in the R-R interval ($P < 0.05$), total power ($P < 0.05$), and the high-frequency component of heart rate variability (HF; $P < 0.05$) and decreases in the low-frequency component (LF; $P < 0.05$) and the ratio of LF to HF ($P < 0.05$). Finally, change in the plasma vitamin E concentration was correlated with change in the LF-HF ratio ($r = -0.43$, $P < 0.04$) independently of changes in the homeostasis model assessment index and plasma catecholamines concentrations.

Conclusions: Chronic vitamin E administration improves the ratio of cardiac sympathetic to parasympathetic tone in patients with type 2 diabetes. Such an effect might be mediated by a decline in oxidative stress. *Am J Clin Nutr* 2001;73:1052-7.

KEY WORDS Vitamin E, heart rate variability, catecholamines, oxidative stress, type 2 diabetes, randomized controlled trial

INTRODUCTION

It is widely accepted that type 2 diabetes is associated with elevated oxidative stress (1–4), which, in turn, is associated with an imbalance in the activity of the cardiac autonomic nervous system (5, 6). Thus, one can hypothesize that antioxidants, such as vitamin E, may have beneficial effects on the cardiac autonomic nervous system. Such effects might be particularly evident in persons with diabetes, who have a higher degree of oxidative stress (2) and are more susceptible to increases in the plasma vitamin E concentration. Only a few of the studies that focused on the relation between oxidative stress and the autonomic nervous system addressed the potential effect of antioxidants such as vitamin E. Vitamin E seems the most appropriate antioxidant to investigate because it is widely used and because several (7–10), although not all (11–13), studies showed it to be useful in lowering the risk of cardiovascular disease in diabetic and nondiabetic subjects. In fact, vitamin E administration can reduce plasma catecholamine concentrations and improve autonomic nervous system balance in diabetic animals (14). In contrast, studies in humans are lacking. Thus, whether vitamin E administration is associated with improvements in cardiac autonomic nervous system balance in humans, particularly in persons with diabetes, needs to be investigated.

Spectral analysis of heart rate variability is a well-accepted, noninvasive tool that allows one to assess autonomic function by studying effects on the cardiac sympathetic and parasympathetic subsystems (15). Several studies showed that the ratio between the low-frequency (LF) component of heart rate variability, which is proposed as an index of vasomotor sympathetic activity and that occurs in synchrony with vasomotor waves, and the high-frequency (HF) component, an index of vagal efferent activity that occurs in synchrony with respiratory acts, reflects the state of sympathovagal balance in numerous physiologic and pathophysiologic conditions (15, 16). Thus, we assessed the effects of chronic supplementation with pharmacologic doses of vitamin E on the cardiac autonomic nervous system in patients with type 2 diabetes and cardiac autonomic neuropathy.

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TABLE 1
Anthropometric, metabolic, and oxidative stress indexes at baseline and after placebo and vitamin E administration[1]

	Baseline		End of study		
	Placebo	Vitamin E	Placebo	Vitamin E	P^2
Age (y)	65.1 ± 3.9	64.3 ± 4.7	65.1 ± 3.9	64.3 ± 4.7	NS
BMI (kg/m^2)	26.4 ± 3.9	26.2 ± 4.3	26.4 ± 3.9	26.2 ± 4.3	NS
Waist-to-hip ratio	0.84 ± 0.01	0.82 ± 0.04	0.84 ± 0.01	0.82 ± 0.04	NS
FPG (mmol/L)	9.2 ± 0.1	9.1 ± 0.3	9.1 ± 0.2	9.0 ± 0.2	NS
FPI (pmol/L)	91.5 ± 0.3	91.2 ± 0.2	91.1 ± 0.2	80.1 ± 0.3	0.05
HOMA index	3.71 ± 0.11	3.68 ± 0.33	3.70 ± 0.21	3.15 ± 0.14	0.05
Hb A_{1c}	0.082 ± 0.005	0.082 ± 0.003	0.081 ± 0.006	0.075 ± 0.003	0.05
MABP (mm Hg)	101 ± 5	100 ± 6	101 ± 4	103 ± 3	NS
FPN (nmol/L)	2.84 ± 0.32	2.86 ± 0.21	2.85 ± 0.22	1.79 ± 0.34	0.03
FPE (pmol/L)	388 ± 42	385 ± 49	387 ± 44	316 ± 37	0.02
FPVE (μmol/L)	7.2 ± 0.3	7.0 ± 0.6	7.3 ± 0.2	24.1 ± 0.6	0.001
TEAC (mmol/L)	1.6 ± 0.3	1.7 ± 0.1	1.5 ± 0.4	2.5 ± 0.2	0.005
TBARS (nmol MDA/L plasma)	0.67 ± 0.02	0.66 ± 0.04	0.67 ± 0.04	0.42 ± 0.03	0.005

[1] $\bar{x}$ ± SD. FPG, fasting plasma glucose; FPI, fasting plasma insulin; HOMA, homeostasis model assessment; Hb A_{1c}, glycated hemoglobin; MABP, mean arterial blood pressure; FPN, fasting plasma norepinephrine; FPE, fasting plasma epinephrine; FPVE, fasting plasma vitamin E; TEAC, Trolox-equivalent antioxidant capacity; TBARS, thiobarbituric acid–reactive substances; MDA, malondialdehyde.
[2] For treatment effect. There were no significant effects of time and no time × treatment interactions.

SUBJECTS AND METHODS

Subjects

Fifty patients with type 2 diabetes and cardiac autonomic neuropathy, as assessed by heart rate variability (17), volunteered for the study. We did not test the effect of pharmacologic doses of vitamin E in healthy subjects because vitamin E—even in pharmacologic doses—only minimally affects the degree of oxidative stress in healthy subjects (18). We hypothesized that the degree of oxidative stress and unbalanced cardiac autonomic tone would have a parallel trend. Because such a trend could be better shown in patients with elevated oxidative stress, we determined that patients with type 2 diabetes would be the best population to include in our study.

None of the patients had any evidence of coronary artery disease as confirmed by an electrocardiogram, echocardiography, and a treadmill test. Insulin resistance was assessed by homeostasis model assessment (HOMA) (19). HOMA is a mathematical model used to estimate insulin resistance and deficient β cell function from a patient's fasting plasma insulin and glucose concentrations. A computer-solved model of insulin-glucose interaction is used to plot the array of fasting plasma insulin and glucose concentrations expected for various degrees of β cell deficiency and insulin resistance. From the array, one can estimate the insulin resistance and deficient β cell function expected to give the fasting plasma glucose and insulin concentrations measured in a patient. The accuracy and precision of HOMA were previously compared with independent estimates of β cell function and insulin resistance (19). Glucose metabolic control was assessed by measuring glycated hemoglobin (Hb A_{1c}) (20). More detailed characteristics of the patients are given in **Table 1**.

All tests were performed in the morning after the subjects had fasted overnight (≥12 h). After hearing a clear explanation of the potential risks of the study, each volunteer gave informed consent to participate in the study. The study was approved by the ethical committee of our institution.

Anthropometric measurements

Weight and height were measured by standard techniques. Body mass index (BMI) was calculated as body weight (in kg)/height^2 (in m). Waist circumference was measured at the midpoint between the lower rib margin and the iliac crest (normally the umbilical level), and hip circumference was measured at the level of the trochanter. Both circumferences were measured to the nearest 0.5 cm with plastic tape and were used to calculate the waist-to-hip ratio. Anthropometric measurements were taken because changes in body composition may significantly affect both insulin resistance and the cardiac autonomic nervous system. Thus, because our study lasted 4 mo, we also hypothesized that changes in heart rate variability indexes and in HOMA could also depend on changes in body composition.

Study protocol

The study was designed as a double-blind randomized controlled trial. At baseline, all subjects were studied at 0800 in a quiet, comfortable room ranging in temperature from 22 to 24°C. A venous blood sample was immediately drawn for measurement of plasma metabolites. Then, each subject rested in the supine position for ≥30 min before the start of the baseline Holter recording, which lasted 60 min. Respiratory frequency was also calculated for 2 min before the Holter recording. If a subject had a respiratory rate <10 breaths/min (ie, <0.15 Hz), he or she was excluded from the study. All patients were then randomly assigned to receive either vitamin E (*all-rac*-α-tocopheryl acetate, 600 mg/d; Evion, Bracco, Italy; $n = 25$) or placebo (sodium citrate; $n = 25$). Each treatment lasted 4 mo. We selected 4 mo because in our previous studies (18, 21, 22) we found that this length of time is necessary to observe any effect of vitamin E. At the end of the treatment period, the patients were reevaluated.

Data acquisition and analysis

The software used for data acquisition and analysis was previously described (23, 24). In brief, the computer program first calculates the interval tacogram. From a section of the tacogram of 512 interval values, simple statistics (mean and variance) are calculated. The computer program automatically calculates the autoregressive coefficients necessary to define the power spectral density estimate and prints out the power and frequency of every spectral component. Two major oscillatory components are usually detectable: HF (≈0.25 Hz and

TABLE 2
Heart rate variability indexes at baseline and after placebo and vitamin E administration[1]

	Baseline		End of study		
	Placebo	Vitamin E	Placebo	Vitamin E	P^2
R-R interval (ms)	763 ± 12	761 ± 15	762 ± 14	847 ± 22	0.05
Total power (ms^2)	2741 ± 446	2695 ± 457	2709 ± 423	2985 ± 491	0.05
Low frequency (NU)	68.2 ± 2.1	67.5 ± 1.3	67.8 ± 1.2	50.4 ± 2.1	0.05
High frequency (NU)	20.3 ± 2.3	19.5 ± 3.1	20.1 ± 1.1	39.8 ± 1.6	0.05
LF:HF	4.3 ± 0.2	4.1 ± 0.4	4.2 ± 0.4	2.1 ± 0.2	0.05

[1] $\bar{x}$ ± SD. NU, normalized units; LF, low-frequency component; HF, high-frequency component.
[2] For treatment effect. There were no significant effects of time and no time × treatment interactions.

varying with respiration) and LF (≈0.1 Hz and corresponding to the slow waves of arterial pressure). Each spectral component is presented in normalized form [normalized units (NU)], by dividing the component by the total power minus the direct current component, if present. Only components >5% of total power were considered significant.

Analytic techniques

Plasma glucose concentrations were measured by the glucose oxidative method (glucose autoanalyzer; Beckman Coulter Inc, Fullerton, CA). Plasma insulin concentrations were measured by commercial double-antibody, solid-phase radioimmunossay (Linco Research Inc, St Charles, MO; CV: 4.8 ± 0.3%; cross reactivity with proinsulin: 0.2%). The total fasting plasma vitamin E concentration was measured by reversed-phase HPLC (25).

The degree of oxidative stress in serum was measured as the reaction product of malondialdehyde with thiobarbituric acid–reactive substances (TBARS) (26). This reaction, although simple and reproducible, is nonspecific because thiobarbituric acid reacts with many other carbonyl-containing compounds. Plasma fatty acids can also oxidize during the 95°C heating step with thiobarbituric acid, generating artificially high results (27, 28). The use of EDTA-containing plasma and HPLC coupled with postcolumn thiobarbituric acid derivitization can, however, identify the specific malondialdehyde–thiobarbituric acid complex, proving a relevant assay for malondialdehyde in biological fluids (29, 30).

The plasma total antioxidant capacity was estimated by the 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid radical cation decolorization assay, with use of the Trolox (St Louis) standard (31). In this assay, 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid is made to react with potassium persulfate in the absence of standards and samples and absorbance is read at 734 nm. A value of 1 Trolox-equivalent antioxidant capacity (TEAC) in a sample is defined as the concentration equivalent to 1 mmol Trolox/L. The impressive sensitivity of this assay may be related to the fact that it not only measures well-known antioxidants such as vitamins C and E, β-carotene, glutathione, cysteine, dihydrolipoate, ubiquinol, and polyphenols, but also determines the contributions to defense against oxidative damage made by lesser known substances such as albumin, uric acid, bilirubin, and other molecules present in the body (31, 32).

Statistical analyses

All results are presented as means ± SDs. Changes in plasma catecholamines, the TEAC, TBARS, vitamin E concentrations, the HOMA index, and the LF-HF ratio, calculated as the difference between pre- and posttreatment values, were used to compare only the changes in (and not just the absolute values of) these variables. Because of the skewed distributions, total power, LF, and HF were logarithmically transformed for statistical testing and back-transformed for presentation in the tables and figures. Two-factor analysis of variance (ANOVA) for repeated measures was used to calculate interactions between treatment and time. Pearson's simple correlations were used to study the association between 2 variables. Partial correlations were used to examine the relation between 2 variables independently of covariates. A P value of 0.05 was chosen as the level of significance. All calculations were made on a personal computer with SPSS 9.0 and SIGMA STAT 2.03 (SPSS Inc, Chicago).

RESULTS

Clinical and laboratory measurements are reported in Table 1. All patients were adults, nonsmokers, normotensive, slightly overweight, and in sufficient metabolic control through use of oral hypoglycemic agents, as shown by Hb A_{1c} measurements.

At baseline, anthropometric, metabolic, and cardiovascular variables and indexes of oxidative stress were not significantly different between the placebo and vitamin E groups. Vitamin E treatment significantly increased plasma vitamin E concentrations and the TEAC and significantly decreased plasma catecholamine concentrations, insulin concentrations, Hb A1c values, the HOMA index, and TBARS concentrations (Table 1). Vitamin E administration was also associated with significant increases in the R-R interval, total power, and HF and significant decreases in LF and the LF-HF ratio (**Table 2**).

Because of significant differences in plasma catecholamines, the TEAC, TBARS, vitamin E, the HOMA index, and the LF-HF ratio before and after treatment with vitamin E, changes in these indexes were calculated. In the vitamin E group, the change in the plasma vitamin E concentration correlated with the change in the plasma TEAC and the change in the plasma TBARS concentration (**Figure 1**). Furthermore, the change in the plasma vitamin E concentration was also significantly correlated with the change in the plasma catecholamine concentration, the change in the LF-HF ratio, and the change in the HOMA index (**Figure 2**). The correlation between the change in the plasma vitamin E concentration and the change in the LF-HF ratio persisted even after adjustment for the change in the HOMA index and the change in the plasma catecholamine concentration ($r = –0.43$, $P < 0.04$).

DISCUSSION

Our results show that chronic vitamin E administration reduces plasma indexes of oxidative stress and plasma catecholamine

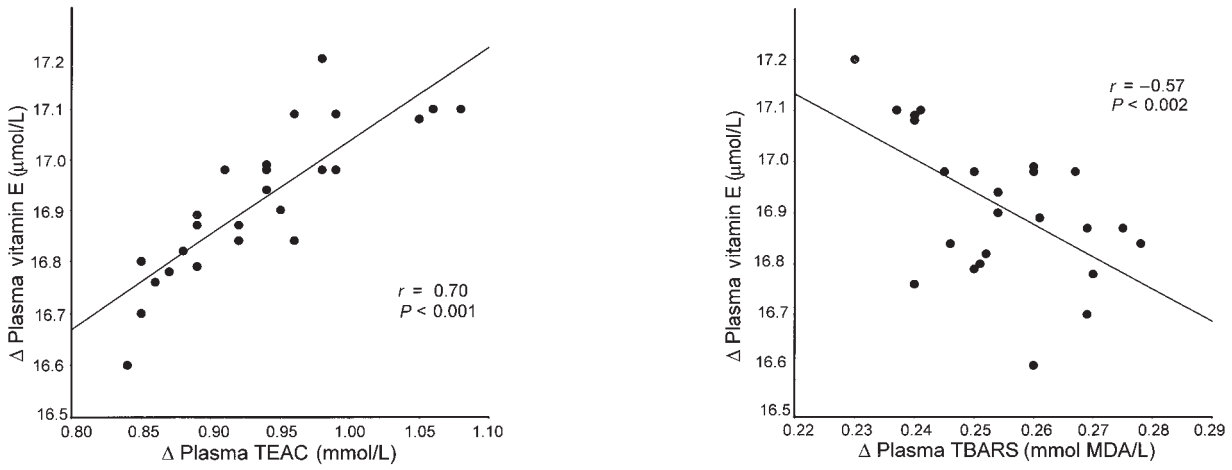

FIGURE 1. Simple correlations between the change in the plasma vitamin E concentration and the change in the plasma Trolox-equivalent antioxidant capacity (TEAC) and the plasma concentration of thiobarbituric acid–reactive substances (TBARS) in vitamin E–treated patients with type 2 diabetes (n = 25).

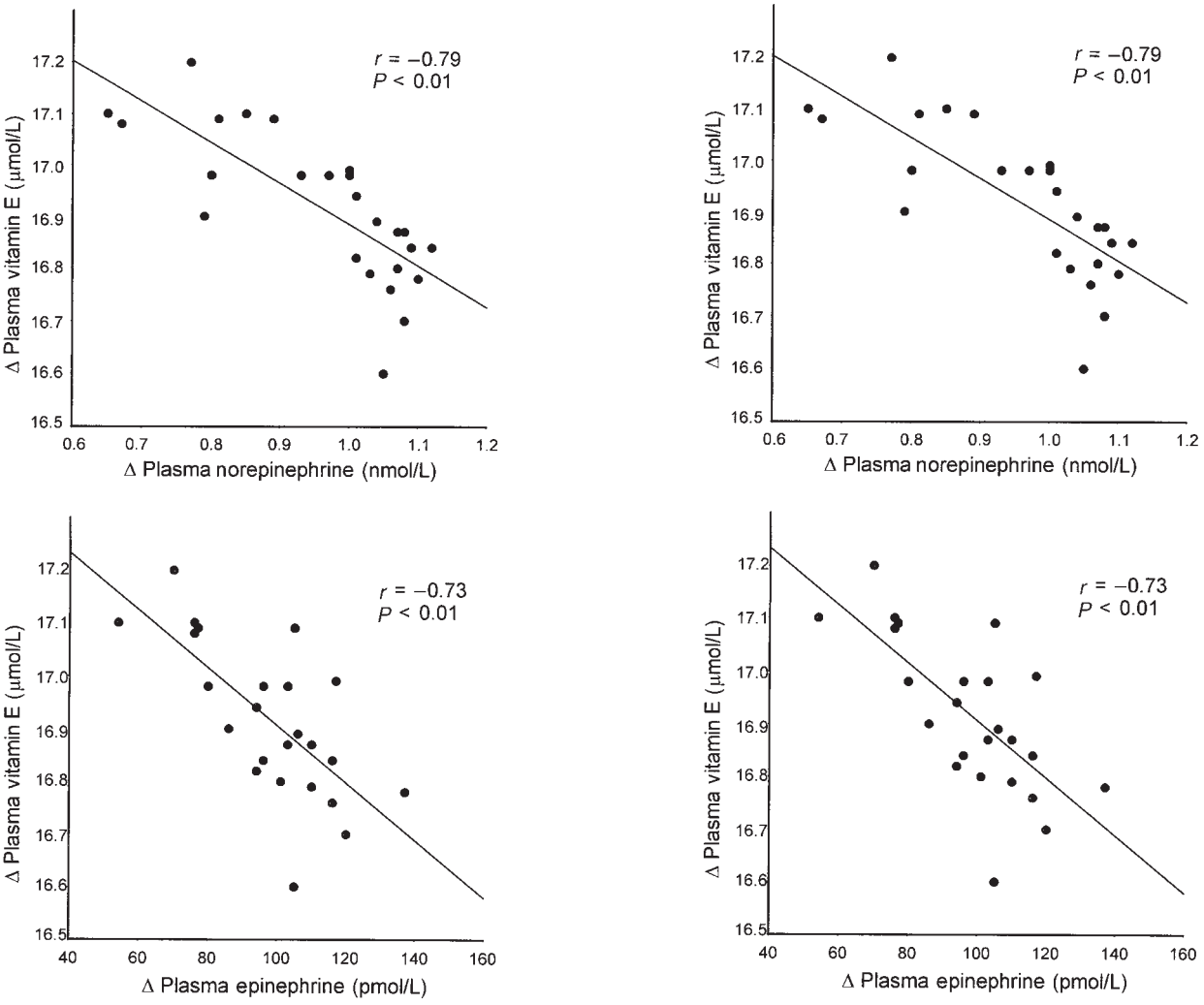

FIGURE 2. Simple correlations between the change in the plasma vitamin E concentration and the change in the plasma norepinephrine concentration, the change in the plasma epinephrine concentration, the change in the ratio of the low-frequency component of heart rate variability to the high-frequency component (LF:HF), and the change in the homeostasis model assessment (HOMA) index in vitamin E–treated patients with type 2 diabetes (n = 25).

concentrations and improves the LF-HF ratio, an index of cardiac sympathovagal balance, in patients with type 2 diabetes. Previous studies showed that patients with type 2 diabetes have impaired cardiovascular autonomic activity, characterized by a reduction in parasympathetic tone and relative sympathetic overactivity (5, 6). Such unbalanced sympathetic-parasympathetic tone seems linked to the degree of oxidative stress (33) and responsible for many cases of sudden death (34, 35) despite the absence of documented preexisting heart disease (34). Therefore, antioxidants, which lower oxidative stress, may exert beneficial effects at the cardiac level by rebalancing autonomic nervous system activity.

To the best of our knowledge, the relation between antioxidants, in particular vitamin E, and the autonomic nervous system has been appropriately evaluated only in animals. In particular, long-term deprivation of dietary vitamin E increases urinary norepinephrine and epinephrine excretion in rats, suggesting that chronic vitamin E deficiency may increase sympathetic nervous system (SNS) activity (36, 37). In contrast, increased amounts of dietary vitamin E decrease the rate constant for the decline of specific activity and the turnover rate of noradrenaline (14). As a whole, such data indicate that elevated amounts of vitamin E in the diet may affect the SNS.

In humans, the relation between vitamin E and the SNS was not previously investigated. Our study is the first to document that chronic, oral administration of vitamin E in pharmacologic doses is associated with a lowering of plasma catecholamine concentrations in patients with type 2 diabetes. It is important to point out that these effects might have a positive influence on cardiac risk. Furthermore, we documented a decline in plasma oxidative stress indexes. Interestingly, changes in cardiac sympathovagal balance and in oxidative stress were correlated. In fact, the change in the plasma vitamin E concentration was weakly but significantly and negatively correlated with the change in the plasma catecholamine concentration and the change in the LF-HF ratio. Although such a correlation does not support a cause-effect relation, our data support the hypothesis that chronic vitamin E administration might be useful for improving cardiac sympathovagal balance and for reducing cardiovascular disease risk.

An unsolved issue is the mechanism of action by which chronic vitamin E administration affects the SNS and thus cardiac sympathovagal balance. Indeed, our study was not designed to assess this. Nevertheless, our findings show that chronic vitamin E administration was associated with a lowering of HOMA values. Because of the strong relations between oxidative stress, antioxidants, insulin resistance, and SNS activity (38–40), we can hypothesize that an improvement in insulin action would be associated with a decline in plasma insulin concentrations, which in turn may have beneficial effects on the SNS. Whether the effect of vitamin E is direct or indirect needs to be addressed in future studies.

One limitation of our study may be the very difficult interpretation of the results. In agreement with previous studies (41–43), we considered the LF component to reflect the response of the SNS or vascular responsiveness (or both) to any mechanical change in blood pressure sensed by the baroreceptor. We considered the HF component to reflect efferent parasympathetic activity at the cardiac level and, at the vascular level, largely the mechanical effects of respiration on cardiac output. Thus, the LF-HF ratio is not a simple result of the balance between cardiac sympathetic and parasympathetic activity, but the result of several other factors as well.

In conclusion, our study showed that chronic vitamin E administration improves oxidative stress in patients with type 2 diabetes and that this effect seems to be associated with a decrease in plasma catecholamine concentrations and cardiac SNS activity. Further studies are needed to investigate the molecular mechanisms of such an effect of vitamin E and whether such an effect is exerted by any other antioxidants.

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