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Original article

α-Tocopherol levels in plasma in new-onset, insulin-dependent diabetes mellitus

Marian J. Muis[a,b], Ronald P. Stolk[a,*], Hans M.G. Princen[c], P.S. Van Dam[b], L.D. Dikkeschei[d], Diederick E. Grobbee[a], Henk J.G. Bilo[d]

[a]*Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, D01.335, PO Box 85500, 3508 GA Utrecht, The Netherlands*
[b]*Department of Internal Medicine, University Medical Center Utrecht, The Netherlands*
[c]*Gaubius Laboratory TNO-PG, Leiden, The Netherlands*
[d]*Isala Clinics Zwolle, The Netherlands*

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Abstract

Background: Diabetic complications have been related to increased oxidative stress. Plasma antioxidant levels may be affected by hyperglycemia-induced oxidative stress as well as by insulin therapy. We evaluated the immediate effect of insulin treatment and improved metabolic control on the important antioxidant α-tocopherol plasma (vitamin E) levels in new-onset, insulin-dependent diabetes mellitus.
Methods: The study was performed in 15 consecutive patients, aged 20–67 years, with new-onset diabetes mellitus requiring acute insulin treatment. Plasma α-tocopherol levels were measured before the start of intensive insulin treatment and monthly for 6 months thereafter. Simultaneously, we studied plasma malondialdehyde (MDA) as a reflection of lipid peroxidation. In addition, comparisons were made to a nondiabetic reference group.
Results: Baseline α-tocopherol levels did not differ from those in nondiabetic subjects. α-Tocopherol decreased significantly, from 33.5±12.1 μmol/l before treatment to 28.11±6.85 μmol/l (−16%) after 1 month of insulin therapy ($p<0.04$) to 26.6±7.03 μmol/l (−20%) after 3 months of insulin therapy ($p<0.02$). This trend did not change after adjusting for variations in cholesterol levels. After 6 months, α-tocopherol was no longer decreased compared to baseline levels (29.6±7.4 μmol/l). MDA concentrations at baseline were significantly higher in the diabetic patients (3.79±2.91 μmol/l) than in the nondiabetic subjects (1.57±0.21 μmol/l, $p=0.006$). MDA concentrations decreased significantly following the start of insulin treatment.
Conclusions: Patients with new-onset, insulin-dependent diabetes mellitus have α-tocopherol levels that are similar to those in normal subjects. Insulin treatment and/or improved metabolic control cause a significant decrease in α-tocopherol levels during the first months.

Keywords: Diabetes mellitus; Vitamin E; Oxidative stress; Antioxidants; MDA

1. Introduction

There is evidence that oxidative stress plays an important role in the development of diabetic complications [1,2]. Results from studies in animals and humans have suggested that oxidative cell injury caused by free radicals contributes to the development of microangiopathy and macroangiopathy [2,3]. Free radical production has been reported to be increased in patients with diabetes mellitus, and hyperglycemia appears to contribute directly to the generation of reactive oxygen species [2]. Extracellular fluids contain several antioxidants that interfere with the oxidative process [4]. Antioxidants such as ascorbic acid, β-carotene, and α-tocopherol may protect LDL cholesterol against oxidative stress-mediated damage [5]. One of the most important natural lipophilic antioxidants is α-tocopherol. α-Tocopherol has the highest biological activity and is the most widely available form of vitamin E in food. The other

* Corresponding author. Tel.: +31 30 250 9305; fax: +31 30 250 5485.
E-mail address: R.P.Stolk@jc.azu.nl (R.P. Stolk).
URL: http://www.juluiscenter.nl

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isomers (β, γ, δ), some of which are more abundant in a typical western diet, are less biologically active than α-tocopherol. α-Tocopherol is an essential fat-soluble vitamin that is principally transported in the blood by plasma lipoproteins.

In patients with type 1 diabetes, increased oxidative stress and lower concentrations of antioxidants have been reported [2]. Published data on the effect of insulin treatment and subsequent glycemic control on serum levels of α-tocopherol in diabetes are contradictory [6–8]. Higher as well as lower serum concentrations of α-tocopherol have been reported. The different results may be explained by differences in metabolic control, or by the presence of lipid disorders of the patients studied, or by the adjustment for changes in lipid levels by insulin treatment.

To evaluate the effect of starting insulin treatment on α-tocopherol plasma levels and lipid peroxidation, we performed a prospective follow-up study in patients with new-onset, insulin-dependent diabetes mellitus.

2. Subjects and methods

2.1. Study population

The present study was performed on 15 patients who sequentially presented at the outpatient clinic with severe hyperglycemia without previously known diabetes mellitus. All patients required immediate insulin treatment at presentation. During the same period, 15 healthy men and women (reference group), who were admitted for minor surgery (e.g., cosmetic surgery, sterilization, or arthroscopy), were invited to participate in the study. These subjects were matched for gender, age, BMI, and smoking habits. All control subjects were in good health, except for the indication for surgery (ASA classification category I), and not using any medication except oral contraceptives. The study was conducted at the outpatient Diabetes Unit of the Isala Clinics in Zwolle, The Netherlands. The study was approved by the hospital Ethical Committee and all patients gave their informed consent.

2.2. Measurements

One trained physician examined the patients according to a standardized protocol. Smoking habits, alcohol use, the use of medication, as well as the use of vitamin supplements were recorded. No patient used any drugs or vitamin supplements that could influence the parameters under investigation. Blood samples were drawn after 30 min of rest in the supine position from the antecubital vein after a normal breakfast and, if started, insulin dose. The blood samples to be used for determination of malondialdehyde (MDA) were collected in EDTA vacutainer tubes, placed on ice immediately, and cooled by oxidation by the addition of 2 mg/ml reduced glutathione and 1.2 mg/ml butylated hydroxytoluene (BHT; final concentrations), as well as by flushing the empty space of the storage tubes with nitrogen.

Plasma malondialdehyde (MDA) concentrations were determined in plasma lipid extracts using 1,3-diethyl-2-thiobarbituric acid, as described by Hoving et al. [9]. Glycosylated hemoglobin A1c (HbA1c) was measured with affinity chromatography (Pierce columns, Glyco test II), (upper limit of normal 6.0%) [10]. Total serum cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides were measured using a Hitachi 717 chemistry analyzer based on commercially available techniques (Boehringer Mannheim, Mannheim, Germany). Low-density lipoprotein (LDL) cholesterol was calculated according to the formula of Friedewald et al. [11]. Plasma α-tocopherol was determined with HPLC according to Zaman et al. [12]. To prevent oxidation of tocopherol during the extraction procedure, (BHT) pyrogallol, vitamin C, and EDTA were added.

2.3. Statistical analysis

Results are given as means±S.D. Baseline and follow-up biochemical data were compared using the paired samples *t*-test. The comparison between subjects with and without diabetes mellitus was performed using the unpaired *t*-test. All analyses were performed using SPSS 9.0 for Windows.

3. Results

Patient characteristics are shown in Table 1. The changes in biochemical parameters during insulin treatment are given in Table 2. Before insulin treatment, plasma α-tocopherol levels in diabetic patients did not differ from those in nondiabetic subjects. α-Tocopherol decreased significantly, from 33.5±12.1 μmol/l before treatment to 28.11±6.85 μmol/l (−16%) after 1 month of insulin therapy ($p<0.04$) to 26.6±7.03 μmol/l (−20%) after 3 months of insulin therapy ($p<0.02$). After 6 months of insulin treatment, α-tocopherol no longer differed significantly from baseline levels. Total cholesterol sharply decreased after insulin initiation. The α-tocopherol/total cholesterol ratio

Table 1
General patient characteristics

	Patients (n=15)	Control group (n=15)
Sex (male/female)	5/10	5/10
Age (years)	29.2±11.9	31.8±9.7
Body mass index (kg/m^2)	22.2±3.7	22.2±2.4
HbA1c (%)	14.3±3.6	4.4±0.5
Systolic blood pressure (mm Hg)	125.5±13.2	122.7±9.8
Diastolic blood pressure (mm Hg)	79.7±6.7	77.3±7.0
Smoking (yes/no)	2/13	2/13

Data represent numbers or means±S.D.

Table 2
Biochemical parameters before and after initiation of insulin treatment

	Before insulin treatment (baseline data)	After 1 month of insulin treatment	After 3 months of insulin treatment	After 6 months of insulin treatment	Control group (compared to baseline data)
HbA1c (%)	14.3±3.65	9.6±1.98**	5.9±1.01*	6.1±0.96*	4.44±0.50*
Total cholesterol (mmol/l)	5.44±1.09	4.79±0.89*	4.80±1.17*	4.67±0.91*	4.45±0.67*
HDL cholesterol (mmol/l)	1.28±0.31	1.34±0.29	1.47±0.33*	1.50±0.33*	1.42±0.26
LDL cholesterol (mmol/l)	3.24±0.91	2.82±0.75*	2.82±0.95*	2.60±0.68*	2.51±0.72*
Triglycerides (mmol/l)	2.00±1.24	1.37±0.99*	1.13±0.93*	1.24±0.99*	1.14±0.58*
α-Tocopherol (μmol/l)	33.52±12.07	28.11±6.85*	26.60±7.03*	29.61±7.45	28.20±4.76
α-Tocopherol/total cholesterol (μmol/mmol)	6.15±1.51	5.78±0.93*	5.69±1.02	6.31±1.61	6.47±1.54
MDA (μmol/l)	3.79±2.91	2.17±0.65*	2.40±0.63*	2.22±0.55	1.57±0.21*

Values represent means±S.D.
MDA=malondialdehyde.
* $p<0.05$.

was only significantly different after 1 month. In addition, the α-tocopherol/LDL ratio showed the same pattern (data not shown). At baseline, the HbA1c and MDA concentrations were significantly higher in the diabetes group than in the control group ($p<0.01$). The MDA concentrations showed a significant decrease after the start of insulin treatment (Table 2). However, after 6 months, the MDA concentrations persisted at a (not significantly) higher level than the reference group. We repeated all these analyses in a subgroup of 12 patients, presumably with type 1 diabetes (age at onset of DM <40 years and BMI <25 kg/m^2). This did not change the magnitude of the results. We also repeated the analyses without the patients and control subjects who smoked. This exclusion of smokers did not change our findings.

4. Discussion

The present study was performed to evaluate the effects of short-term insulin treatment and subsequent glycemic control in new-onset, insulin-dependent diabetes mellitus on plasma α-tocopherol levels and lipid peroxidation (measured as MDA). Our data clearly show a significant decrease in plasma α-tocopherol levels during the first months of insulin treatment, even when the data are corrected for the concomitant reduction in plasma cholesterol levels. After 6 months, plasma α-tocopherol levels showed a trend towards the initial levels in the untreated patients, which were similar to those in a group of nondiabetic controls. We simultaneously measured plasma MDA levels as a parameter for lipid peroxidation. Plasma MDA was significantly elevated in uncontrolled diabetic patients ($p=0.006$), reflecting increased oxidative stress. The decline we observed during insulin treatment was significant. However, the MDA levels at month 6 in the patient group were still elevated compared to those in controls.

To appreciate these findings, some issues need to be addressed. First, our study was based on a small sample of 15 patients with new-onset, insulin-dependent diabetes who sequentially presented at the outpatient clinic. Only 12 patients were likely to have type 1 diabetes. Second, direct measurement of oxidative stress in vivo is a very complex matter since free radicals are highly reactive, have a very short half-life, and are present in very low concentrations only. Indirect methods for measuring secondary products of oxidative stress are considered rather unspecific and may give conflicting results [13]. Extracellular fluids contain several antioxidants that interfere with the oxidative process [4]. One of the most important antioxidants is α-tocopherol, which was measured in our study. However, the measurement of antioxidants may even be less specific because it reflects indirectly the reaction of the whole organism to increased oxidative stress. Consequently, we think that plasma MDA levels reflect oxidative stress better than plasma α-tocopherol levels.

We found significantly elevated plasma MDA levels in uncontrolled diabetic patients, representing increased oxidative stress. This is in agreement with findings from other investigators, who reported that systemic oxidative stress was increased in new-onset, young diabetic patients, as measured by changes in the antioxidant defense systems and in the parameters of lipoperoxidation, like plasma MDA levels [14]. Another study evaluated the presence of oxidative stress in patients with poor metabolic control [15]. It showed that glycemic control may reduce oxidative stress, but total normalization of the parameters of oxidative stress was not achieved, indicating continued oxidant injury despite optimal metabolic control of the diabetes. We similarly found that, in the hyperglycemic state, the MDA concentrations were elevated and that after improved metabolic control they decreased, but not to normal levels.

Published studies have reported different effects of insulin treatment on serum levels of α-tocopherol in type 1 diabetes. One study showed that insulin infusion acutely depletes α-tocopherol in plasma regardless of insulin resistance [7]. This is in line with our findings. We observed a significant decrease in α-tocopherol levels after

the start of insulin therapy during the first months. This effect was still present after 6 months, but it was no longer significant. It may be caused directly by insulin or indirectly by the improved metabolic control. These findings are opposite those of another study showing an increase in α-tocopherol levels after 3 days of euglycemic insulin therapy in patients with type 2 diabetes with chronic hyperglycemia [8].

The mechanism by which insulin or improved metabolic control influences plasma α-tocopherol levels has yet to be elucidated. Studies examining mechanisms for regulation of plasma α-tocopherol have led to the recognition that the liver controls plasma α-tocopherol concentrations [16]. The liver seems to be the most important organ in the processing of dietary α- and γ-tocopherols. Whether insulin or improved metabolic control influences this processing of α-tocopherol by the liver is not known.

What may be the implications of our findings for clinical practice? In our study, the decline in α-tocopherol levels was temporary, lasting only a few months. Whether this may have clinical consequences is unclear. When α-tocopherol levels are below normal for a longer period of time, there is an inverse relation with vascular disease incidence and mortality, as reported by several observational studies [17]. Therefore, it has been proposed that diabetic patients be given vitamin E in order to prevent or delay the development of microvascular complications [2,15,18]. However, recently published results have shown that daily administration of 400 IU vitamin E for an average of 4–5 years to middle-aged and elderly patients with diabetes and/or increased CVD risk has no effect on CV outcome or nephropathy [19,20]. Hence, at this moment, recommending vitamin E supplementation is difficult to justify.

In conclusion, our findings support the view that in patients with untreated, new-onset diabetes mellitus, oxidative stress is increased, as reflected by elevated MDA levels. In addition, our results indicate that the start of insulin treatment and/or improved metabolic control cause a significant decrease in α-tocopherol levels during the first months. This finding needs further investigation because there are still questions about the mechanism and significance of insulin action on α-tocopherol metabolism that the present study was not able to answer.

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