

# Double-blind, randomised study of the effect of combined treatment with vitamin C and E on albuminuria in Type 2 diabetic patients

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## Abstract

**Aims** Elevated levels of urinary albumin excretion rate (AER) predict high risk for progressing to end-stage renal disease. In streptozotocin-induced diabetes, supplementation with vitamin C or E reduces albuminuria and glomerular hypertrophy. We tested the hypothesis that supplementation of both vitamin C and E in pharmacological doses lowers AER in Type 2 diabetic patients with persistent micro/macroalbuminuria.

**Methods** Thirty Type 2 diabetic patients with AER 30–300 mg/24 h were included in a double-blind randomised, cross-over trial. Patients received vitamin C (1250 mg) and vitamin E (680 IU) per day or matching placebo for 4 weeks with a 3-week wash-out period between treatment periods in random order.

**Results** Combined treatment with vitamin C and E reduced AER by 19% (95% CI 6–34%) ( $p = 0.04$ ), geometric mean 197 mg/24 h (95% CI 114–341 mg/24 h) vs. 243 mg/24 h (146–404 mg/24 h). No changes were seen in serum creatinine, haemoglobin A<sub>1c</sub> or blood pressure. Fasting plasma concentrations of vitamin C and E increased in all patients during active treatment (mean vitamin C 79.4  $\mu\text{mol/L}$  (SD 27.8) vs. 41.9  $\mu\text{mol/L}$  (18.4) and vitamin E 47.0  $\mu\text{mol/L}$  (19.8) vs. 29.5  $\mu\text{mol/L}$  (15.3),  $P < 0.000001$ ). Except for two patients that started additional blood pressure lowering treatment during the run-in period, no changes in medication, food and exercise habits or in the number of smokers occurred during the study.

**Conclusion** Short-term treatment with vitamin C and E in pharmacological doses lowers AER in Type 2 diabetic patients with micro/macroalbuminuria. Further long-term, large-scale studies of this albuminuria reducing treatment modality are warranted.

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**Keywords** Type 2 diabetes, albuminuria, vitamin C, vitamin E, kidney function

**Abbreviations** ACE, angiotensin converting enzyme; AER, urinary albumin excretion rate; ELISA, enzyme linked immunosorbent assay; HPLC, high pressure liquid chromatography

## Introduction

Elevated levels of urinary albumin excretion rate (AER) predict high risk for progression to end-stage renal disease in Type 2 diabetes mellitus [1]. Several studies provide

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support for the concept that a reduction in AER is a valid renoprotective treatment goal [2]. Treatment with angiotensin converting enzyme (ACE) inhibitors reduces AER, however, normalization of AER does not occur in the majority of patients, encouraging research in supplementary treatment modalities.

Oxidative stress may be a common mechanism for the pathogenesis of complications in Type 2 diabetes [3]. Thus, plasma concentrations of ascorbic acid are decreased in both human and experimental diabetes [4–6]. Similarly, plasma vitamin E levels are reduced in Type 2 diabetic patients [7,8]. Protective effects of treatment with vitamin C or E on experimental renal injury in diabetic animals have been reported [9,10]. Since vitamin C, a water-soluble antioxidant, and vitamin E, a lipid-soluble antioxidant, act synergistically *in vitro* [11,12], we decided to test the hypothesis that supplementation of both vitamin C and E in pharmacological doses lowers AER in Type 2 diabetic patients with micro/macroalbuminuria.

**Patients and methods**

**Patients**

Patients were recruited from the outpatient clinic at Steno Diabetes Center. Inclusion criteria were Type 2 diabetes according to 1985 WHO definition, and an albumin excretion rate in the range 30–300 mg in two out of three consecutive sterile 24 h urine collections prior to enrolment in the study. Since ACE-inhibitors may reduce AER as well as lowering blood pressure [13,14], treatment with ACE-inhibitors was stopped, and blood pressure strictly monitored, for at least eight weeks before randomization (*n* = 28) to exclude any prolonged effect of this treatment. In cases where blood pressure was >180/100 mmHg, additional blood pressure lowering treatment with diuretics, calcium antagonists or  $\beta$ -blockers was initiated during run-in (*n* = 2). Patients taking vitamin C or E supplementation were asked to stop at least eight weeks before randomization (*n* = 3) [15]. Exclusion criteria were systolic blood pressure higher than 200 mmHg or diastolic blood pressure higher than 110 mmHg, glomerular filtration rate less than 40 mL/min/1.73 m<sup>2</sup>, prior myocardial infarction or congestive heart failure. The study protocol was in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Copenhagen County. All patients gave informed consent. Of the 37 eligible patients, four had prior myocardial infarction and three refused to participate, thus 30 patients entered the study. One withdrew during the eight-week run-in period due to an acute spinal injury causing paresis of both legs. Thus 29 patients were randomised and all of these completed the study. Patients who were not randomised did not differ from the rest of the patients in clinical and biochemical variables.

**Cross-over design**

After the run-in period, patients entered a double-blind, randomised, cross-over trial. Patients were instructed to take

five tablets of vitamin C and E (250 mg of vitamin C and 136 units of d- $\alpha$ -tocopherol per tablet (CellaVie, Ferrosan A/S, Copenhagen)) giving a total dose of 1250 mg vitamin C and 680 IU of d- $\alpha$ -tocopherol per day, or five matching placebo tablets, in the morning. The rationale for the selected doses was based upon *in vivo* and *in vitro* studies. An improvement in endothelium-dependent vasodilatation after intravenous administration of 1000 mg vitamin C has been reported in Type 2 diabetic patients [16], and 400 IU of  $\alpha$ -tocopherol effectively decreases oxidation of LDL-cholesterol *in vitro* [17]. Furthermore, a decrease in the number of nonfatal myocardial infarctions in patients with established coronary-vessel disease has been reported with vitamin E doses of 400–800 IU [18]. After four weeks of treatment there was a three-week washout period before patients crossed over to the opposite treatment for another four weeks [15]. Fifteen patients were given active treatment as the initial intervention, and 14 were given placebo (Fig. 1).

**Measurements**

Measurements were done at the start of the run-in phase, at randomization, and at the end of each treatment period of the double-blind crossover phase, and consisted of blood pressure, three consecutive sterile 24 h urine collections, and blood sampling for measurement of biochemical variables. All measurements were done after a 12 h fast. Supine blood pressure values were measured twice in both arms with a Hawksley random zero sphygmomanometer, and the average of these measurements was used.

**Assays**

AER was measured by ELISA [19]. HbA<sub>1c</sub> was measured with ion-exchange high-performance liquid chromatography (Bio-Rad VARIANT, California, USA) and the nondiabetic reference

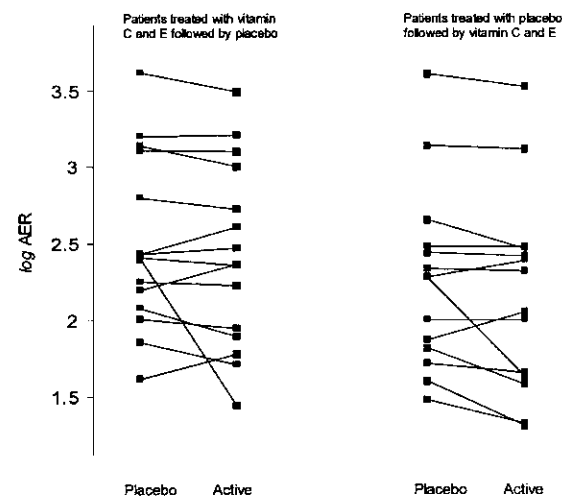


Figure 1 Individual log urinary albumin excretion rate during placebo and active treatment.

range in our laboratory was 4.1–6.4%. Plasma  $\alpha$ -tocopherol, ascorbic acid and dehydro-ascorbic acid were measured by HPLC [20,21].

### Statistical analysis

Results are expressed as mean with SD or 95% confidence intervals, when appropriate. AER is expressed as geometric mean (antilog 5%-percentile; antilog 95%-percentile for the average of the logged values) due to a positive skewness of the distribution. We used paired Student's *t*-test to compare differences between the variables during the two phases of the crossover part of the study. The mean of the logged values of AER has been used for comparison of this variable, and the difference is expressed in percent reduction with active treatment. Pearson correlation analysis was used to study associations between AER to changes in vitamin C and E concentrations. A prestudy sample-size calculation indicated that at least 30 patients would be needed to detect a 20% change in AER, assuming a coefficient of variation of 30% for AER with a two-sided  $\alpha = 0.05$  and  $\beta = 0.9$ .

## Results

### Run-in phase

Twenty-eight of the 30 patients who entered the study were being treated with ACE-inhibitors which had to be stopped at least eight weeks before randomization. Geometric mean for AER before treatment with ACE-inhibitors was stopped was 112 (68–182) mg per 24 h and mean blood pressure was 151 (17)/89 (10) mmHg. During run-in, AER increased to 231 (122–373) mg per 24 h ( $P = 0.0001$ ) and mean blood pressure to 155 (18)/91 (10) mmHg ( $P = 0.01$ ). The mean age at randomization was 58.7 (SD 7.3) years with average known diabetes duration of 12.2

(4.4) years. Mean glycosylated haemoglobin  $A_{1c}$  was 8.6 (1.2) % with a mean fasting blood glucose of 8.7 (3.4) mmol/L. Four patients were treated with insulin as monotherapy, 10 patients with insulin as well as oral hypoglycaemic agents (OHA), 14 patients with OHA, and one patient with diet alone. Nine patients were smokers. During the run-in period, nine patients had albuminuria above 300 mg per 24 h. Two patients started additional blood pressure lowering treatment (one with a calcium antagonist and one with a  $\beta$ -blocker) during this period. No significant differences were found between patients starting with placebo treatment, and patients starting with active treatment with vitamin C and E, at baseline for any of these variables.

### Randomised, double-blind cross-over phase

Nine females and 20 males completed the study. Table 1 shows the results after each of the two treatment periods. There was a 19% (95% CI 6–34%) reduction in AER with active treatment,  $p = 0.04$ . There was no significant order-of-treatment effect ( $P = 0.83$ ), as illustrated by Fig. 1.

No significant changes were seen for systolic or diastolic blood pressure, glycemic control, serum creatinine or serum total cholesterol. No change in medication, except shift from active to placebo tablets and vice versa, was recorded in any patient during this period. The number of smokers remained unchanged and no change in food and exercise habits was reported in interviews throughout the cross-over period.

The reduction in AER was not significantly associated with the increase in fasting plasma  $\alpha$ -tocopherol concentrations ( $r = -0.03$ ,  $p = 0.9$ ) or the obtained fasting plasma concentrations of  $\alpha$ -tocopherol during active treatment ( $r = 0.06$ ,  $p = 0.8$ ). Similarly, the increase in fasting plasma

Table 1 Blood pressure and biochemical variables.

Variable	Randomised double-blind crossover		Difference (95% CI)	P for difference
	Placebo	Active		
Urinary albumin excretion rate (mg/24 h)*	243 (146–404)	197 (114–341)	19% (6–34)†	0.04
S-creatinine ( $\mu$ mol/L)	86 (19)	85 (20)	0.8 (–1.9–3.4)	0.55
Systolic blood pressure (mmHg)	153 (17)	151 (14)	1.4 (–3.1–5.9)	0.52
Diastolic blood pressure (mmHg)	89 (11)	88 (11)	1.3 (–2.0–4.4)	0.43
Haemoglobin $A_{1c}$ (%)	9.0 (1.4)	9.0 (1.4)	0.0 (–0.3–0.2)	0.78
Fasting p-glucose (mmol/L)	9.3 (3.2)	8.9 (3.2)	–0.4 (–1.2–0.5)	0.36
Fasting s-total cholesterol (mmol/L)	5.1 (0.9)	5.0 (0.8)	–0.1 (–0.3–0.1)	0.44
Fasting p- $\alpha$ -tocopherol ( $\mu$ mol/L)	29.5 (15.3)	47.0 (19.8)	17.4 (12.0–22.8)	< 0.000001
Fasting p-ascorbic acid ( $\mu$ mol/L)	41.9 (18.4)	79.4 (27.8)	38.8 (29.9–47.1)	< 0.000001
Fasting p-dehydroascorbic acid ( $\mu$ mol/L)	3.0 (2.2)	6.3 (3.7)	3.5 (2.1–4.8)	0.00001

Data are mean (SD). S denotes serum and p denotes plasma.

\*Geometric mean (95% confidence interval) of three consecutive 24 h urine collections

†Difference in percentage. Calculated as  $100\% - 10^{(\log_{10}(\text{active}) - \log_{10}(\text{placebo}))} \times 100\%$

ascorbic acid or plasma dehydroascorbic acid was not correlated to the decrease in AER ( $r = 0.21$ ,  $p = 0.3$  and  $r = 0.09$ ,  $p = 0.6$ , respectively).

Simple counting of the tablets indicated that adherence was high, with more than 99% of the tablets being taken. Fasting values of plasma  $\alpha$ -tocopherol concentrations and of plasma ascorbic acid also increased in all patients (Table 1).

### Discussion

Our study shows that short-duration treatment with vitamin C and E in pharmacological doses in Type 2 diabetic patients with micro/macrolalbuminuria significantly lowers AER, thus suggesting a potential renoprotective effect in these patients [2]. This effect was seen despite no significant changes in known confounders such as haemoglobin A<sub>1c</sub>, blood pressure, and smoking.

Protective effects of treatment with vitamin C or E on renal injury in diabetic animals have been reported [9,10]. Vitamin C effectively reduced glomerular hypertrophy and albuminuria in rats with streptozotocin-induced diabetes [9]. Similarly, administration of vitamin E prevented glomerular hyperfiltration, albuminuria and glomerular hypertrophy in the early phase of glomerular injury in a comparable streptozotocin-induced diabetes animal model [9,10].

A study in Type 1 diabetic patients, primarily designed to investigate the effect of vitamin E supplementation on retinal blood flow, also showed an improvement in renal function, as indicated by creatinine clearance being normalized during treatment with 1800 IU vitamin E per day compared to placebo, with no significant changes in AER [22].

Intake of 1000 mg vitamin C daily has been reported to decrease AER after 9 months of treatment in 20 patients with Type 1 or Type 2 diabetes and with microalbuminuria or retinopathy [23]. The decrease in AER correlated to the rise in plasma values of vitamin C. This is in contrast to our study, where no correlation between the reduction in AER and the increases in, or the obtained fasting plasma concentrations of, vitamin C were seen.

A subgroup analysis of 3654 primarily Type 2 diabetic patients participating in the Heart Outcomes Prevention Evaluation study (HOPE) demonstrated no effect for a combined endpoint of diabetic microvascular complications (nephropathy, dialysis, and laser therapy) in patients receiving vitamin E (400 IU) daily for 4.5 years [24]. Yet, no information on compliance and plasma values of vitamin E has been reported. Despite the negative result of this study, it is, however, still possible that vitamin E supplementation requires cosupplementation with other antioxidants to have beneficial effects.

Treatment with vitamin C or E has been reported to reduce blood pressure [25,26], however, as seen in Table 1,

active treatment did not lower blood pressure significantly in the present study, and thus the reduction in AER cannot be explained by a systemic effect on blood pressure.

In our study, all patients stopped treatment with ACE-inhibitors eight weeks before randomization to exclude interaction with this treatment modality. The increase seen in AER during this period is in accordance with results from other studies in Type 2 diabetes [27]. Whether patients already receiving ACE-inhibitors also benefit from combined treatment with vitamin C and E remains to be elucidated. However, a recent study in Type 2 diabetic patients showed marked effect on progression to diabetic nephropathy with intensified multifactorial treatment, including vitamin C and E, as well as ACE-inhibitors, as compared to standard multifactorial treatment, even though half of the patients in the standard group were treated with ACE-inhibitors [28].

Patients did not report any side-effects during the course of the study. It should, however, be noted that excessive intake of vitamin C has been reported to be associated with renal oxalosis in patients with chronic, as well as acute, renal failure [29].

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