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# Impact of Vitamin E supplementation on lipoprotein peroxidation and composition in Type 1 diabetic patients treated with Atorvastatin

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## Abstract

*Objective:* To investigate the impact of Vitamin E on lipids and peroxidation during statin treatment. *Research Design and Methods:* T1DM patients with high cholesterol received Atorvastatin 20 mg with either placebo (group AP, $n = 11$) or d-$\alpha$-tocopherol 750 IU (group AE, $n = 11$) daily. They were monitored for blood biochemistry, low-density lipoprotein (LDL) subfractions and lipid peroxidation at inclusion and after 3 and 6 months. *Results:* Serum cholesterol and triglycerides decreased to the same extent (29 and 21% respectively) in both groups. Serum tocopherol decreased by 18% in AP and increased by 50% in AE ($P < 0.0001$, between-group comparison by repeated measures ANOVA) but relative to lipids it increased by 15% in AP and by 100% in AE. Copper-induced production of thiobarbituric reactive substances in the LDL + VLDL fraction increased by 18% in AP and did not change in AE ($P = 0.02$). The lagtime for the production of fluorescent products was prolonged by 13 min only in group AE ($P = 0.028$). Plasma malondialdehyde decreased by 35% in both groups ($P = 0.002$) but not when adjusted for lipids. *Conclusions:* In T1DM Vitamin E supplements do not affect the lowering of lipids and plasma malondialdehyde achieved by Atorvastatin. They reverse the increase of in vitro peroxidation caused by Atorvastatin but do not achieve the decreases observed in patients not receiving lipid-lowering drugs. These results indicate that the antioxidant effect of Vitamin E is attenuated when given in conjunction with this statin.
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## 1. Introduction

Patients with diabetes mellitus have a two- to four-fold increased risk of both developing and dying from cardio-vascular disease [1,2]. This increased risk is independently associated with diabetes-induced abnormalities in plasma lipids and lipoprotein metabolism [3]. In addition, hyperglycemia directly causes cellular dysfunctions [4,5], glycation [6] and oxidative stress [7]. All these factors further aggravate the consequences of the diabetic dyslipidemia by increasing susceptibility to lipid peroxidation [8,9]. This leads to accumulation of products of oxidative damage to lipids [10], foam cell formation and eventually increased atheroma plaque deposition [11].

These diabetes-related abnormalities thus exaggerate the risk of even moderately elevated low-density lipoprotein (LDL) cholesterol [12]. For these reasons, more aggressive lipid-lowering targets are currently recommended for these patients [13]. One of the preferred therapies is statins, not only as effective lipid lowering drugs, but because of several other pleiotropic anti-atherogenic effects [14]. Indeed, wide-scale studies have unequivocally demonstrated that statins can be successfully used to correct dyslipidemia and to prevent coronary heart disease in these patients [15]. However, most of these studies were secondary prevention

trials in diabetic patients who were predominantly Type 2 (T2DM) [16]. Although the same lipid-lowering guidelines are applied to Type 1 diabetes (T1DM), less information is available on the effects of statins in these patients [17]. This question is relevant when considering the use of statins in the prevention of atherosclerosis in diabetic patients, since in T1DM, in contrast to T2DM, the dyslipidemia (high triglycerides, small dense LDL and low HDL cholesterol) is less pronounced and is closely associated with the presence of nephropathy [18,19]. Thus, the effects of statins on lipoprotein composition and properties could differ in the various types of diabetic patients.

With regard to lipoprotein peroxidation, we have shown in previous studies that susceptibility of LDL + VLDL to in vitro copper-induced peroxidation is higher in T1DM patients than in non-diabetic subjects [8,20] and that supplementation with α-tocopherol decreases this susceptibility in a saturable and reversible manner [21]. When T1DM patients with hypercholesterolemia were treated with Atorvastatin 40 mg daily for up to 12 weeks, serum α-tocopherol decreased and susceptibility to peroxidation increased significantly despite marked decreases in serum cholesterol and triglycerides [22]. This observation is relevant when planning long-term and especially primary prevention in these patients. Indeed, lipid peroxidation plays its fundamental role in the early stages of atheroma plaque formation and this may take place years before the appearance of clinical episodes.

We therefore aimed to investigate whether the Atorvastatin-induced changes in peroxidation can be reversed by anti-oxidant supplementation. For this purpose we studied the effects of α-tocopherol when given in conjunction with the lipid-lowering therapy.

## 2. Materials and Methods

### 2.1. Patient population and study design

Type 1 diabetic patients attending the outpatient diabetes clinic of the Antwerp University hospital and having history of high serum cholesterol were asked to take part in the study. After a washout (lipid-lowering medication-free) period of 4–6 weeks, 32 patients were screened for blood lipids. Inclusion criteria were total cholesterol > 4.9 and LDL cholesterol > 3.0 mmol/L but Triglycerides < 4.5 mmol/L) and normal blood levels of thyroxin (9.7–23.4 pmol/L) and TSH (0.47–4.7 μU/mL). Twenty-four patients satisfying the inclusion criteria were randomised in blocks of 4, matched for sex and age, to 2 groups. Group AE ($n = 12$) received 20 mg Atorvastastin® daily together with 250 IU (168 mg) d-α-tocopherol 3 times daily (Omega Pharma, NV, Nazareth, Belgium) and group AP ($n = 12$) received 20 mg Atorvastastin® with placebo (280 mg soybean oil containing 0.25 mg tocopherol per capsule and identical in taste and appearance to the Vitamin E capsules). Patients

were monitored at randomisation (visit 1), after 3 (visit 2) and after 6 months treatment (visit 3). During the course of the study one patient from each group dropped out, one due to thyroid dysfunction and one due to an accident. These two patients were not included in the statistical analyses.

The experimental protocol was in accord with the Helsinki declaration and was approved by the ethical commission of the University Hospital. Participating subjects signed an approved consent form.

All patients were on a standard diet for diabetes recommending 7.5 to 8.5 MJ/day (50% of the energy as carbohydrates, 20% as protein and 30% as fats). This diet assures a daily intake of at least 3 mg Vitamin E, 3000 μg Vitamin A, 150 mg Vitamin C and 26 mg flavonoids.

### 2.2. Analytic methods

Fasting blood samples were collected at each visit. *Routine blood tests* (blood count, lipids and creatinphosphokinase) were analysed in the hospital laboratory. Total analytical variability (coefficient of variation CV) was 2, 1.9 and 0.9% for total cholesterol, HDL cholesterol and triglycerides respectively. LDL-cholesterol was calculated according to the Friedewald equation [23]. Glycated haemoglobin ($HbA_{1c}$) was measured using a HPLC cation exchange column (Modular Diabetic monitoring system BIO–RAD, California USA), CV was 1.5%.

*Oxidant-antioxidant balance* was evaluated by measuring the concentrations of Vitamins E and A in serum using HPLC (Shimadzu, Bio–Rad reverse phase C18 with 100% methanol mobile phase) and detection at 292 and 325 nm and CV of 10 and 13% respectively [24]. The susceptibility of LDL + VLDL to copper catalysed oxidation was measured by isolating these two groups of lipoproteins (non-HDL) by dextran sulphate/$MgCl_2$ precipitation and incubation of a suspension containing 200 μg/ml of cholesterol with 46 μM $CuSO_4$ for up to 180 min at 37 °C, during which aliquots were taken every 30 min for the measurement of thiobarbituric acid reactive substances (TBARS). Fluorescence at 360 nm excitation and 440 nm emission was monitored continuously. Three phases were measured: (1) the lagtime (expressed in minutes), during which fluorescence does not increase significantly; it quantifies the capacity of the antioxidants within the lipoproteins to retard the initiation of oxidation; (2) the slope of the propagation phase, during which fluorescence increases rapidly and which indicates the velocity of oxidative changes in the apo B and (3) the saturation phase during which fluorescence reaches a plateau and which gives an estimate of the total amount of lipid oxidised. CVs of these parameters ranged from 3 to 11% [20]. Plasma malondialdehyde (MDA) was analysed by HPLC using reverse phase LiChrospher RP C18 (Alltech, Deerfield, IL), methanol/$KH_2PO_4$ 10 mM (40/60 v/v) as mobile phase and detection at 532 nm [25]; CV was 9%.

*Composition of the non-HDL fraction* was determined by measuring the content in cholesterol (CHOD–PAP kit,

Merck, Germany), triglycerides (GPO–Trinder kit, Sigma Diagnostics, St. Louis, MO, USA) and Vitamins A and E.

*LDL subfractions* were measured in plasma by polyacrylamide gel electrophoresis (Lipoprint LDL Kit, Quantimetrix, CA, USA). Up to 7 LDL subfractions can be separated as well as one VLDL band, 3 Midbands and one HDL band. When the VLDL position is taken as 0.00 and HDL as 1.00, the "Large LDL" bands have the relative positions 0.25, 0.30 and 0.36. "Small LDL" bands are at the positions 0.41, 0.46, 0.51, 0.56 and 0.61. The presence or absence of each band was noted by comparison with a template. In order to quantify the subfractions, the optical density of each LDL subfraction was scanned (Appraise densitometer, Beckman Instruments, Inc., Diagnostic System Group, Brea CA) and expressed as a percent of the total LDL (sum of all the fractions).

### 2.3. Statistical methods

Data were analysed using SPSS software (version 10, Chicago, IL). Results are expressed as means ± S.D. and two-tailed $P$ values $<0.05$ were considered significant. Between-group comparisons at inclusion were done by $t$-test or Mann–Whitney test for the non-gaussian variables. The effect of medication versus placebo during the course of the three visits was analysed by repeated measures ANOVA. Data was analysed for the change over time during the course of the various visits (within group comparison) and for differences between the 2 groups with regard to these time-related changes i.e. for the interaction between medication and time (between-group comparison of the change over time).

## 3. Experimental results

Randomisation after screening resulted in two comparable groups of patients in terms of clinical characteristics and routine biochemical parameters (Table 1). Five patients had moderate hypertension (defined as $>130/85$ mm Hg) and three had intermittent microalbuminuria ($<20\,\mu g/min$). One-third to one-half of the patients in both groups had some degree of retinopathy, peripheral arteriopathy and coronary heart disease but neuropathy was more frequent in group AP (nine patients versus four in group AE, $P = 0.018$).

Treatment with 20 mg/day Atorvastatin decreased serum total cholesterol by 29%, from $6.08 \pm 0.68$ mmol/L before treatment to $4.34 \pm 0.77$ after 3 months and $4.41 \pm 0.71$ after 6 months, LDL cholesterol decreased by 41% from $3.91 \pm 0.52$ to $2.32 \pm 0.58$ and $2.33 \pm 0.56$ mmol/L ($P < 0.0001$ for the time-related changes). Triglycerides decreased by 21% from $1.17 \pm 0.57$ to $0.93 \pm 0.39$ and $0.93 \pm 0.43$ mmol/L ($P = 0.005$). These decreases in serum lipids were the same in both groups (the between-group comparison of the change over time was not significant) and there was no further significant change between 3 and 6 months.

Table 1
Patient characteristics before treatment

| Characteristic | Group AE (n = 11) | Group AP (n = 11) |
|---|---|---|
| Age (years)[a] | 43 (21–69) | 59 (23–66) |
| Sex (male/female)[b] | 10/1 | 9/2 |
| Duration of diabetes (years)[a] | 20 (6–41) | 16 (6–34) |
| HbA$_{1c}$ (%) | 7.8 ± 0.9 | 8.1 ± 1.2 |
| Insulin dose (U/day) | 54 ± 19 | 43 ± 18 |
| Insulin dose (U/kg body weight) | 0.70 ± 0.18 | 0.56 ± 0.24 |
| BMI (kg/m$^2$) | 25.2 ± 2.8 | 26.0 ± 2.1 |
| Blood pressure systolic (mmHg)[a] | 130 (120–170) | 130 (105–150) |
| Blood pressure diastolic (mmHg)[a] | 80 (70–105) | 80 (70–85) |
| Total cholesterol (mmol/L) | 6.06 ± 0.79 | 6.09 ± 0.60 |
| LDL cholesterol (mmol/L) | 3.94 ± 0.49 | 3.88 ± 0.58 |
| HDL cholesterol (mmol/L) | 1.65 ± 0.37 | 1.61 ± 0.42 |
| Triglycerides (mmol/L) | 1.03 ± 0.41 | 1.32 ± 0.69 |

Values are expressed as means ± S.D.

[a] As median (minimum−maximum) for non-Gaussian distributed data.

[b] As number of observations. There were no significant differences between the two groups.

HDL cholesterol and glycated hemoglobin did not change in either group. Creatine phosphokinase tended to increase in both groups and to the same extent (from $132 \pm 69$ U/L before treatment to $136 \pm 70$ U/L after 3 months and $171 \pm 113$ U/L after 6 months, $P = 0.07$ when comparing inclusion and 6 months).

Serum α-tocopherol at inclusion was strongly related to serum cholesterol ($r = 0.59$, $P = 0.004$) but not to triglycerides. As shown in Table 2, in group AE serum α-tocopherol increased by 48% after 3 months and by 62% after 6 months, whereas in group AP it decreased by 18% ($P < 0.0001$ for the between-group comparison). However, when expressed relative to total lipids (sum cholesterol + triglycerides), α-tocopherol doubled in group AE and it also increased slightly (by 15%) in AP ($P = 0.015$ for the time-related change within this group and $P < 0.0001$ for the between-group comparison). Serum retinol did not change in either group.

Susceptibility to peroxidation in vitro was monitored in the LDL + VLDL fraction which was subjected to a 3 h incubation with copper. Total production of TBARS increased by 18% in group AP ($P = 0.05$ for the change over time within this group). These changes were significantly different from the changes in group AE ($P = 0.02$ for the between-group comparison). In group AE total TBARS production did not change after 3 months and decreased slightly (by 13%) after 6 months, albeit not significantly. Nevertheless, when analysing the TBARS production at the various time points of the in vitro incubation (shown in Fig. 1), it was noted that the decrease of TBARS in the Vitamin E supplemented group (AE) occurred only in the early stages of the in vitro peroxidation (60–120 min). TBARS production after 150 min incubation was not affected by Vitamin E supplementation. In contrast, the increase in TBARS seen in the unsupplemented group (AP) was evident at all time points. The changes in group AE were thus only significantly

Table 2

Serum vitamins, LDL + VLDL composition and lipid peroxidation in Type 1 diabetic patients before and after receiving Atorvastatin 20 mg/day with either d-a-tocopherol 750 IU/day or Placebo

| Variable | Group AE: Atorvastatin + α-tocopherol (n = 11) | | | Group AP: Atorvastatin + Placebo (n = 11) | | | P-value |
|---|---|---|---|---|---|---|---|
| | Inclusion | After 3 months | After 6 months | Inclusion | After 3 months | After 6 months | |
| Serum α-tocopherol (μmol/L) | 39.3 ± 9.7 | 58.2 ± 14.3 | 63.5 ± 25.5* | 45.1 ± 9.5 | 37.0 ± 5.2 | 39.8 ± 8.4 | <0.001 |
| Serum α-tocopherol (μmol/mmol total lipid) | 5.55 ± 1.22 | 11.33 ± 1.82 | 12.3 ± 3.97** | 6.08 ± 1.02 | 7.01 ± 1.10 | 7.20 ± 1.02* | <0.001 |
| Serum retinol (μmol/L) | 3.34 ± 0.74 | 3.09 ± 0.52 | 3.14 ± 0.44 | 3.13 ± 0.80 | 3.15 ± 1.24 | 3.42 ± 1.07 | ns |
| Plasma malondialdehyde (μmol/L) | 1.27 ± 0.39 | 0.78 ± 0.14 | 0.79 ± 0.16** | 1.17 ± 0.31 | 0.76 ± 0.17 | 0.80 ± 0.21** | ns |
| Plasma malondialdehyde (μmol/mmol total lipid) | 0.174 ± 0.045 | 0.158 ± 0.023 | 0.150 ± 0.028 | 0.152 ± 0.038 | 0.138 ± 0.031 | 0.144 ± 0.045 | ns |
| Plasma malondialdehyde (μmol/mmol LDLcholesterol) | 0.317 ± 0.102 | 0.383 ± 0.086 | 0.361 ± 0.084 | 0.306 ± 0.066 | 0.317 ± 0.077 | 0.325 ± 0.104 | ns |
| *In vitro lipid peroxidation*[a] | | | | | | | |
| Total TBARS production | 5345 ± 1515 | 5271 ± 1020 | 4654 ± 1110 | 5551 ± 1690 | 6566 ± 2197 | 6527 ± 1696* | 0.020 |
| Fluorescence lagtime (min) | 106 ± 27 | 121 ± 12 | 118 ± 17* | 116 ± 18 | 116 ± 29 | 106 ± 18 | 0.028 |
| Fluorescence slope (FU/min) | 0.35 ± 0.08 | 0.39 ± 0.06 | 0.36 ± 0.07 | 0.40 ± 0.19 | 0.39 ± 0.09 | 0.42 ± 0.06 | ns |
| Composition non-HDL fraction | | | | | | | |
| Content in cholesterol (mmol/L) | 3.97 ± 0.90 | 2.47 ± 0.56 | 2.57 ± 0.81** | 4.04 ± 0.91 | 2.61 ± 0.65 | 2.69 ± 1.11** | ns |
| Content in triglycerides (mmol/L) | 0.61 ± 0.35 | 0.56 ± 0.25 | 0.59 ± 0.24 | 0.88 ± 0.42 | 0.68 ± 0.32 | 0.75 ± 0.37 | ns |
| Content in α-tocopherol (μmol/L) | 15.4 ± 6.2 | 20.6 ± 11.1 | 24.8 ± 12.6* | 17.4 ± 4.0 | 14.0 ± 3.5 | 16.6 ± 5.8 | 0.022 |
| Content in α-tocopherol (μmol/mmol × total lipid) | 3.37 ± 0.69 | 7.36 ± 2.52 | 7.84 ± 3.17* | 3.57 ± 0.66 | 4.23 ± 0.73 | 4.98 ± 1.02 | 0.019 |

Values are expressed as means ± S.D. The groups were not significantly different at inclusion. *Denotes $P < 0.05$; **$P < 0.001$. For the overall change over time (during the course of the three visits) within the group. The P-values denote the between-groups comparison of the changes over time (repeated measures ANOVA).

[a] In vitro lipid peroxidation of LDL + VLDL (non-HDL) was determined by incubating with copper and measuring the concentration of TBARS every 30 min for a total of 3 h. Total TBARS production was calculated as area under the curve (see Fig. 1) and expressed as nmol MDA equivalents/mg non-HDL cholesterol × minutes. The formation of fluorescent products was monitored continuously at 360/440 nm excitation/emmission to measure lagtime and velocity of formation (slope).

different from those in group AP at 60 and 90 min ($P < 0.05$).

The lagtime for the production of fluorescent products of peroxidation correlated with serum α-tocopherol (at inclusion, $r = 0.46$, $P = 0.03$). The lagtime did not change in AP but was prolonged by 13 min in group AE, $P = 0.028$.

In order to investigate whether these differences in peroxidability were associated with changes in the composition of the lipoprotein particles, lipid and tocopherol content was measured in the LDL + VLDL fraction (Table 2). Content in cholesterol decreased to the same extent (by 35%, $P < 0.001$) in both groups and content in triglycerides did not change. Whereas tocopherol content did not change in AP, in AE it increased by about 50% and doubled when expressed relative to total lipids.

With regard to the LDL subfractions, at inclusion only one patient in each group had peaks at the position 0.41, which is defined as the biggest of the "small LDL" subfractions. Within the range 0.25–0.36 defined as the "large LDL", five patients in group AE showed peaks at the least dense position 0.25 (compared to no patient in AP, $P = 0.002$) and only three had peaks at the densest of the large LDL, at position 0.36 (compared to 10 patients in AP, $P = 0.007$). After 3 months, there was a shift towards the less dense region of the large LDL in eight patients of group AP (compared to three in AE), this being mainly due to a shift from position 0.36 towards position 0.25. In contrast, in group AE a shift in the opposite direction from position 0.25 towards 0.30 occurred in six patients compared to none in AP ($P = 0.014$ for the between-groups comparison). Between 3 and 6 months both groups evolved similarly with half the patients not changing pattern, one-third shifting towards the less dense region and one-fifth towards the denser region.
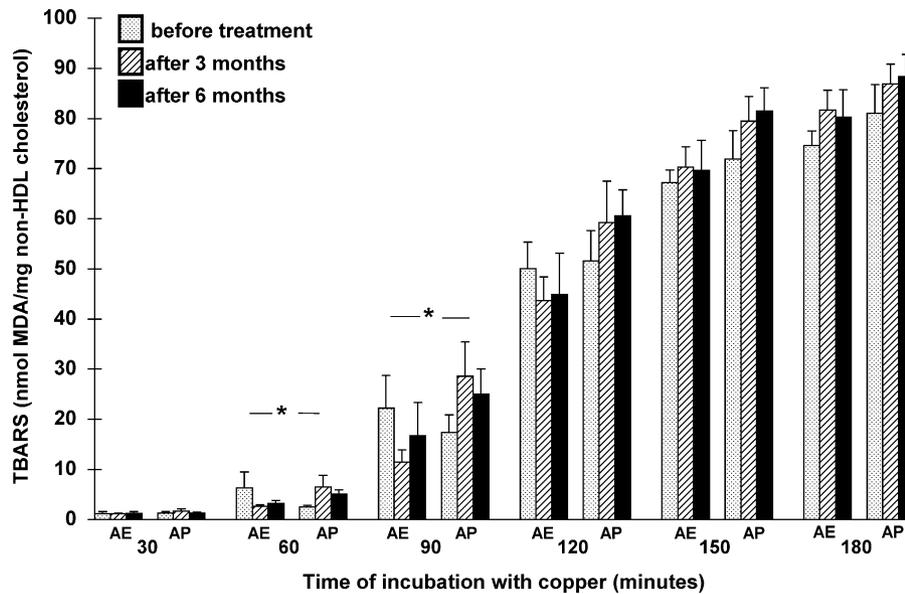
Fig. 1. Time course of the copper-induced production of thiobarbituric–reactive substances (TBARS) during in vitro incubation of LDL and VLDL from patients receiving Atorvastatin 20 mg daily. Group AE received 750 IU d-a-tocopherol in addition and group AP received placebo. Shown are mean ± S.E.M. Pointed bars represent results before treatment, diagonal stripes after 3 months and black after 6 months. *$P < 0.05$ for the between-group comparison of the change over the three visits.

In order to monitor in vivo lipid peroxidation, malondi-aldehyde was measured in plasma. Values at inclusion were positively related to age ($r = 0.55$, $P = 0.05$) but did not correlate with either serum lipids, Vitamins A or E or with parameters of in vitro lipid peroxidation. Treatment with Atorvastatin caused a 35% decrease which was the same in both groups ($P = 0.002$ for the change over time within the groups but ns when comparing the two groups). When expressed relative to total lipids, the decrease of about 10% was not statistically significant. When expressed relative to LDL cholesterol, however, there was even a tendency for increase especially in group AE (by 21% after 3 months and 14% after 6 months, $P = 0.058$ when compared to the values before treatment but ns between-groups). However the power to detect a significant change was only 0.24 in this small group of patients.

## 4. Discussion

In this study, we compared the evolution of serum lipids, lipoprotein composition and peroxidation in T1DM patients treated with Atorvastatin 20 mg daily for 6 months and receiving either placebo or d-α-tocopherol (750 IU) in addition.

Atorvastatin treatment led to similar decreases in serum LDL cholesterol (by 40%) and triglycerides (by 20%) as seen in our previous study, conducted in a comparable group of patients who received a higher dose (40 mg daily) for a shorter period (up to 3 months) [22] and in other studies conducted in T1DM [26]. Supplementation with α-tocopherol did not affect this decrease. Identical doses of α-tocopherol

given for up to 12 months to normolipemic T1DM patients did not affect serum lipids either [21,27]. In other studies, triglycerides either decreased (in T1DM children with abnormally high baseline values) [28] or increased (in healthy older individuals [29] and in young women [30]. Regarding the effects on cholesterol, HDL levels were not low in our patients and were not affected by treatment with Atorvastatin in combination or not with α-tocopherol. In contrast, in CAD patients with low HDL cholesterol and treated with a combination of statin–niacin, α-tocopherol (in an antioxidant cocktail also containing high doses of ascorbate, β-carotene and selenium) blunted the increase in $HDL_2$ which was achieved by the lipid lowering drugs [31].

In this group of patients, baseline serum α-tocopherol was high (40 μmol/L). Atorvastatin treatment (group AP) led to a 18% decrease, but relative to lipids, it increased slightly (by 15%). In our previous study, Atorvastatin 40 mg daily led to a more masked decrease (by 30%) but when adjusted for total serum lipids the increase was similar (12%). Supplementation with d-α-tocopherol (group AE) reversed this situation and increased serum levels by about 50% and by 100% when adjusted for lipids. A 100% increase of serum α-tocopherol was also seen in normolipemic T1DM patients receiving the same dose tocopherol but no lipid-lowering therapy [21]. These observations and the strong correlation with cholesterol levels agree with other studies [32–34] and corroborate that α-tocopherol levels reached in serum are largely determined by the transporting capacity of the lipoproteins. In addition, the liver tocopherol binding protein which packages α-tocopherol into the VLDL particle [35] is saturable. This prevents plasma tocopherol from rising more than three-fold after oral supplements at doses

ranging from 10 to 2000 IU/day. In this study, saturation was reached at levels of 12 and 8 μmol/mmol lipid in serum and the LDL + VLDL respectively. The highest levels reported in the literature are 8–15 mol α-tocopherol per LDL particle [36]. Incubation of lipoproteins with tocopherol in vitro can force levels up to 30 mol tocopherol/LDL particle [37].

Human supplementation studies with the various forms of α-tocopherol have invariably shown that ex vivo copper-induced peroxidation of LDL decreases and that oxidation is delayed by 15–20 min [38] corresponding to a delay of 4 min per mol tocopherol increase per LDL particle [37]. Daily dosages needed for this effect are 400 IU for healthy controls [39,40] and at least 750 IU for T1DM and T2 DM patients [21,41]. In this study we observed that Atorvastatin did not affect the lagtime and increased the total production of TBARS by 18%. Supplementation with α-tocopherol prolonged the lagtime by 13 min and counteracted the increase in total production of TBARS but did not achieve any significant decrease. This is in stark contrast to the 50–60% decreases observed in the above-mentioned studies conducted in patients receiving similar tocopherol dosages but no lipid-lowering therapy [21,41]. It is also interesting to note that α-tocopherol supplementation only decreased peroxidation in the early stages of the incubation with copper (30–120 min). It did not counteract the Atorvastatin-induced increases in TBARS at later time points (after 150 min). Furthermore, Atorvastatin did not affect the capacity of the particle to transport tocopherol because its content in the LDL+VLDL fraction did not decrease in AP and in group AE it increased to the same extent as in patients not receiving statins (a doubling relative to lipids). These observations suggest that Atorvastatin, by altering lipoprotein composition, affects stages or factors of the peroxidation cascade that are not modulated by α-tocopherol. For instance, copper-induced peroxidation is determined by the content in hydroperoxide, cholesterol [42], PUFA [43,44], the linoleic to oleic ratio [45] and by the antioxidant composition [46,47]. Of particular interest are observations showing a decrease in coenzyme Q10 levels after statins [48] and that increased lipid peroxidation in myocardial ischemia patients treated with pravastatin was reversed by ubiquinone supplementation [49].

Another factor affecting susceptibility to peroxidation is particle density. Small dense LDL is more prone to peroxidation [50]. Atorvastatin treatment has been shown to lead to an increase in LDL particle diameter in combined dyslipidemia and T2DM [51]. Our previous study with T1DM patients, confirmed that Atorvastatin induced a shift of LDL fractions towards the less dense regions [22,52]. In this study, the same tendency was observed after a lower dose statin given for a longer period, but not when α-tocopherol supplements were added. However, the limited power and poor matching with regard to this parameter did not allow any conclusions on treatment effects.

Evidence on the clinical relevance of the lag phase of in vitro oxidation stems from the significant correlations with global coronary atherosclerosis scores [53]. However, it should be stressed that such in vitro methods only give a partial insight on the peroxidation cascade and may therefore not reflect the global in vivo situation. In order to gain an insight into the in vivo lipid peroxidation status we measured plasma malondialdehyde (MDA), one of the many end-products of lipid peroxidation which is largely derived from polyunsaturated fatty acids with more than two double bonds (e.g. arachidonic acid) [54]. Despite the fact that it can represent as little as 10% of all peroxidation products [37] and that its clinical relevance is still controversial [55], plasma levels of MDA are still currently taken as a marker of in vivo lipid peroxidation [56]. We, among others, have observed higher levels in diabetic patients and they were negatively related to levels of endogenous antioxidants such as glutathione and uric acid [57,10]. Treatment with Atorvastatin caused a 35% decrease in plasma MDA levels. Although initial MDA in our patients did not correlate with serum lipids, this decrease seems to parallel the decrease in serum lipids because, relative to lipids, MDA did not decrease significantly in either group. Moreover, α-tocopherol supplementation did not modify the Atorvastatin-induced decrease in plasma MDA. Some studies have shown that α-tocopherol supplements lead to MDA decreases in healthy controls [58], young T1DM [59], in hemodialysis patients [60] and smokers [61]. Other studies, however, did not find any changes in MDA [62] or in other lipid peroxidation products [63]. These conflicting observations support the increasing consensus that Vitamin E is only effective in subgroups of patients defined by factors such as age, sex, type of diabetes, complication profile, presence or not of nephropathy, baseline lipids, oxidative stress status and medications [64]. Our study thus suffers from limitations such as a preponderance of males since it is known that the response to Vitamin E can differ between the sexes [30,58]. Furthermore, the validity of markers such as MDA and isoprostanes has to be confirmed [65] before claiming that determined doses of Vitamin E can affect in vivo oxidative stress [64].

Although lipid peroxidation plays a fundamental role in the formation of the fatty streaks of the atheroma plaque, its impact (and that of α-tocopherol) on the clinical events that are precipitated by plaque progression and rupture is less well known. It is worth noting that the conflicting results of intervention trials with Vitamin E may in part be due to differences in the stage of the atherosclerotic process in the study subjects [66–69,70].

Despite these limitations, our results are relevant when considering the use of lipid lowering drugs with or without Vitamin E supplements for the long-term primary prevention of atherosclerosis in T1DM. They demonstrate that the antioxidant efficacy of α-tocopherol is attenuated when given together with Atorvastatin but that the degree of lipid-lowering is not affected.

## Acknowledgements

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